ISBN 978-83-66216-38-9

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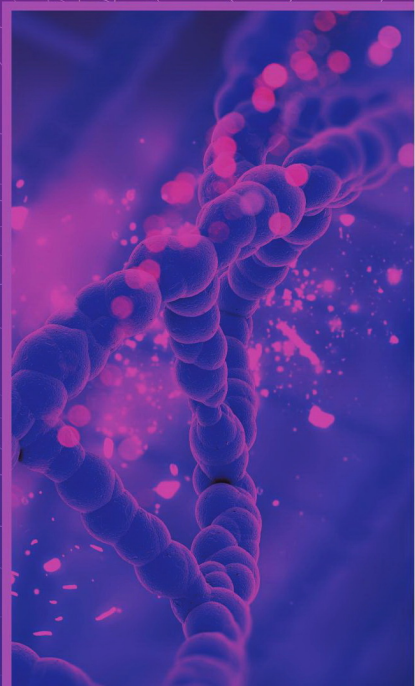
MONOGRAPH



iScience
Warsaw, Poland - 2020

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Warsaw-2020

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S. Kh. Ikramova: The effect of hydrocortisone on apoptosis and proliferation of normal and tumor cells in experimental carcinogenesis. Monograph. – Warsaw: iScience Sp. z o. o., 2020 – 79 p.

Hormone replacement therapy is one of the often-used therapeutic methods, designed to change the proliferating activity of normal or transformed cells. Frequently, it is applied to the so-called "hormone-dependent tissues", that is, cells whose division or death processes directly depend on the concentration of certain hormones, as well as the presence or absence of receptors for them.

However, in some patients, estrogen and progesterone receptors (most often used in the treatment of breast cancer) are absent.

Overcoming the resistance of tumor cells to traditional hormone therapy contributes to the search for alternative ways of regulating cell division or death, using other hormonal drugs, which was chosen as the hormone hydrocortisone. Hydrocortisone stimulates bone marrow hematopoiesis - erythropoiesis, the formation and maturation of a neutrophilic series of leukocytes and platelets.

This monograph is intended for use by oncologists as well as for self-training of students and master's degree students of medical universities.

ISBN 978-83-66216-38-9

CONTENTS

INTRODUCTION

In recent years, many studies have appeared on the possibility of improving hormone replacement therapy for malignant neoplasms. This is due to new data on the mechanism of regulation of transformed cells, the possibility of replacing the main regulators of cell proliferation and death, with a genetic malfunction in their expression or function, by other biologically active compounds. In most cases, special attention in identifying such agents is given to the natural regulators of metabolism - hormones.

Hormone replacement therapy - is one of the most commonly used therapeutic interventions that can alter the proliferating activity of normal or transformed cells. Most often, it is applied to the so-called "hormone-dependent tissues", the processes of cell division or death in which directly depend on the concentration of certain hormones, as well as the presence or absence of receptors for them.

In connection with the introduction of methods for detecting hormone receptors used in hormone therapy of tumors, such as estrogens, progesterone, steroids, a problem arose because some of the transformed cells (breast, thyroid gland, epithelium of the large and small intestines, etc.) do not have pharmacoprerogative hormones on their surface due to deep genetic rearrangements. There are many of such mutant tumor cells, for example, breast tumor cells with the absence of estrogen and progesterone receptors were found in 15% of patients. Therefore, overcoming the resistance of cells of such tumors to traditional hormone therapy promotes the search for alternative ways of regulating cell division or death using other hormonal drugs that can take on the function of proliferation inhibitors or apoptosis inducers of transformed cells, while not adversely affecting normal cell processes.

Scientific research on the discovery of new properties in the "old" hormones began recently, but great success has already been achieved in this direction. For example, the main glucocorticoid hormone formed in humans and mammals is hydrocortisone (cortisol). It has a multifaceted effect: it controls protein, carbohydrate and fat metabolism. Under physiological conditions, hydrocortisone acts as a regulator of homeostasis, exercising control over immunocompetent systems, preventing a hyperactive reaction during the immune response and inflammation.

Hydrocortisone stimulates bone marrow hematopoiesis - erythropoiesis, the formation and maturation of a neutrophilic series of leukocytes and platelets. The molecular mechanisms of action of hydrocortisone are determined by:

- Binding to DNA regions located in the promoter part of the steroid-responsive gene;

• Interaction with transcription factors (AP-1, NF - kB) and inhibitory proteins (1kB) of gene regulators of immunomodulatory and anti-inflammatory cytokines, and their receptors, adhesion molecules, etc.

A change in the level of endogenous hydrocortisone is often manifested in the processes of malignant transformation of cells. The risk of malignant neoplasms increases with adrenal hyperfunction.

It has been established that glucocorticoid hormones are regulators of cell proliferation and differentiation and slow down the growth of some tumors of the colon and cell lines. The action of glucocorticoids is regulated at the proreceptor level through the expression of 11 beta isoforms - hydroxysteroid dehydrase (11 beta HSD 1,11 beta HSD 2), which are responsible for the mutual conversion of hormone-active hydrocortisone into cortisone. Comparison of the therapeutic efficacy of two inducers of apoptosis - hydrocortisone and adriamycin on AKR lymphoid cell lines and B-16 melanoma in young and old animals. When using adriamycin against AKR lymphoma and B-16 melanoma, the results were opposite.

In patients with progressive malignant neoplasms and in the pre- and postoperative period, immunological suppression is observed. Hydrocortisone causes the redistribution of immunocompetent cells in various organs and tissues, which is especially important for surgical intervention.

The main factor determining the individual resistance of tumor cells is the genetic features that are manifested in the over- or hypoexpression of proteins or receptors (76). Certain oncogenic proteins, proliferation, and apoptosis are related factors. Success in understanding the mechanisms of the emergence of drug resistance and ways to overcome it help to improve approaches to the treatment of malignant neoplasms. First of all, this is when there are no specific receptors on the surface of tumor cells for conducting pharmacological therapy with conventional chemotherapy drugs, which does not give a positive result and leads to side effects.

Therefore, the goal of replacement therapy in order to overcome drug resistance is to search for alternative mechanisms of inhibiting the proliferation of transformed cells and inducing their death. Apparently, hormones, in particular hydrocortisone, can be such natural factors in the regulation of metabolic processes in the body, which is of great importance both scientifically and in practice.

The purpose of the monograph is to study the possibility of inhibiting the proliferation and induction of apoptosis of tumor and normal cells when exposed to hydrocortisone and the mechanism of its cytotoxic effect on transformed cells.

Purpose of monography - in this regard, the following tasks had to be solved:

1. Identification of transformed breast cells with no estrogen receptors on their surface and quantitative characteristics of HER - 2 / neu receptors;

2. The study of the cytotoxic effect of hydrocortisone on transformed breast cells in an in vitro experiment;

3. The study of the induction of hydrocortisone apoptosis in the cells of an experimental tumor of the colon adenocarcinoma (AKATOL) in vitro experiments;

4. Antitumor activity of hydrocortisone in in vivo experiments on laboratory animals (BALB / c mice);

5. The study of the morphological features of tumor cells (AKATOL) when exposed to hydrocortisone;

6. Inhibition of proliferation and induction of apoptosis by hydrocortisone in experimental tumor tissue and in bone marrow cells of experimental animals.

The scientific novelty of the work. For the first time, the possibility of including processes of inhibiting the proliferation and induction of apoptosis of tumor cells through the action of various doses of hydrocortisone is theoretically substantiated, and the mechanism of the cytotoxic activity of this hormone on transformed and normal cells is described.

The practical significance of the work. In vitro studies have established that reducing the dose of the therapeutic effect (to 0,025 mg) does not change the quantitative expression of HER - 2 / neu on the surface of breast tumor cells, and the most effective cytotoxic concentration of hydrocortisone for tumor cells was 0,05 mg / 1,6.10 cells.

It was established that in vivo experiments, hydrocortisone has antiproliferative activity and inhibits the growth of tumor cells.

Hydrocortisone has a negative effect on the metabolism and vital activity of tumor cells. Low doses affect it more sparingly on the body as a whole, but with a pronounced effect on tumor cells.

High doses of hydrocortisone reduce the proliferation of normal hematopoietic cells, and lower doses (1.25 and 2.5 mg / kg) did not have a toxic effect on them. The most optimal dose is 2.5 mg / kg, which, while maintaining high antitumor activity, does not cause resistance of tumor cells. However, it has side effects in the form of a decrease in the proliferation of hematopoietic elements.

CHAPTER I.
HYDROCORTISON: BIOLOGICAL EFFECTS, MECHANISM OF ACTION, RESEARCH IN EXPERIMENTAL CARCINOGENESIS

The morphology and physiology of the adrenal glands, the value of their hormones in humans and animals, since 1948, has been the subject of a large number of studies. In the adrenal cortex, with the participation of many enzymes, a complex chain of biochemical transformations occurs, as a result of which, 3 main biologically active compounds are ultimately formed and secreted into the blood: aldosterone, glucocorticoid hormone (hydrocortisone, cortisol) and androgens.

Aldosterone is one of the regulators of water and electrolyte metabolism. It contributes to the delay in the body of sodium, water and the elimination of excess potassium.

Androgens still regulate the formation of the external genital organs in utero, and subsequently participate in the control of the appearance and development of secondary sexual characteristics. Androgens have anabolic effects, especially in the bones and muscles, and regulate the growth of the child. On their basis, anabolic hormonal drugs were created - methandrostenolone, nerobol, dianabol, nerobolil, retabolil and others, which have a weakened androgenic effect and enhanced anabolic properties.

Glucocorticoids (HA) are lipophilic substances that are distributed throughout the body. The approximate volume of distribution of prednisolone in the human body is 0,35 – 0,7 l / kg. This is due to the very high ability of glucocorticoids to bind to serum proteins. Steroid-binding proteins include albumin and transcortin. At a low concentration of 80 - 70%, HA is associated with protein. However, when using high doses of glucocorticoids, their protein-binding ability is reduced to 60 - 70% due to saturation of transcortin-binding sites and a greater amount of free drug diffuses into peripheral tissues. This leads to a higher volume distribution of glucocorticoids and increases the risk of side effects. Similar processes can be observed in patients with hypoalbuminemia, in which it is recommended to use lower doses of the drug.

Glucocorticoid metabolism occurs in the liver by hydroxylation and conjugation. Less than 15% of glucocorticoids are excreted unchanged in the urine. With an increase in the volume of distribution and concentration of the drug, its clearance increases. However, a violation of the overall clearance is noticeable only when using high concentrations of prednisolone (about 70 mg / day). Factors leading to a decrease in the clearance of glucocorticoids

include liver and kidney failure, the elderly patients (over 65 years), the combined use of certain drugs (ketoconazole, oral contraceptives, etc.). An increase in clearance is observed in individuals who have been receiving glucocorticoids for a long time, suffering from hyperthyroidism, as well as when using certain medications (phenytoin, rifampin and barbiturates, etc.). Glucocorticoids mainly differ in the duration of biological effects and in the ratio between glucocorticoid and mineralocorticoid activities. The glucocorticoids used in the clinic are conditionally divided into short, medium and long-acting drugs. This division is based on the duration of ACTH suppression after a single dose of the drug in a dose equivalent to 50 mg of prednisolone. The half-life of hydrocortisone in blood plasma is 80 - 115 minutes, and for other glucocorticoids this indicator is: 30 minutes for cortisol, 3,4 -3,8 hours for prednisone, 2,2-3,5 hours for prednisone, 1,3 – 3,1 hours - for methylprednisolone (MP), 1,8 – 4,7 hours - for dexamethasone. Differences in the half-life of glucocorticoids depend on the pharmacokinetics of the drugs, which in turn is closely related to the doses of hormones used. The change in the kinetics of glucocorticoids depending on the dose is a consequence of the nonlinear binding of hormones to plasma proteins: with an increase in the dose, the number of glucocorticoids unrelated to the protein increases. The relationship between half-life and biological activity is also not absolute. For example, prednisolone and dexamethasone have the same half-life, but dexamethasone has a more pronounced glucocorticoid activity.

Based on data on the duration of ACTH suppression, glucocorticoids are divided ***into 2 main groups****:*

- with short activity - hydrocortisone, cortisone, prednisone, methylprednisolone, triamsinolone;
- with prolonged activity - dexamethasone, betamethasone.

The main glucocorticoid hormone formed in humans is hydrocortisone (cortisol), it has a multifaceted effect in the body; control of protein, carbohydrate and fat metabolism. The regulatory effect of hydrocortisone is carried out by its effect on the activity of many enzymes outside and intracellular. Hydrocortisone has a catabolic effect - it delays the synthesis of its own proteins and contributes to their breakdown, especially in bones and muscles. Under physiological conditions, the action of hydrocortisone in relation to all types of metabolism is regulatory, i.e. it delays protein synthesis, for example, then and to the extent that the body needs it at any given moment. The "excess" amino acids under the action of hydrocortisone are spent on the synthesis of carbohydrates (gluconeogenesis)

and fats. Under the influence of large doses of hydrocortisone, administered as drugs or formed in diseases of the adrenal glands (tumors), muscle tone and even their volume are reduced. Hydrocortisone can inhibit the formation of connective tissue and delay scarring.

Suppressing the activity of connective tissue enzymes - hyaluronidase and collagenase, hydrocortisone reduces the permeability of membranes and, thereby, reduces the processes of exudation. This is his property and was regarded as anti-inflammatory. However, the anti-inflammatory effect of the hormone includes a number of mechanisms that become more and more complicated as they are studied. In particular, hydrocortisone, affecting intracellular enzymes, reduces the activity of lymphocytes and plasma cells, inhibits activity and reduces the volume of lymph nodes and thymus, thus, reduces the formation of antibodies. Hydrocortisone modulates thymocyte selection and cytokine secretion. It inhibits the release of cytokines (interleukin 1 and 2) from lymphocytes and macrophages, inhibits the release of inflammatory mediators by eosinophils. Glucocorticosteroids stimulate steroid receptors and induce the formation of specific proteins - lipocortins, which have the ability to reduce the formation of superactive radicals, prostaglandins E2 and leukotrienes, and reduce exudation processes, i.e. have anti-inflammatory effect.

Pharmacological doses of glucocorticoids can inhibit the transcription of enzyme genes that are involved in the formation of lipid mediators. They inhibit the transcription of the cytosolic form of phospholipase A2 induced by cytokines, inhibit the expression of cytokininduced cyclooxygenase-2 (COX-2) genes in monocytes and directly reduce the synthesis of COX-2. GCS also suppress the expression of collagenase genes, the expression of adhesion molecules, and inhibit the synthesis of humor increase apoptosis of T and B lymphocytes. Thus, hydrocortisone inhibits the synthesis of prostaglandins that are involved in the development of inflammation both at the level of phospholipase A2 and the formation of arachidonic and other eicosanoic acids, and at the level of cyclooxygenase and the direct formation of prostaglandins (eicosanoids). Hydrocortisone, exercising control over immunocompetent systems, prevents a hyperactive reaction during the immune response and inflammation.

Hydrocortisone inhibits the activity of mast cells of connective tissue, which leads to a decrease in the formation and release of histamine and heparin by them. Both of these biologically active substances are involved in the inflammatory reaction, their reduction is another element of

the anti-inflammatory effect of hydrocortisone. Its antihistamine effect is stronger than that of many antihistamines. Hydrocortisone stimulates bone marrow hematopoiesis - erythropoiesis, the formation and maturation of a neutrophilic series of leukocytes and platelets. Reducing the production of heparin by mast cells, as well as affecting platelet enzymes, hydrocortisone can enhance blood coagulation and thrombosis.

Hydrocortisone regulates carbohydrate metabolism, controlling gluconeogenesis - the formation of carbohydrates due to amino acids. The effect on carbohydrate metabolism led to the designation of hydrocortisone as a glucocorticosteroid, and drugs created on its basis - as glucocorticosteroid drugs.

In addition, hydrocortisone regulates fat metabolism, contributing to the formation of fat due to amino acids. In the presence of larger than normal amounts of hydrocortisone in the body, a peculiar deposition of fat occurs on the face, back, stomach. The effect on fat metabolism also consists in regulating the formation of eicosanoic fatty acids and prostaglandins by acting on the enzymes involved in their synthesis.

Hydrocortisone is involved in the regulation of the transport of butyrate and other short-chain fatty acids (8CPA) located in the colon and colon. It should be noted that butyrate is the preferred energy source for colonocytes and is necessary for the regulation of electrolytes in the colon and colon. It is known that the process of anionic exchange of HCO (3) (-) / 8CPA (-) is one of the main mechanisms of oil transport through the apical membrane vesicles and through the apical membrane of the colon and colon epithelium. It is known that the process of anionic exchange of HCO (3) (-) / 8CPA (-) is one of the main mechanisms of oil transport through the apical membrane vesicles and through the apical membrane of the colon and colon epithelium. Studies have also been conducted to study the incorporation of hydrocortisone and phorbol 12-myristate-13-acetate (PMA) in the possible regulation of the butyrate / anion exchange process in the cells of the Caco-2 colon adenocarcinoma strain. The butyrate / anion exchange process was evaluated by measuring the butyric acid susceptibility in Caco-2 cells. The results showed that a 24-hour incubation of cells with PMA (1 micromole) did not significantly increase oil acid susceptibility compared to incubation with 4a-PMA (inactive form). In contrast, incubation with hydrocortisone had a significant effect on the butyric acid susceptibility in Caco-2 cells compared to a carrier vehicle (ethyl alcohol).

Large amounts of hydrocortisone, endogenously formed in diseases (Cushing's syndrome, etc.) or introduced into the body, contribute to the

excretion of potassium, the retention of sodium and water. Modern synthetic drugs have a much smaller effect on water-electrolyte metabolism than hydrocortisone. Hydrocortisone is involved, along with many other factors, in maintaining blood pressure.

The adrenal cortex is under the regulatory control of the pituitary gland. Adrenocorticotropic hormone (ACTH), secreted by the anterior pituitary gland, stimulates the production and excretion of hydrocortisone and androgens by the adrenal cortex. On the production of aldosterone, ACT has no effect or almost no effect. There is a feedback system between hydrocortisone and ACTH: ACTH, entering in increased amounts into the bloodstream, stimulates the release of hydrocortisone, while the latter accumulates in sufficient quantities for this situation, its level leads to a decrease in ACTH production.

This circumstance is proved by the study of hormone levels, as well as by clinical and morphological observations. After an adrenectomy due to Cushing's disease, an increase in the pituitary gland with increased production of ACTH and an increase in the size of the Turkish saddle (Nelson's syndrome) often develops. In some patients, hypothalamic dysfunction is observed. The pituitary gland and its ACTH products are controlled by the neurohormones of the hypothalamus, which, in turn, is under the regulatory influence of cortical structures.

The molecular mechanisms of action of hydrocortisone are determined by the following: 1) binding to DNA regions located in the promoter region of the steroid-responsive gene; 2) interaction with transcription factors (AP-1, HP-kV) and inhibitory proteins (1kV): gene regulators of immunomodulatory and pro-inflammatory cytokines (TNF-, IL-1, IL-2, IL-6, IL-3, IL- 8 and GM-CSF), their receptors (IL-2P, etc.), adhesion molecules, proteinases (collagenase, stromelysin), etc.

Hydrocortisone exerts its biological activity by binding to cytoplasmic GK receptors located inside target cells that regulate the transcription of a wide range of genes. It is believed that each cell sensitive to hydrocortisone contains from 10 to 100 steroid-response genes. Genes encoding the synthesis of HK receptors belong to the superfamily, which includes genes for the cytosolic receptors of other steroid hormones (progesterone, estrogens, as well as thyroid hormones, retinoic acid and 1.25 (OH) 2 D-GK receptors are expressed almost all cells, however, their density in different cells is not the same. Using the cloned cDNA, the primary structure of the HA receptor gene, consisting of 800 amino acid residues, was established. There is one class of HA receptors that do not differ in structure

and degree of affinity for hydrocortisone in various tissues. However, the mineral-corticoid receptor also binds to HA with high affinity. The C-terminal portion of the inactive GK receptor is associated with a large protein complex (approximately 300 W), which includes 2 subunits of the stress protein Нзр90. This protein ensures the correct assembly of the GK receptor, contributing to the acquisition of a conformation that is optimal for binding to hydrocortisone, and preventing the appearance of a non-hydrocortisone receptor in the nucleus. After the binding of hydrocortisone to the receptor, Нзр90 dissociation occurs, which allows the activated HA receptor to very quickly localize in the nucleus and bind to DNA. The expression of HA receptors is regulated by various factors at the transcriptional, translational and post-translational levels. Long-term GC therapy suppresses the expression of GK receptors in circulating monocytes and white blood cells.

GK receptors regulate the transcription of GK-dependent genes, and due to two main mechanisms: direct and indirect.

Firstly, inside the cell, GK receptors form a dimer that binds to DNA regions called glucocorticoid-responsive elements (g1-corticold receptors-GRE) located in the promoter region of the steroid-responsive gene.

Secondly, GK receptors interact with various transcription factors or nuclear factors (nuclage factor - NF). Nuclear factors, such as the transcription factor activator protein (AP-1 and NF-kB), are regulators of several genes involved in the immune response and inflammation, including genes for cytokines, their receptors, adhesion molecules, proteinases, etc. [30].

AP-1, a heterodimer of Fos and Jun proteins, which are products of the proto-oncogenes c-fos and c-jun, forms a protein complex with activated GK receptors in the nucleus, causing repression of DNA binding. It is believed that glucocorticoids can suppress the effects of those cytokines whose action is due to the activation of AP-1. For example, TNF-alpha and phorbol ester increase the binding of AP-1 to DNA, which is inhibited by HA. This effect is mediated by interaction with GRE, which causes repression of the corresponding cytokine genes. HA are potent inhibitors of transcription of the collagenase gene induced by TNF-a and phorbol ester, which is also regulated by factor AP-1. Stimulation of T-lymphocytes is mediated by activation of AP-1, which leads to the induction of several genes encoding IL-2, IL-2P, and cell proliferation. This activation is also suppressed by hydrocortisone. Besides, hydrocortisone, as already noted, can inhibit the expression of cytokine genes indirectly. The genes of some cytokines have been found to be particularly sensitive to the inhibitory effect of

hydrocortisone. For example, the IL-6 gene has at least 4 negative GREs located very close to the promoter site, and 1 GRE located near the site from which transcription begins. In addition, hydrocortisone enhances the mRNA destruction of cytokines such as IL-1, IL-3 and GM-CSF. Hydrocortisone not only blocks the synthesis of cytokines, but can also cancel their effects. One of the mechanisms of its action is associated with the suppression of the synthesis of cytokine receptors, in particular IL-2P.

Recently, another important mechanism of action of hydrocortisone has been discovered, associated with the influence on the transcriptional activation of the cytoplasmic inhibitor NF-kB - lkBa. NF-kB is a natural transcription factor involved in the expression of genes of various pro-inflammatory cytokines (IL-8, IL-6, IL-2, etc.). In unstimulated NF-kB cells, the heterodimer is in the cytoplasm in the form of an inactive complex with inhibitory proteins related to family 1kV (IkVA, 1kVV, IkV-P), and in the form of an inactive precursor molecule (MP-kV1 and No.-1 <B2). Upon stimulation of immunocompetent IL-1 cells, TNF of 1 kV is rapidly destroyed, and the free NF-kB dimer translocates to the nucleus, where it activates target genes, including the gene encoding the synthesis of its inhibitor (1 kVA). There is evidence that the binding of the activated HA-receptor complex to NF-kB prevents the interaction of the latter with DNA and an increase in gene transcription. In addition, it has recently been shown that HAs have the ability to enhance transcription and 1 kVA synthesis [43, 47].

Adrenal cell proliferation causes 3-7% of malignant neoplasms of the kidneys. Mostly these are mild adrenocortical adenomas, which can be functional or functional. More rarely, this leads to primary adrenal cancer. It has been established that mild functional adenomas or hyperplasias cause overexpression of aldosterone. Progression from adrenal adenoma to cancer involves monoclonal proliferation of cells that, among other defects, underwent chromosomal duplication at 11p15.5, leading to overexpression of the IGF2 gene and inhibition of the work of CDKNIC and H19 genes. The TP53 gene is expressed during cancer progression in most patients. Some key oncogenes and genes that suppress tumor growth remain unidentified, although the chromosomal places in which they lie may be located 17p, 1p, 2p16, and 11q13 for genes that suppress the growth of transformed cells on chromosomes 4, 5, and 12 for oncogenes.

Thus, the adrenal glands are part of a complex neuroendocrine complex that implements the body's adaptation processes. Hydrocortisone, changing the metabolic processes to the side necessary for the body at any

given moment and modulating the immune response, implements direct adaptation processes.

A change in the expression of hydrocortisone is often manifested in the processes of malignant transformation of cells. In itself, adrenal gland metastases of tumors are quite common. So, it was noted that metastases of breast cancer affect the adrenal glands in 58% of cases, bronchogenic lung cancer in 3640%, and melanoma in 33% of cases. At the same time, 1-NN in this case develops very rarely, since, as indicated, the destruction of 90% of the cortex of both adrenal glands is necessary. The most common tumor, the metastases of which cause the development of the clinical picture of hypocorticism, is non-Hodgkin's large cell lymphoma, and metastases of bronchogenic lung cancer are somewhat less likely to cause the disease.

For many decades, glucocorticoid function of the adrenal glands was evaluated by the level of daily excretion of 17-hydroxycorticosteroids with urine (Porter-Zilber reaction). Despite the fact that 70-80% of the excreted metabolites of hydrocortisone belongs to the fraction of 17-ketogenic steroids (cortol, cortolone), 17-ACS contains the most physiologically important steroids - cortisol, cortisone, 11-deoxycortisol and their tetrahydroforms. Normally, the level of 17-ACS excretion in adults is approximately 8.2–22 μmol (3–8 mg) per day [26]. Despite the rather low diagnostic value of this study, its results, for comparison with the literature data of previous years, continue to be described in modern works. Even less informative is the assessment of the daily excretion of 11-hydroxycorticosteroids, the fraction of which, in addition to hydrocortisone and cortisone, includes corticosterone. Excretion of 11-ACS with urine is normally 250-830 nmol per day. In addition, there are various options for studying the plasma level of corticosteroid metabolites, including as part of dynamic tests.

Glucocorticosteroid drugs are widely used therapeutic agents in pediatric practice. The history of the introduction of glucocorticosteroids presents a bright page in pharmacology and clinical pediatrics. In 1948, P. Hench and his staff first used hydrocortisone to treat patients with rheumatoid arthritis and received a pronounced positive effect. This event was the beginning of a detailed study of the adrenal glands and the pituitary gland, the widespread use of drugs created on the basis of glucocorticoid hormones of the adrenal glands, with a number of diseases, the creation of new synthetic hormonal drugs.

In the first decade, the use of glucocorticosteroids, their appointment gave great hope for the success of the treatment of rheumatoid arthritis, bronchial asthma, glomerulonephritis and a number of other diseases.

However, with the accumulation of observations, significant, often health-threatening unwanted effects and complications of glucocorticosteroid therapy (hormonal dependence, inhibition of the adrenal cortex, osteoporosis, gastrointestinal ulcer, etc.) were revealed. These circumstances made it possible to minimize the use of glucocorticosteroids for bronchial asthma, and for rheumatoid arthritis in 1970-1980 they almost abandoned their use. But the development of pharmacologists, the creation of new dosage forms of glucocorticosteroids and the modification by clinicians of the methods for the use and prevention of complications, led to a return to the use of glucocorticosteroids in a number of diseases, including bronchial asthma and rheumatoid arthritis. To date, the indications and contraindications for the administration of glucocorticosteroids have been quite clearly formulated, the methods for their appointment have been developed, although improvement continues.

Glucocorticosteroids - refers to potent drugs that affect all types of metabolic processes, the immune status of the body. With a number of diseases, it is not possible to obtain an improvement in the patient's condition without the use of these drugs, which served as the basis for calling these drugs "the disease with controlling agents." In tablet form, hydrocortisone is rapidly absorbed. The bioavailability of the drug ranges from 50% to 100%. It depends on the individual characteristics of the patient, the state of the gastrointestinal tract and metabolic processes. The time to reach maximum concentration is also subject to significant fluctuations and is 1.5-3 hours. Upon receipt in the circulation of 80-90% hydrocortisone binds to proteins - albumin and transcortin. Transcortin delivers hydrocortisone to the cells.

Only part of the drug that is not related to protein has biological activity. When prescribing large doses of hydrocortisone, a significant part of the drug remains unrelated to the protein and is distributed in the tissues. The binding ability of transcortin changes during the day.

Long-term treatment with hydrocortisone suppresses adrenal cortex function and ACTH production by the anterior pituitary gland, and also reduces the ability of transcortin to bind hydrocortisone. In tissues, hydrocortisone binds to cytoplasmic glucocorticosteroid receptors, which are located inside the cells and regulate gene transcription. Glucocorticosteroid receptors are expressed in almost all cells of the body, however, the density of receptors in the cells is different. Long-term therapy with large doses of hydrocortisone inhibits the expression of glucocorticosteroid monocyte receptors. Hydrocortisone remains bound to the corresponding receptors in the cells and after it disappears from the circulation.

All glucocorticosteroid drugs inhibit the secretion of glucocorticosteroid hormones by the adrenal cortex and the production of the anterior pituitary gland ACTH. However, the degree and duration of this effect is different for the drugs. The most pronounced suppression of ACTH secretion is observed in dexamethasone.

The metabolism of hydrocortisone, like natural hormones, occurs in the liver. Most hydrocortisone is excreted by the kidneys in the form of esters of glucuronic and sulfuric acids. Hydrocortisone metabolism was monitored by administering labeled drugs to volunteers. For 48 hours, up to 90% of intravenously administered hydrocortisone is excreted in the urine, a small part of the drug is excreted in the feces. With renal and liver failure, as well as with ketoconazole (nizoral), the clearance of hydrocortisone decreases. The intake of barbiturates and rifampicin increases the clearance of hydrocortisone.

Glucocorticosteroid drugs, along with a therapeutic effect, cause a number of undesirable manifestations. They are called "side effects" conditionally, since these effects are characteristic of natural glucocorticosteroid hormones and synthetic glucocorticosteroid drugs. There is no doubt that the development of undesirable effects is caused, on the one hand, by the dose of the drug, and on the other, by the individual characteristics of the patient, genetic factors on which the state of the immune status, sensitivity of the receptors, the development of a disease, its course and reaction to therapy depend.

Undesirable manifestations of hydrocortisone therapy can be divided into 2 groups.

I - group - frequent, but not hazardous to health, depending, first of all, on the dose of the drug. These are manifestations of exogenous hypercorticism - increased appetite, weight gain, skin and trophic changes: dry skin, thinning, striae, acne, increased capillary pattern on the palms of the hands. With large doses of hydrocortisone and its prolonged use, these phenomena occur in almost all children. A common reaction to hydrocortisone treatment is leukocytosis. Hypokalemia and enlargement of the liver, in which hydrocortisone metabolism occurs, can be observed.

II - a group of undesirable manifestations can be considered as complications of hormonal therapy. They are found infrequently and depend not so much on the dose as on the individual characteristics of the patient, his genetic and constitutional predisposition. These are suppression of the function of the adrenal cortex and ACTH production, infectious complications, increased blood pressure, thromboembolic complications,

osteoporosis, ulcerative process in the gastrointestinal tract, hyperglycemia and glucosuria, posterior subcapsular cataract, mental disorders, myopathy, growth retardation.

Suppression of adrenal cortex function and ACTH production was clinically confirmed in the first decade of hydrocortisone administration. Descriptions of cases of development of acute adrenal insufficiency during surgical interventions or injuries in people who received hydrocortisone were published. However, with the accumulation of clinical experience and the study of the function of the adrenal glands and the pituitary gland, they learned to avoid such a complication by taking measures to restore the function of the adrenal cortex and pituitary gland.

The higher the doses and the longer the course of treatment, the slower, more gradual should be the withdrawal of the drug, which ensures a gradual restoration of adrenal and pituitary function. According to E. G. Garber, a full recovery of function requires a long time, possibly up to a year.

Infectious complications of hormone replacement therapy are currently infrequent, since antibiotics are usually prescribed during the administration of a large dose of the drug.

Thromboembolic complications are due to the ability of hydrocortisone to suppress the formation of heparin by mast cells. Such a complication threatens when using large doses of hydrocortisone against the background of hypovolemia and hypercoagulation, therefore, in severe patients, primarily with nephrotic syndrome, constant monitoring of the circulating blood volume, correction of hypovolemia and frequent administration of anticoagulants (heparin) and antiplatelet agents (pentoxifylline, ticlide, dipyridamole and etc.).

Steroid osteoporosis develops as a result of exposure to bone tissue of an excessive amount of glucocorticosteroids. Hydrocortisone treatment of certain diseases is accompanied by the development of osteoporosis, while the duration of use, dose and age of the child are important. The development of steroid osteoporosis is also observed with high endogenous overproduction of hydrocortisone (Cushing's syndrome). According to statistical studies conducted by the Japanese Ministry of Health and Fertility, in 1999, 2016 cases of Cushing's syndrome were noted.

Osteoporosis during treatment with glucocorticosteroid drugs is due to the biological effects of the natural hormones of glucocorticosteroids, which are based on molecular mechanisms of interaction with glucocorticosteroid receptors of osteoclasts and osteoblasts.

Disturbances in the expression of hydrocortisone can cause malignant transformations of a wide variety of body cells. The most common adrenocortical adenomas. The risk of malignant neoplasms is high with adrenal hyperfunction (1,7 % of cases), and the risk is higher in patients with tumors greater than 3 cm. Increased secretion of hydrocortisone is found in approximately two thirds of cases of adrenal gland tumors.

It should also be noted the development of hormonal resistance in target cells of hydrocortisone. Most often, such cell tolerance develops with nephrotic syndrome. In the development of hormone resistance, pronounced metabolic disturbances (significant hypoalbuminemia) may be important, requiring correction; persistent viral infection, which is an indication for the use of interferon (leukinferon, viferon, interferon, etc.): foci of bacterial infection requiring rehabilitation, as well as the presence of allergies. However, the main cause of hormone resistance is the morphological features of glomerulonephritis. Hormone-resistant variants of the nephrotic syndrome are an indication for a kidney biopsy.

The therapeutic effect and undesirable effects of hydrocortisone, therefore, depend on: the dose of the drug that enters the circulation; sensitivity of specific receptors and drug interactions with them; the duration of the circulation of the drug and the time of its binding to receptors; the severity of the underlying disease. Hydrocortisone has a multifaceted effect on bone tissue, which in total activates bone remodeling. The latter leads to an increase in the rate of resorbtion without compensatory growth of bone formation and the development of osteoporosis.

In recent years, studies have intensified on the possibility of improving hormone replacement therapy for malignant neoplasms. This is primarily due to new data on the mechanism of regulation of cell transformation, the possibility of replacing the main regulators of tissue proliferation during genetic failure in their expression or work with other biological active compounds. In most cases, special attention when searching for such agents is given to the natural regulators of the metabolism of body cells - hormones.

Hormone replacement therapy is one of the often used therapeutic effects designed to change the proliferating activity of normal or transformed cells. Most often, it is applied to the so-called "hormone-dependent tissues", that is, cells, the processes of division or death of which directly depend on the concentration of certain hormones or the presence or absence of a receptor for them.

The most common method of using hormones is "pulse therapy." "Pulse therapy" - therapy in excess of high doses of hormones administered intravenously, is used for the extremely severe course of some diseases. First of all, these are crisis states in case of systemic diseases of connective tissue, processes of malignant transformation of cells, in some cases, hormone-resistant forms of the nephrotic form of glomerulonephritis. "Pulse therapy" with hydrocortisone was first used to combat the rejection crises of transplanted organs, which are based on immune processes. Large doses of the hormone affect the state of B-lymphocytes, which leads to a significant decrease in the production of immunoglobulins, autoantibodies and CEC. The synthesis of pro-inflammatory interleukins-1, -6, -8 is reduced, and the degradation of genes controlling the synthesis of interleukins and their receptors is enhanced.

In recent years, in connection with the introduction into general clinical practice of methods for detecting hormone receptors that are traditionally used in hormone replacement therapy for tumors, such as estrogens, progesterone, steroids, another problem has arisen that has not previously occurred in the appointment of hormone therapy. Part of the transformed cells, and most often these are cells of hormone-dependent tissues (mammary gland, thyroid gland, epithelium of the large and small intestines, etc.) do not have receptors for pharmacoprogressive hormones on their surface due to deep genetic rearrangements that occurred during cell transformation. At the same time, such tumors mutant in hormonal receptors are very significant. Thus, the absence of estrogen and progesterone receptors on the surface of breast cancer cells occurs in approximately 15% of patients. One way to overcome the resistance of such tumors to traditional hormone replacement therapy is to find alternative ways to regulate the processes of their division or death using other hormonal drugs, which in this case can take on the function of proliferation inhibitors or apoptosis inducers of transformed cells.

Scientific research on the discovery of new properties in "old" hormones is conducted in the world relatively recently, but great successes have already been achieved in this direction. Hydrocortisone is no exception in such studies. The possible use of this hormone in cancer therapy has been the subject of many studies.

At the Frankfurt-Hoechst City Clinic (Frankfurt, Germany), studies were conducted on the use of a synthetic derivative of hydrocortisone in combination therapy for metastatic breast cancer. The results showed a positive trend in similar therapy. Of the complications associated with the use

of high doses of hormone therapy, the development in some cases of severe multiform erythema, a pathological reaction of the skin to the administration of drugs, is noted.

Glucocorticoid hormones have been shown to regulate cell proliferation and differentiation and slow down the growth of certain colon tumors and adenocarcinoma cell lines. The action of glucocorticoids is regulated, in particular, at the proreceptor level through the expression of 11 beta isoforms - hydroxysteroid dehydrase (11 Beta HSD I, 11 Beta HSD 2), which are responsible for the mutual conversion of hormone-active hydrocortisone into cortisone. Since both of these isoforms are expressed in colon cells in mammals, studies have been conducted to study the expression of 11 beta HSD1 and 11beta HSD 2 in the epithelium in colorectal cancer in humans. It was found that 11beta HSD isoforms are expressed in colon adenocarcinoma, however, their representation is not identical in tumor and non-tumor tissue. There is a significant decrease in 11beta HSD 2 prevalence, a decrease in mRNA amounts, and enzyme activity in tumor tissue. In contrast, 11 beta HSD1 activity and mRNA abundance were increased in some, but not all, tumor samples. The results demonstrate that malignancy is associated with a decrease in steady-state levels of 11beta HSD 2 mRNA and enzyme activity and, in some cases, also with an increasing expression of 11beta HSD1. It is also noted that colorectal tumor cells have a reduced ability to autocrine inactivation of glucocorticoids.

A case of the use of combined hormonal therapy with steroids and glucocorticoids in combination with other chemotherapeutic drugs for the treatment of Hodgkin's disease, one of the common tumor diseases in pediatric oncology, is described. The authors describe a patient with Cushing's syndrome who suppressed the development of Hodgkin's disease concomitant with this syndrome. After removing his pituitary tumor, Hodgkin’s disease began to progress rapidly, which forced doctors to use combination therapy with hydrocortisone.

The efficacy of co-therapy with ketoconazole (300 mg three times daily) and progesterone in 38 patients with prostate cancer was investigated. Of these 38 patients, 21 (55.3%) showed a decrease in PSA antigen> 50% and their average life expectancy was up to 6 months (range 3-48 months). A decrease in PSA> 50% was observed in 21 of 34 patients (61.8%) with established metastases. Thirteen patients (34.2%), all who had metastases, showed a decrease in PSA> 80% on average, their life expectancy up to 9 months (range 3-48 months). Overall, 12 patients (31.6%) showed toxicity

associated with an intermediate dose of ketaconazole, but only 6 patients (15.8%) discontinued therapy due to unbearable side effects.

In patients with pancreatic cancer with a large loss in mass, as a result of the progressive course of the disease, there is a decrease in leptin ($P < 0.05$), but a higher expression of hydrocortisone, interleukin 6, a decrease in energy expenditure, an increase in fat oxidation than in healthy subjects ($n = 6$, $P < 0.05$). According to observations during 4 hours, during the nursing period, the areas of the curves for glucose, hydrocortisone, and interleukin 6 were larger ($P < 0.05$), and less for leptin in groups of patients with pancreatic cancer ($P < 0.05$). A low concentration of leptin, increased fat oxidation, and insulin resistance are associated with increased concentrations of hydrocortisone and interleukin 6, which indicates the participation of these biologically active substances in the body's defense reactions during malignant transformation of pancreatic cells.

4 patients with periocular hemangioma underwent hormone therapy with steroids triamcylon and betamethasone. Changes in serological concentrations of hydrocortisone and a reaction to synactene were monitored. Long-term suppression of circulating serological concentrations of hydrocortisone and hormone responses to synactene was noted in 3 cases. Moreover, in all 4 cases there was a tendency to proliferation of capillary hemangioma. Thus, adrenal suppression of hydrocortisone expression after steroid injection for periocular capillary hemangioma is a potentially life-threatening complication. The proliferation of transformed cells is a frequent side effect with such therapy, which indicates the inherent property of hydrocortisone to regulate cell proliferation in eye pathologies. The therapeutic efficacy of two apoptosis inducers was compared - hydrocortisone and adriamycin on AKR lymphoma and B-16 melanoma cell lines in young and old mice. Administration of hydrocortisone acetate slowed tumor growth only in old mice. Similar effects were obtained with adriamycin in relation to AKR lymphoma, but opposite results were noted in tumor tissue of B-16 melanoma. Thus, age-related antitumor efficacy of two apoptosis-inducing agents was demonstrated. Fifty-five patients with proven leptomeningitis multiple solid cancerous tumors have undergone monitoring studies to determine their pathological reactions. Group M. ($n = 29$) received methotrexate (15 mg) and the MHA group ($n = 26$) received methotrexate (15 mg), hydrocortisone (15 mg / m2) and aga-C (30 mg / m2) twice a week subcutaneously until a cytological reaction occurs. The primary foci of the tumor were in the lungs ($n = 33$), chest ($n = 13$) and stomach ($n = 5$); adenocarcinoma was detected in 45 patients. The cytological response to

subcutaneous administration of chemotherapy was significantly higher in the MNA group compared with the M group (38.5% and 13.8%, respectively, P = 0.036). The mean life span of tumor cells was 18.6 weeks (MHA group) and 10.4 weeks (M group) (P = 0.029). Thus, the combined subcutaneous administration of methotrexate, 2-hydroxy-6-aminopyrimidine with arabinoside and hydrocortisone showed more beneficial effects than methotrexate monotherapy for leptomeningitis multiple solid cancerous tumors.

Two isozymes of 11beta-hydroxysteroid dehydrase (11-HSD) are responsible for the mutual conversion of hydrocortisone (F) to cortisone (E). Type 1 isozyme, 11-HSD1, acts mainly as an in vivo reductase, activating the translation of E into F, while type 2, 11-HSD2, acts as a dehydrase, inactivating the translation of F into E. 11-HSD1 is most abundant in the liver, and 11-HSDB2 in the kidney. Studies have been conducted to determine which isozyme and organs primarily contribute to the equilibrium of plasma F and E in peripheral circulation and to determine differences in the action of 11-HSD in adrenocortical disorders. The values for the F / E ratios in the peripheral vein were the same as those from the adrenal and renal veins. The double reciprocal graph between the peripheral plasma F and E in patients with various adrenocortical tumors was almost identical to that in healthy people. The F / E ratio in peripheral blood was higher in patients with Cushing's syndrome and was lower in patients with primary aldosterosis and hormone-inactive adrenocortical adenoma than in healthy people. These results suggest that renal 11-HSD2 is the main factor controlling the equilibrium of plasma concentrations of F and E and that hydrocortisone and an excess of aldosterone do not alter the equilibrium of plasma concentrations of F and E, and can also change the content of 11-HSD2.

An excess of androgen in women with polycystic ovary syndrome (PCOS) can be of ovarian and / or adrenal origin due to a change in the metabolism of hydrocortisone. A decrease in the peripheral metabolism of hydrocortisone can be caused by a significant inactivation of hydrocortisone with 5alpha reductase (5a1rpa-R) or a weakened regeneration of hydrocortisone from cortisone 11beta-hydroxysteroid dehydrase (11beta-HSD1), which leads to a decrease in the suppression of the negative pressure-response hormone response and normal hormone response due to excess androgen. First, in order to avoid the obesity-related effects of hydrocortisone, overweight women with PCOS were compared with 19 women from the control group, which closely corresponded to body mass index (BM1). Second, the effects of obesity were studied in 42 women with

a wide range of BM1 PCOS. Sterilization of urinary metabolites of steroids was monitored by gas chromatography and mass spectrometry. Urinary excretion of androgens (androsterone (P = 0.003), etiocholanolone (P = 0.02), C19 sulfate (sulfate cellulose), steroids (P = 0.009)), cortisone metabolites (tetrahydrocortisone (P = 0.02), alpha-cortolone (P <0.001), beta-cortol + beta-cortolone (P <0.001), cortolones (P <0.001), E metabolites (P <0.001), and TCM (P = 0.002) increased in overweight women with PCOS compared with control groups. The ratio of 5 a1rpa-tetrahydrocortisol (5a1rpa-TGF) and 5 beta-THf (P = 0.04) also increased and the ratio of TGF + THF + a1rha-sogto1 / THE + cortolones (P = 0.01) significantly decreased in women without excess weight with PCS according to compared with the control groups of women. Thus, an increase in the rate of production of hydrocortisone and androgens in vivo in women without excess weight with PCOS has been established. Insulin leads to a decrease in steroids with PCOS, but this is not associated with an increased rate of production of hydrocortisone.

Carboplatin (SR) is a commonly used broad-spectrum chemotherapeutic drug in the treatment of malignant neoplasms. The use of SR in 12% of patients leads to allergic reactions of hypersensitivity. The pathophysiological mechanisms of these reactions are not completely known to date. Apparently, reactions of increased sensitivity to SR can be IgE-mediated and are caused by low molecular weight platinum compounds acting as haptens. Platinum salts are also able to initiate the release of histamine from basophilic and mast cells. The case of induction of tolerance to SR in a 65-year-old man is described. During the third course of taking SR, he experienced an anaphylactic reaction. Suspecting the possibility of an anaphylactic reaction due to the release of histamine, therapy was performed to withdraw the patient from a critical condition. After that, a new tactics of treating the patient with CP was compiled. The new treatment regimen consisted of preliminary intravenous administration of hydrocortisone and antihistamines (60 ml / hour for 30 minutes), then CP was administered (100 ml / hour for the next 60 minutes, followed by another 120 ml / hour). Thus, when using drugs based on platinum compounds, combination therapy with hydrocortisone and antihistamines is indicated.

A relationship was found between the occurrence of steroid-dependent ovarian cancer and hydrocortisone secreted by the adrenal adenoma. In a 49-year-old woman, after removal of the left adrenal adenoma, the level of free hydrocortisone in the urine decreased and approached normal values, but the level of marker T (specific oncoprotein during ovarian cell

transformation) increased within the tumor range (3,04 ng / ml). After bilateral ovarian ectomy, the level of marker T decreased and the patient's condition improved. Thus, an association was established between hydrocortisone concentrations and an increase in ovarian cell proliferation.

In patients with progressive malignant neoplasms and in the pre- and postoperative period, immunological suppression is observed. Hydrocortisone causes the redistribution of immunocompetent cells in various organs and tissues, this becomes especially important after surgery. Studies have been conducted to establish serological levels and the circadian rhythm of hydrocortisone in patients with colorectal cancer in the period before and after surgery, as well as to establish the immune status in these patients. In 21 patients with colorectal cancer who underwent surgery, the ratio of the subpopulation of lymphocytes, cortisolemia, and the circadian rhythm of hydrocortisol (23:00 p.m. and 8:00 a.m.) were evaluated. An increase in cortisolemia, a decrease in the total number of lymphocytes and T-helpers in the postoperative period was statistically significant compared with the time before surgery. Patients with altered circadian rhythms were 47% and 36% on days 3 and 7 after surgery, respectively. Before surgery, 19% of patients had an altered circadian rhythm of hydrocortisone, and this was in patients with a central tumor growth site ($p < 0.05$) and with metastases ($p < 0.01$). No association was found between lymphocytopenia, a shift in cortisolemia, and the circadian rhythm of hydrocortisone in patients before and after surgery.

Thus, lymphocytopenia in patients with colorectal cancer is not associated with the level of hydrocortisone and a shift in its circadian rhythm. However, a shift in the circadian rhythm of hydrocortisone, found in 19% of patients, is associated with the detection of metastatic foci in them and with a worse prognosis of the course of the disease.

The efficacy of combination therapy was evaluated, which included the combined use of hydrocortisone, metatrexate (MTX), teniposide, carmustine, methylprednisolone (MBVP) and radiation therapy in patients with primary lymphoma (PCNSL). Combination therapy consisted of two cycles of MVVP and MTX (3 mg / m2), temiposide (100 mg / m2), carmustine (100 mg / m2), methylprednisolone (60 mg / m2), hydrocortisone (25 mg) and two intrathecal injections MTX 15 mg, cytarabine 40 mg and hydrocortisone 25 mg, followed by irradiation of 40 Gy. Sequential therapy lasted 27 months and was performed on 52 patients. One patient died before the start of combination therapy and 5 patients died during its course. 4 patients received radiation therapy after one cycle of chemotherapy and 42

patients completed the full therapy. Hematological signs of toxicity with respect to leukocytes were observed in 78% and in relation to platelets in 24% of patients. Two patients who did not find criteria for an objective reaction survived more than a 1-year period, one of them is still alive without a sign of illness. Eighteen patients died: 11 deaths were due to tumor progression, five cases were probably related to the treatment, and leukocephalopathy resulting from intercurrent disease was detected in each of them. The total survival time was 46 months on average. In general, the effectiveness of the combination therapy was noted, however, a 10% indicator of toxic mortality during therapy emphasizes the need for an individual approach to the appointment of such treatment.

The pituitary adenoma increasing expression of adrenocorticotropic hormone is known as the clinically silent corticotrophic adenoma. To study the mechanism of inducing a corticotrophic adenoma, the concentration of the prohormone hydrocortisone 1/3 (PCI / 3) prohormone convertase was studied using this type of adenoma, as well as with Cushing's disease. All studied samples obtained with Cushing's disease showed a very positive reaction to PCI / 3. On the contrary, the representation of PCI / 3 was very weak in samples obtained with corticotrophic adenoma. The absence of PCI / 3 in corticotrophic adenomas indicates that similar adenomas arise in various types of cells sharing hydrocortisone prohormone (POMC), but the signal from this hormone is inadequate due to an excess of adrenocorticotropic hormone.

23 patients with gastrointestinal cancers were monitored for free hydrocortisone in the urine. Free hydrocortisone in urine (UFC) was compared with blood parameters, tumor size, concomitant diseases, treatment, nutritional status and quality of life. Significant positive correlations were between endogenous hydrocortisone levels and loss of appetite, fatigue, and nausea or vomiting. The results confirm the idea of chronic conditions in violation of hormonal status and cancer that has arisen. The interaction between cytokines and the hypothalamic-pituitary-adrenal (NRA) axis is also important in the treatment and diagnosis.

Studies have been conducted comparing baseline levels of salivary hydrocortisone, daily expression of hydrocortisone, and this dependence in women with breast cancer and a group of healthy women. The study participants were 33 women with cured breast cancer (after 3-5 years) and 21 healthy women. The results showed that women with breast cancer showed higher levels of hydrocortisone than the control group. There were no differences among the groups in the daily expression of the hormone or the

responses to hydrocortisone upon awakening. The results of this experimental study show that women with breast cancer have elevated levels of basic hydrocortisone.

An HPB-AML-1 (AML-1) cell line was obtained from peripheral blood monocytes taken from a patient with acute myeloid leukemia (AML-M1). Morphological and phenotypic studies of AML-1 cells have demonstrated that they contain lipid inclusions and express antigens on their surface, similar to those on the surface of bone marrow stromal cells (MSC). It was found that it is possible to cause the differentiation of AML-1 cells into adipocytes with a mixture of hydrocortisone, methyl isobutylxanthine and indomethacin. In contrast, peroxisome activation of proliferation receptor (PPARamma), which plays a key role in lipid metabolism and is expressed in AML-1 cells, reduced the number of lipid droplets in these cells. Such studies confirm the possibility of the participation of hydrocortisone in the processes of differentiation of blood cells, both transformed and normal.

CHAPTER II.
MATERIALS AND METHODS USED IN MONOGRAPHY

During the research, a commercial preparation of hydrocortisone was used (Gedeon-Richter, Hungary).

Surgical material for the isolation of tumor cells was obtained at the Republican Cancer Research Center of the Ministry of Health of the Republic of Uzbekistan.

We used BALB / c line mice weighing 20-22 g contained in plastic cages (5 per cage) under standardized conditions of relative humidity (50-60%), temperature (22 ° C) and light mode (12 hours of darkness and light). Mice received standard commercial food and drinking water "ab libitum". (Table 2.1. shows data on the type and number of animals used in the experimental work.

All operations when working with growth media and preparations were carried out under sterile conditions using a laminar box. The buffers were prepared on bidistilled water, filtered through membrane filters (0.22 μm Millipor, Germany) and autocanned at 1.2 atm for 30 minutes.

Used organic solvents (ethanol, methanol, phenol, chloroform, acetone) were previously distilled. Glassware before use is pre-sterilized at 160 C for 120 minutes. Equipment, devices, utensils made of polymer materials were irradiated with ultraviolet light for 30 minutes. The experiment used transformed mammary gland cells isolated from surgical tumor material obtained from a patient born in 1926 with a diagnosis of gynecomastia of the right breast.

2.1. Determination of the cytotoxic activity of hydrocortisone on transformed breast cells in an in vitro experiment

Experiment goal	Used animals			Number of animals
	kind	line	sex	
Determination of antitumor activity on a tumor strain of colon adenocarcinoma (AKATOL)	mouses	BALB/c	males	40
The definition of mitotic tumor tissue activity colon adenocarcinoma (AKATOL)	mouses	BALB/c	males	40
Determination of the apoptotic index of tumor tissue of the colon adenocarcinoma (AKATOL)	mouses	BALB/c	males	

Determination of the mitotic index of bone marrow cells	**mouses**	**BALB/c**	**males**	**40**
The study of apoptosis in the cells of the tumor tissue of the colon adenocarcinoma (AKATOL)	**mouses**	**BALB/c**	**males**	**6**
TOTAL NUMBER OF USED MOUSE				**166**

Table 2.1. Type and number of animals used in the experimental work

The tumor tissue was washed with a solution of 0,9 % sodium chloride and trypsinized with a 0,25% trypsin solution according to FS 42-3321-96, heated to 37 ° C.

The resulting cell suspension was washed repeatedly (3-4 times) in 199 medium, containing 100 IU / ml of penicillin and 50 IU / ml of streptomycin, by centrifugation at 1500 rpm for 10 minutes.

The resulting washed cell suspension was divided into 4 groups of $1,6 \times 10^6$ cells each and the volume of each group of cells was adjusted to 1 ml with 199 medium.

Hydrocortisone in doses of 0,025 mg, 0,5 mg and 1,0 mg dissolved in physiological saline was added to three experimental groups of cells. The drug was incubated with a suspension of cells for 1 h at a temperature of 37°C, after which the cells were applied in a thick drop on a glass slide, stained with an equal volume of 0,1% trypan blue solution.

To quantitatively take into account the cytotoxic effect of hydrocortisone using light microscopy, the number of stained (dead) cells was counted under a 400-fold increase. The value of the cytotoxic effect (CTD) was determined by the formula:

$$\mathbf{CTD\ (\%) = (A / B) \times 100}$$

where A - is the number of dead cells;
B - is the total number of cells examined.

2.2. The study of apoptosis in the cells of the tumor strain of colon adenocarcinoma (AKATOL)

AKATOL strain cells were isolated from tumor material taken from BALB / c mice on day 16 after implantation of a tumor. The isolated tumor tissue was washed with a solution of 0,9% sodium chloride and trypsinized with a 0,25% solution of trypsin heated to 37°C. The resulting suspension of cells was washed in 199 medium, containing 100 IU / ml of penicillin and 50 IU / ml of streptomycin, by centrifugation at 1500 rpm for 10 minutes.

After centrifugation, the cell suspension was divided into 4 g. 1,6 • 106 cells in each, the volume of each group of cells was adjusted to 1.0 ml with medium 199, stained with 0,1% trypan blue solution and placed in the form of a thick drop on glass slides. Hydrocortisone in doses of 0,025 mg, 0,5 mg and 1,0 mg dissolved in physiological saline was added to three experimental groups of cells. The drug was incubated with a suspension of cells for 1 h at room temperature, while simultaneously conducting visual observation of selected groups of cells using a light microscope.

The occurrence of apoptosis in tumor cells under the action of hydrocortisone was recorded using morphological features, such as plasma membrane bleeding, nuclear chromatin condensation along the periphery of the nucleus, reduction of its size (pycnosis), formation of high molecular weight DNA fragments (karyorexis), DNA splitting into oligonucleosomal fragments (ladder type), compaction of cellular organelles, a decrease in the volume of the cytoplasm, the acquisition of a bubble-like membrane, budding of cell fragments with the formation of discrete apoptotic bodies surrounded by a membrane and containing compacted remains of organelles and nuclei.

All morphological changes in each experimental group of cells were recorded on film using an EOS300 camera (Canon).

2.3. Immunohistochemical method for determining the expression of estrogen receptors and HER-2 - human epidermal growth factor receptor

The expression of the HER-2 gene (c-erbB2) and estrogen receptors was determined on the membranes of breast tumor cells when exposed to hydrocortisone in vitro at doses of 0,025 mg / 1,6 • 10^6, 0,05 mg / 1,6 • 10^6 and 0,1 mg / 1,6 • 10^6 cells.

Breast cancer cells were obtained from the surgical material of patient X., born in 1957, with a diagnosis of $T_2N_0M_0$ left breast cancer receiving hypoxiradiotherapy at a dose of SOD 7 Gy. Tumor tissue was treated with the solution as in 2.1 and 2.2.

In the obtained suspension and distributed into 4 groups, the number of cells was counted using a Goryaev chamber, the volume of each group of cells was adjusted to 1 ml with medium 199. Hydrocortisone was administered in three experimental groups of cells at doses of 0,025 mg, 0,5 mg, and 1,0 mg each 1,6 • 10^6 cells. The incubation of the drug with a suspension of cells was carried out for 1 h at a temperature of 37°C.

After the end of the incubation time, the cells of each group were fixed for 24 h in a 10% neutral buffered formalin solution, subjected to

conventional wiring, and embedded in paraffin. An immunohistochemical study was performed with streptavidin-biotin by the peroxidase method according to the generally accepted scheme on paraffin sections 3-5 microns thick. Murine anti-estrogen receptor antibodies, clone 6F11 (NovoCastra) and monoclonal antibodies to c-ebB2 proteins (NCL-C-erB, DAKO, Denmark) were used.

Sections after dewaxing were treated with 3% hydrogen peroxide solution to inhibit endogenous peroxidase. Then they were placed in serum to reduce background staining.

Primary antibodies were detected using the streptavidin-biotin kits LSAB ("DAKO") and NegersTest ™ ("DAKO").

Incubated with secondary biotinylated rabbit antibodies (30 min) and the avidin-biotin-peroxidase complex (30 min) at room temperature. Peroxidase was visualized with diaminobenzidine (solution with hydrogen peroxide - 5 min). The nuclei were stained with hematoxylin and the preparations were enclosed in Canadian balsam.

The results of immunohistochemical reactions were evaluated by a semi-quantitative method using a Leisa light microscope (Germany).

The HER-2 gene encodes a transmembrane protein homologous to the epidermal growth factor receptor. In approximately 25-30% of cases in breast cancer patients, there is amplification of the HER-2 gene, which leads to over-expression of the encoded protein and increased cell proliferation. Interpretation of HER.-2 expression: 0 points - no staining or staining <10% of tumor cells; 1+ point - incomplete staining of the membrane, stained> 10% of tumor cells; 2+ points - complete staining of the membrane, weak or moderate staining> 10% of tumor cells; 3+ points - complete staining of the membrane, strong staining of tumor cells.

The results are processed on an H-score scale and the staining intensity is estimated from 0 to 3. The H-scores of each preparation are calculated by the formula:

$$\mathbf{H = \%3+\%2 + \%1+‰}$$

where 0 - is the lack of staining;
1 - weak;
2 - moderate;
3 - strong.

2.4. Determination of the antitumor activity of hydrocortisone

The antitumor activity of hydrocortisone was studied on a transplantable tumor strain of colon adenocarcinoma (AKATOL) in BALB / s mice. Tumor strains were transplanted according to the instructions.

Before the introduction and at the end of the experiment, animals were weighed, the mass of animals at the end of the experiment was determined by subtracting the mass of the tumor. Tumors were measured (in 3 projections) after slaughter. At the end of the experiment, the tumor mass was determined.

The average tumor volume was found by the formula:

$$V_{av} = \left\{ \frac{\pi}{6} \right\} ABC$$

where - A, B, C - the length, width and height of the tumor; V_{av} - the average tumor volume in cm^3.

Animals were slaughtered under ether anesthesia no earlier than 16 days after the last administration of the test compounds. The control was a group of animals with the introduction of solutions used to dissolve the investigated substances - physiological saline.

The percentage inhibition of tumor growth was determined at the end of the experiment by the formula:

$$T\% = \left\{ \frac{B_K - B_0}{B_K} \right\} 100$$

where B_k - is the average tumor mass in animals of the control group,

B_0 - is the average tumor mass in animals of the experimental group.

The data obtained were subjected to statistical processing by the Student-Fisher method at a 95% significance level.

2.5. Morphological analysis of tumor tissue of the colon adenocarcinoma (AKATOL)

Samples of the tumor material for morphological analysis were taken from BALB / c mice with an implanted AKATOL tumor after 16 days

after tumor inoculation. Samples were poured into paraffin blocks, sections were made 3-5 microns thick and stained with hematoxylin and eosin. The analysis of the obtained preparations was carried out under a light microscope Leica (Germany).

The degree of morphological changes in tumor tissue in the experimental and control groups was distinguished by the following features.

Organoidity. The tumor consists of parenchyma and stroma. Parenchyma - the tumor's own tissue, which makes up its main mass and determines its growth and character. The stroma consists of connective tissue; in it pass the vessels and nerves that feed the tumor.

Atypism is a combination of biological properties that distinguish newly formed tissue from the original tissue. The acquisition by a tumor cell of new properties that are not inherent in a normal cell is called anaplasia (from Greek ana - back, placis - education) or cataplasia (from Greek cata - top to bottom, plasis - education). The term cataplasia is most accepted in modern literature. There are morphological, functional, antigenic atypism and metabolic atypism (metabolic). Morphological atypism is divided into tissue and cell.

Tissue atypism is characterized by a violation of the size, shape and relationship of tissue structures. For example, in epithelial, in particular, glandular tumors, the size and shape of the glands is disturbed, the lobular structure of the organ is lost, the ratio of parenchyma and stroma varies widely - in some cases, the parenchyma prevails over the stroma, in others, the stroma prevails over the parenchyma. Violation of the relationship of tissue structures in tumors from the integument epithelium is manifested in the fact that the epithelial layer of the skin can be located in the thickness of the dermis, and not on the surface. In tumors of mesenchymal origin (connective tissue, muscle), bundles of fibers differ in length, thickness, and random arrangement. Atypism of the stroma can be manifested by quantitative and qualitative characteristics of the fibrous component, as well as the ratio of cellular and fibrous components. Vessels can also be atypical. Usually they are thin-walled, often represented by a single layer of endothelial cells, or tumor cells form their wall. Their lumen is wide. Vascular atypism creates the prerequisite for the development of secondary changes in tumors caused by circulatory disorders. Tissue atypism is most characteristic of mature, benign tumors.

Cellular atypism at the light-optical level is expressed in the polymorphism of cells, nuclei and nucleoli, polyploidy, a change in the nuclear cytoplasmic index in favor of nuclei, and the appearance of many

mitoses. Tumor cells are distinguished by a variety of sizes, shapes and densities of nuclei. Often the nuclei are large hyperchromic, contain several nucleoli, sometimes hypertrophied. A change in the size of the nuclei of tumor cells to a certain extent can be associated with a shift in the number of chromosomes (amount of DNA) in them. Tumor cells are characterized by aneuploidy, that is, the amount of DNA that is different from the diploid set of chromosomes, most often it is increased and may correspond to a triploid or polyploid set of chromosomes. However, it is necessary to know that the diploid normal number of chromosomes can sometimes occur in neoplasms of a high degree of malignancy. In addition, no relationship was found between the degree of ploidy and the histological structure of the tumor, its proliferative ability, or other neoplasm properties. Cellular atypism can be expressed in varying degrees. With the proliferation of benign or slowly growing malignant tumors, neoplastic cells tend to differentiate. For example, the cells that make up a lipoma (a benign neoplasm from adipocytes) are similar to mature adipocytes under microscopic examination. As the degree of malignancy increases, the degree of differentiation decreases. Sometimes cell polymorphism is so significant that the tumor cells in appearance become unlike the cells of the original tissue or organ. Sometimes the histological structure of a malignant tumor is simplified and it becomes monomorphic (for example, in low-grade mesenchymal tumors). When the origin of the cell cannot be established by microscopic examination, that is, the neoplasm cells have no analogues among normal cells, the neoplasm is called undifferentiated or anaplastic. Anaplastic tumors of various organs are very similar to each other, which makes morphological differential diagnosis very difficult.

In malignant neoplasms, differentiation disorders are determined both in the cytoplasm and in the cell nucleus. These changes are similar to those with dysplasia, but here they are more pronounced. They include pleomorphism (a variety of cell shapes), an increase in the size of the nucleus, an increase in the nuclear-cytoplasmic ratio, hyperchromia of the nuclei, an increase in the nucleoli, a violation of the distribution of chromatin in the nucleus, a violation of the structure of the nuclear membrane, etc. The severity of these cytological disorders increases as the degree of malignancy increases.

Neoplastic cells can sometimes differentiate in a different way than the cells from which they evolved. For example, in the neoplastic glandular epithelium of the endometrium, sometimes glandular and keratinizing

epithelial cells (adenosquamous cancer) are formed. The term "tumor metaplasia" is used to refer to this phenomenon.

An important manifestation of the morphological atypism of the tumor cell is the ***pathology of the mitotic regimen***. The mitotic regime encompasses a number of parameters characterizing mitosis from various angles: the mitotic index reflecting mitotic activity, that is, the percentage of dividing cells from the entire population; the percentage of dividing cells located at different stages of mitosis; the relative number of all pathological mitoses; the percentage of certain types of pathological mitoses. It was found that in the cells of the tumor, the production of ceylons is disrupted, which under normal conditions regulate the mitotic activity of cells and act as inhibitors of cell division. The pathology of mitosis in tumor cells confirms the effect of oncogenic factors on the genetic apparatus of the cell, which determines the unregulated growth of the tumor. The main feature of the mitotic regime of malignant tumor cells is a sharp increase in the number of pathological mitoses and the diversity of their types. The metaphase suffers mainly, the percentage of K-metaphases with adhesion or scattering of hyperspiral chromosomes is high, lagging of chromosomes and their fragments in the metaphase. Often met three-group metaphases, metaphases with scattering and mass fragmentation of chromosomes, asymmetric, multipolar and monocentric mitoses. Certain types of mitosis pathology may be characteristic of certain types of tumors, which can be used as an additional criterion for the differential diagnosis of tumors of different histogenesis and clarification of the histogenetic affiliation of the neoplasm.

The atypism of ultrastructures revealed by electron microscopy is expressed in an increase in the number of predominantly free-lying ribosomes, polysomes, and the appearance of abnormal mitochondria. The cytoplasm is sparse, the nuclei are large, round or irregular in shape with a marginal or diffuse chromatin arrangement. Numerous membrane contacts of the nucleus, mitochondria, and endoplasmic reticulum are detected, which are very rarely detected in a normal cell. All these signs are characteristic of immature undifferentiated cells. However, by electron microscopy, cells with specific differentiation can be detected for the tissue from which the tumor originates. This symptom is often used to establish the histogenesis of the tumor.

2.6. Determination of mitotic activity of tumor tissue of the colon adenocarcinoma (AKATOL)

Mice weighing 20-22 g were injected intraperitoneally with hydrocortisone in physiological saline on days 3, 5, 7, and 9 after transplantation of the AKATOL tumor in doses: 1.25 mg / kg body weight, 2.5 mg / kg body weight, 5 mg / kg body weight. Animals of the control group received saline.

On day 16, animals were killed by decapitation and 1 cm3 of the tumor was taken for histological examination. This sample was fixed in 10% neutral formalin. Then the tissue was embedded in paraffin and sections 4–5 μm thick were prepared by staining them with a hematoxylin + eosin mixture, in which the number of cells in the division was counted under a microscope, from which the mitotic index (MI) was calculated.

$$MI \text{‰} = A/1000 \times 100$$

a - the number of cells in the division

For each case, the mitotic index was calculated in 20-25 areas of the tumor, in total, 1000 cells per 1 animal should be counted.

2.7. Determination of apoptosis in tumor cells of the colon adenocarcinoma (AKATOL)

Also, as in the study of the mitotic activity of tumor cells, mice weighing 20-22 g were injected intraperitoneally with hydrocortisone in physiological saline on 3, 5, 7 and 9 days after transplantation of the AKATOL tumor in doses: 1.25 mg / kg body weight, 2, 5 mg / kg body weight, 5 mg / kg body weight. Animals of the control group received saline.

On day 16, animals were killed by decapitation under ether anesthesia and 1 cm^3 of the tumor was taken for histological examination. The resulting region was fixed in 10% neutral formalin. The fixed tissue was embedded in paraffin and sections of histological preparations 4-5 μm thick were prepared by staining them with a hematoxylin + eosin mixture. In them, cells under apoptosis were counted under a microscope according to the following criteria: plasma membrane bleeding, nuclear chromatin condensation along the periphery of the nucleus, reduction of its size (pycnosis), formation of high molecular weight DNA fragments (karyorexis), DNA splitting into oligonucleosomal fragments (ladder type), compaction of cellular organelles, a decrease in the volume of the cytoplasm, a bubbly appearance of the cell membrane, budding of cell fragments with the

formation of discrete apoptotic bodies surrounded by a membrane and containing compacted remains of organelles and nuclei. The number of apoptotic cells was expressed as a percentage relative to the total number of counted cells.

2.8. Determination of proliferative activity of bone marrow cells in experimental tumor-bearing animals

Hydrocortisone was administered intraperitoneally to mice weighing 20-22 g in physiological saline on days 3, 5, 7, and 9 after transplantation of an AKATOL tumor in doses: 1.25 mg / kg body weight, 2.5 mg / kg body weight, 5 mg / kg body weight. Animals of the control group received saline.

On day 16, animals were sacrificed by decapitation, and 199 cells of the bone marrow were washed out of the femoral cavity with nutrient medium.

To determine the proliferative activity of bone marrow cells, the direct hourly cell culture method was used for FitzGerald chromosome analysis.

The principle of the method is that dividing bone marrow cells located at different stages of the mitotic cycle are accumulated using colchicine.

Bone marrow cells were resuspended in 199 nutrient medium with colchicine at a concentration of 0,3-0,4 μg per I ml of medium. After 2 hours, a suspension of cells was fixed in a mixture of ethyl alcohol with glacial acetic acid in a ratio of 3: 1. After 3-fold fixation, the precipitate was deposited on glass slides and stained with Romanovsky-Giemsa dye. The obtained preparations were analyzed under a Leica microscope (Germany), eyepiece 10^x, lens 100^x. To determine the mitotic index (formula 2.5) per 1000 bone marrow cells, all dividing cells were counted.

Statistical processing of the obtained data was carried out according to the Student-Fisher method, in relation to biological research. Differences were considered statistically significant at $p \leq 0.05$.

CHAPTER III.
RESEARCH RESULTS

3.1. The effect of hydrocortisone on the change in the representation of the oncoprotein NER-2 / neu on the surface of breast cancer cells in in vitro experiments

The problem of drug resistance of tumor cells to therapeutic effects has become urgent with the advent of effective chemotherapy drugs that can inhibit not only the growth of malignant neoplasms, but also dispense with previously traditional radiotherapy and surgical intervention. Particularly acute is the problem of drug resistance in the treatment of hormone-dependent tumors. The peculiarity of the treatment of these neoplasms is that in order to suppress the proliferation of malignant cells and to prevent relapse, natural regulators are used - hormones, and in the presence of some genetic characteristics of an individual individual, such therapy is often ineffective.

The absence of estrogen and progesterone receptors on the surface of breast cancer cells does not allow adhering to the traditional therapy of this disease and requires a change in pharmacological effects, while this pathology occurs in approximately 15% of patients. The main factor determining individual resistance is the genetic features that are manifested in the over- or hypoexpression of proteins or receptors in cells prone to transformational changes. Concomitant factors are the presence of certain oncogenic proteins, proliferation and apoptosis of tumor cells.

Despite the recent successes achieved in understanding the mechanisms of the emergence of drug resistance and the outlined ways to overcome it, there are a number of problems, the overcoming of which can help to improve existing approaches to the treatment of malignant neoplasms. First of all, this is the lack of a full replacement for hormonal therapy, which is widely and successfully used to treat a wide range of tumor pathologies. It is clear that if, for example, estrogen receptors are absent on the surface of breast cancer cells, pharmacological treatment with generally accepted chemotherapy drugs will not give the result in terms of minimizing side effects and positive dynamics of therapy, such as the use of hormones. Based on this, one of the ways to overcome drug resistance during hormone replacement therapy is to launch alternative mechanisms for inhibiting the proliferation of transformed cells, and this is possible only when using natural factors regulating metabolic processes in these cells. First of all, such biologically active substances are hormones, but if in the case of genetic

disorders there are no receptors on the surface of tumor cells, for example, estrogens, other hormones, in particular, hydrocortisone, can take on the function of regulating their proliferation.

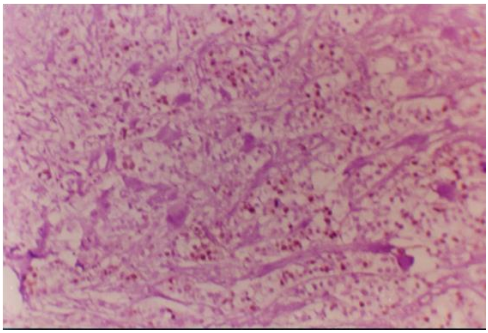
We conducted studies to identify transformed mammary gland cells with no estrogen receptors on their surface. The purpose of such experiments was to study the inclusion of mechanisms for inhibiting the proliferation of tumor cells through alternative hormonal exposure to hydrocortisone.

Using immunohistochemical analysis using murine anti-estrogen receptor antibodies (clone 6F11, NovoCastra), we examined samples of 6 breast tumors of different genesis, obtained from the surgical material of patients of the Russian Oncology Center of the Ministry of Health of the Republic of Uzbekistan.

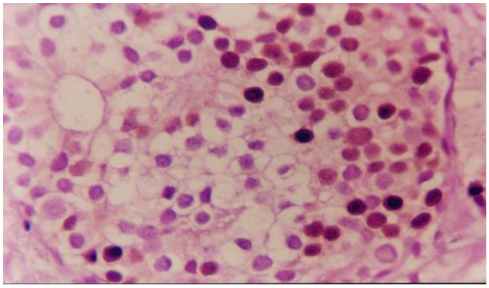
In 4 out of 6 samples of breast tumor tissue, the number of estrogen receptors corresponded to 2+ and 3+ points, that is, in these cells there were no genetic disturbances in the expression of estrogen receptors (Fig. 3.1, 3.2, 3.3, 3.4). In 1 of 6 tumor samples, the representation of estrogen receptors was insignificant and corresponded to a 1+ score (Fig. 3.5, 3.6). And only 1 sample of transformed breast cells showed a complete absence of estrogen receptors - 0 points (Fig. 3.7).

For further experiments, we took tumor material with a complete absence of estrogen receptors. As a marker for identifying the inhibition of proliferation of transformed mammary cells under the action of hydrocortisone, we selected the HER2 / neu gene, the expression product of which is oncoprotein under the same name.

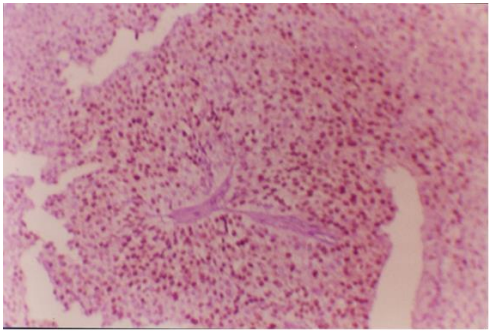
HER2 / neu (human epidermal growth factor receptor 2, synonyms: HER2, cbb2) is an oncogenic protein weighing 185 kDa, belonging to the epidermal growth factor receptor (EGFR) family. This family includes four homologous receptors EGB-1 (EGFR, HER1), EGB-2 (HER2 / neu), EgbB-3 (EE), and EGB-4 (HER4)]. The proto-oncogene HER2 / neu is located on chromosome 17 at q21. Activation of HER2 / neu expression, its homo- or heterodimerization causes intracellular signaling events, which are critical for cell growth, differentiation, and survival.



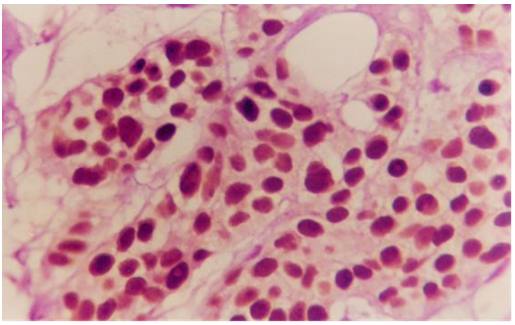
Picture 3.1. Mammary cancer. Positive immunohistochemical reaction to estrogen receptors (2+). Immunoperoxidase method. Ocular 10^x, lens 20^x.



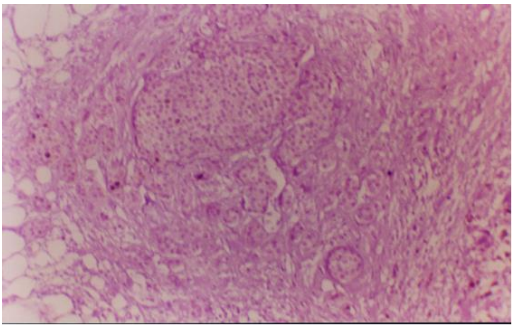
Picture 3.2. Mammary cancer. Positive immunohistochemical reaction to estrogen receptors (2+). Immunoperoxidase method. Ocular 10^x, lens 40^x.



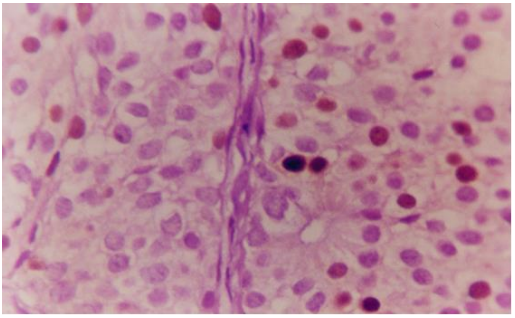
Picture 3.3. Mammary cancer. Sharply positive immunohistochemical reaction to estrogen receptors (3+). Immunoperoxidase method. Ocular 10^x, lens 20^x.



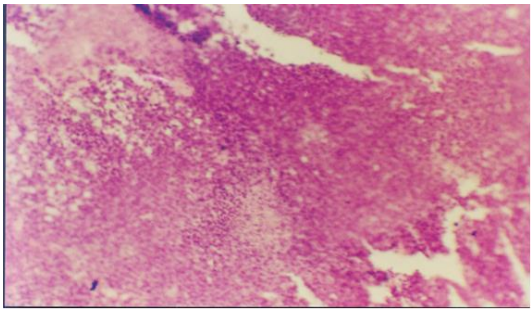
Picture 3.4. Mammary cancer. Sharply positive immunohistochemical reaction to estrogen receptors (3+). Immunoperoxidase method. Ocular 10^x, lens 40^x.



Picture 3.5. Mammary cancer. Weak positive immunohistochemical reaction to estrogen receptors (1+). Immunoperoxidase method. Ocular 10^x, lens 20^x.



Picture 3.6. Mammary cancer. Weak positive immunohistochemical reaction o estrogen receptors (1+). Immunoperoxidase method. Ocular 10^x, lens 40^x.



Picture 3.7. Mammary cancer. Negative immunohistochemical reaction to estrogen receptors (1+). Immunoperoxidase method. Ocular 10^x, lens 20^x.

Overexpression of HER2/neu is most often detected with the development of malignant neoplasms in humans.

By laboratory methods, HER2/neu overexpression is determined in 25-30% of patients diagnosed with breast cancer.

Moreover, the detection of this protein in breast cancer indicates a poorer prognosis of the course of the disease.

Thus, an increase in the number of oncogenic protein HER-2/neu on the surface of epithelial cells in case of cancer of the intestine and breast requires the use of higher doses of cytostatics and indicates an increased resistance of these cells to chemotherapeutic effects.

Thus, the change in the quantitative representation of the oncoprotein HER-2/neu on the surface of breast cancer cells in the direction of reduction indicates:

firstly, the effectiveness of chemotherapeutic or, as in our case, hormonal effects;

secondly, about serious changes in the work of the gene apparatus of transformed cells under the influence of a biologically active substance.

We investigated the quantitative characteristics of the location of HER-2/neu receptors on the membrane of breast tumor cells with no estrogen receptors when exposed to hydrocortisone in vitro at doses of 0,025 mg / 1,6 • 10^6 cells, 0,05 mg /1,6 • 10^6 cells and 0,1 mg / 1,6 • 10^6 cells (table. 3.2).

Exposure to hydrocortisone in all doses led to a decrease in the number of HER-2 / neu receptors on the membranes of transformed breast cells by an average of 27,25 ± 1,14%, $p < 0,001$.

Moreover, the quantitative characteristics of changes in the expression of this protein between the experimental groups did not

statistically significantly differ (Table 3.1). Significant changes in the expression of HER-2 / neu on the surface of breast cancer cells when hydrocortisone is used may be associated with the inhibition of the proliferative activity of the studied cells by this hormone by signaling from hydrocortisone-mediated receptors. In turn, it should be assumed that similar processes affect the genetic apparatus of the cell, which leads to a change in the expression of certain genes, in particular those responsible for mitotic activity, for example, HER-2 / neu. It should also be noted that the use of hormonal drugs in physiologically significant concentrations leads to undesirable side effects. Our studies have established that reducing the dose of the therapeutic effect of hydrocortisone does not affect the effectiveness of inhibition of the expression of HER-2 / neu on the surface of breast cancer cells.

Concentration hydrocortisone mg / cell	**Number of cells**	**Exposure time, h**	**Number HER-2 / neu, H-points**
0,025	**1,6 - 106**	**24**	**217,2±8,13***
0,05	**1,6 - 106**	**24**	**205±10,83***
0,1	**1,6 - 106**	**24**	**206,4±7,85***
(control)	**1,6 - 106**	**-**	**288±2,21**

Table 3.1. Representation of HER-2/neu on the membranes of breast cancer cells when exposed to hydrocortisone in vitro * $p < 0.001$

3.2. Investigation of the cytotoxic effect of hydrocortisone on transformed breast cells in an in vitro experiment

In recent years, many researchers are revising previously considered the main areas of application of glucocorticosteroid drugs, as, in particular, in the treatment of rheumatoid arthritis, bronchial asthma, and inflammatory processes. Establishment of the mechanisms of the influence of glucocorticosteroids on the suppression of the activity of connective tissue enzymes - hyaluronidase and collagenase, a decrease in the permeability of membranes and thereby a decrease in exudation processes, a suppression of activity and a decrease in the volume of lymph nodes and thymus, and secretion of cytokines - all this suggests an antiproliferative and apoptosis inducing effect against various cells of the body, including transformed.

In our study, we studied the cytotoxic effect of hydrocortisone, one of the glucocorticosteroid hormone family, on transformed mammary cells in an in vitro experiment.

Cell cultivation, used to screen studies of the effects of drugs, are less expensive and less time consuming than in vivo testing, although assessing the individual sensitivity of the tumor has several difficulties. This, in particular, the selection of an adequate concentration of drugs and the definition of criteria for cell damage.

The concentrations of the studied substances in vitro were selected on the basis of randomly selected exposure doses, however, this choice was made using data obtained in experiments with a unicellular suspension of homogenized tissue without long-term cultivation and incubation for 1 hour with preparations at 37 ° C followed by staining trypan blue to detect the number of dead (stained) and living (unpainted) cells. As a rule, we investigated three different concentrations: 1/10, 1/100 and 1/1000 of the therapeutic dose of the same drug, plotted the dependence of cell survival on the dose, and subsequently used the one that was most effective. As a result of a series of experiments with various drugs and cells, both normal (lymphocytes, chicken fibroblasts) and tumor (mammary, thyroid), we came to the conclusion that the most optimal is 1/1000 of the therapeutic dose (in terms of 1 kg body weight).

Another difficult task that arises when testing the effects of drugs in vitro is the choice of exposure duration. Although it is believed that the main action of most drugs is during the first hour of exposure, we decided to increase it to 24 hours (the duration of the first mitoses).

The experiment used transformed mammary gland cells isolated from surgical tumor material obtained from a patient born in 1926 with a diagnosis of gynecomastia of the right breast. The results of the experiments are shown in table. 3.2.

As can be seen from the results of the studies (Table 3.2), hydrocortisone in experiments in vitro showed high cytotoxic activity against transformed mammary cells. There is a dose-dependent effect of exposure to hydrocortisone.

In the lowest dose studied (group I), hydrocortisone suppressed the viability of transformed breast cells by 42%, while no apoptotic cells were observed. This suggests that the effect of a dose of 0,025 mg of this hormone on the metabolic processes of the studied cells was reduced to a violation of the integrity of the cytoplasmic membrane.

At a dose of 0.05 mg (group II), hydrocortisone suppressed the viability of breast cells by 69%, which indicates a high cytotoxic activity of this hormone at a given concentration. At the same time, we observed the appearance of apoptotic cells, their number was 3% of all the studied breast

cells. This suggests that with an increase in the active substance, the hydrocortisone preparation changes its effect on the course of metabolic processes. With an increase in active hormone molecules, the processes of violation of biochemical reactions affect other vital organelles of the cell, in particular, it can be a nuclear apparatus. Such a shift in the effect of hydrocortisone towards increased disorganization processes in the cells and an increase in the cytotoxic properties of hydrocortisone, simultaneously with an increase in the dose of exposure, can be explained by an increase in the number of molecules capable of penetrating the nuclear membrane, as well as the possibility of the interaction of the hormone with a large number of receptors.

In the largest dose studied (0,1 mg - group III), hydrocortisone suppressed the viability of breast cells by 60%, which was slightly ($p > 0,05$) lower than that when using the hormone at a concentration of 0,05 mg.

The number of apoptotic cells also decreased slightly, up to 2%, when compared with group II. Such changes in the quantitative indicators of the cytotoxic effect of hydrocortisone can be explained in two ways. On the one hand, the cytotoxic activity of hydrocortisone exceeds 50%, compared with the control group, and the number of apoptotic cells remains significantly higher compared to group I (the lowest dose of hydrocortisone exposure is 0,025 mg) and control.

Dose of action hydrocortisone (mg)	Number of cells	The number of dead cells,%	The number of living cells,%	The number of apoptotic cells,%
0,025	1,6 - 106	42±0,03*	58±0,03*	-
0,05	1,6 - 106	69±0,03*	28±0,03*	3±0,01
0,1	1,6 - 106	60±0,03*	38±0,03*	2±0,01
(control)	1,6 - 106	2±0,01	98±0,01	-

Table 3.2. Cytotoxic effect of hydrocortisone in relation to transformed breast cells * - $p < 0,05$

On the other hand, the change in the quantitative indicators of the cytotoxic effect of the hormone, in comparison with other experimental groups and the control, is homogeneous and correlates with possible adequate changes in the case of an increase in the anti-stress response of cells to toxic effects.

When analyzing the results in the III experimental group, we assume that a decrease in the cytotoxic activity of hydrocortisone is associated with several factors.

The first factor that can reduce the cytotoxic activity of hydrocortisone with an increase in its active concentration is receptorocytosis, when receptors for the hormone, with its hyperconcentration, are invaded deep into the cytoplasmic or nuclear membrane. The second factor, also associated with the signal transmission mechanism, is the excess of the physiologically permissible norm of the number of hormone molecules when active mechanisms of anti-stress protection of the cell against possible toxic effects of hyperconcentration of biologically active substances are activated, including inactivation of the active substance by its deposition by protein complexes and prohibition of active intermembrane transport hormones. And finally, the competitive interaction of a large number of hydrocortisone molecules with an insufficient number of receptors, which can lead to some decrease in the effects inherent in glucocortecosteroids.

3.3. Hydrocortisone induction of apoptosis in cells of an experimental tumor of the colon adenocarcinoma (AKATOL) in an in vitro experiment

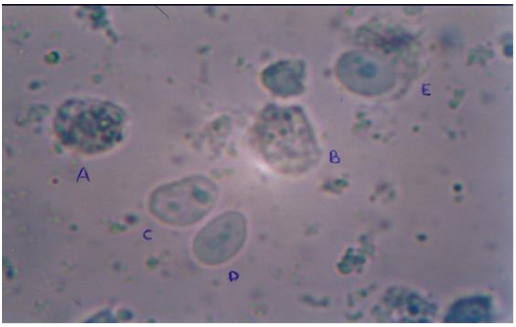
Apoptosis is a special form of cell death, the onset of which is embedded in the genetic program of the cell. The involvement of apoptosis in the most diverse processes occurring in the body of higher animals and humans is justified: the formation of organs and tissues in embryogenesis, hematopoiesis, the creation of an antigen-recognizing repertoire of lymphocytes and the implementation of their killer function, the development of tumors, the implementation of the cytotoxic effect of ionizing radiation and a number of drugs, etc. d. There is no doubt the important role of apoptosis in the pathogenesis of many diseases. Increased readiness for apoptosis leads to diseases associated with the development of atrophic processes in the nervous, muscle and other tissues, and when the processes of malignant transformation of tissues occur, the same readiness of cancer cells indicates tumor regression. Therefore, the detection of apoptotic death of tumor cells allows not only to judge the antitumor effect of the test substance, but also to talk about the mechanism of its antiproliferative effect, to illustrate the depth of metabolic rearrangements, including the genetic apparatus of the cell.

Currently, in clinical and laboratory practice and during scientific research, various tests are used to quantify the apoptosis of eukaryotic cells, which can be divided into several groups: morphological (by

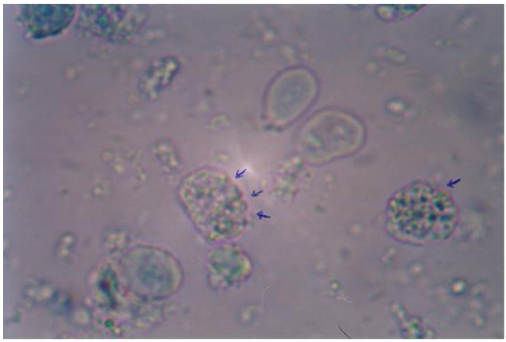
morphostructural characteristics), biochemical (internucleosomal DNA degradation), immunological (using antibodies to Fas receptors), TUNEL-method (Therminal desoxiribosyl transferase (TdT) mediated dUTP Nick End Labeling) - characterizing CD8-dependent apoptosis. In our study, we observed the occurrence of apoptotic processes in the cells of the transplantable strain of colon adenocarcinoma (AKATOL) according to morphological characteristics (plasma membrane blebbing, condensation of nuclear chromatin along the periphery of the nucleus, reduction of its size (pycnosis), formation of DNA fragments with high molecular weight (karyorexis) DNA splitting into oligonucleosomal fragments (ladder type), compaction of cellular organelles, reduction of cytoplasm volume, acquisition of a bubble-like membrane, budding of cell fragments with the formation of discrete apoptotic bodies surrounded by a membrane and containing compacted remains of organelles and nuclei). In an in vitro experiment, in living cells, morphostructural changes were recorded within 1 h. Immediately after application of the drug, an intravital analysis of the state of the nucleus and cytoplasm of several selected and fixed in the field of view cells was performed. A total of 3 concentrations of hydrocortisone were tested during the experiments: 0,025 mg / $1,6 \cdot 10^6$ cells (group I); 0,05 mg / $1,6 \cdot 10^6$ cells (group II); 0,1 mg / $1,6 \cdot 10^6$ cells (group III).

In group I of the studied cells in the field of view, we observed 2 tumor cells (A and B) and 3 lymphoid cells (C, B, E) (Fig. 3.8).

Cells A and B are prone to swelling, bulges are formed on the surface of the cell wall. The distribution of coarse chromatin is uniform throughout the entire core volume (diffuse distribution). After 20 min of incubation of cells with hydrocortisone, destruction of the cell membrane was detected in places of critical swelling (Fig. 3.9). Following this, chromatin consolidation (karyorexis) and nuclear destruction (karyolysis) took place. 35 minutes after the introduction of the hormone, the integrity of the surface membrane of cell B occurred, in cell A there was also a violation of the integrity of the surface membrane, but with the release of the contents of the cytoplasm into the extracellular space. In this case, it should be noted that the lymphoid cells C, D, and E maintained their integrity during the experiment (1 h) and did not have any visible morphological changes in their structure.

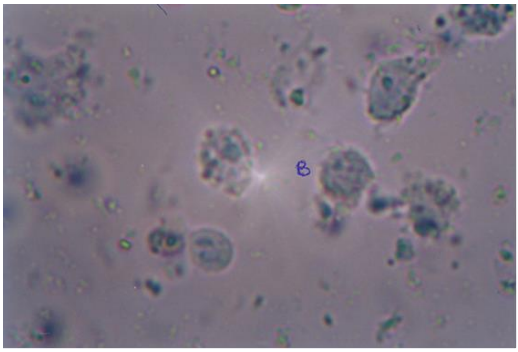


Picture 3.8. Group I. The effect of hydrocortisone on AKATOL tumor cells. Initial phase. Coloring by - trypan blue. Ocular 10^x, lens 100^x.

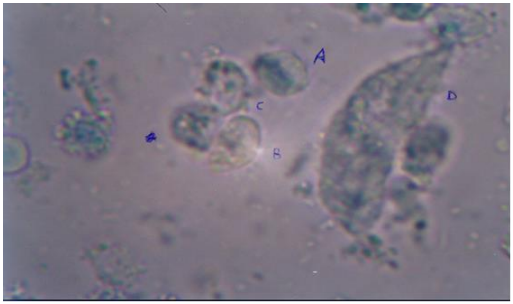


Picture 3.9. Group I. The effect of hydrocortisone on AKATOL tumor cells, h/w 20 minutes. Coloring by -trypan blue. Ocular 10^x, lens 100^x.

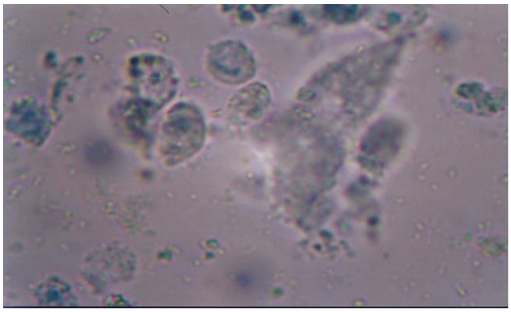
Picture 3.10. Group II. The effect of hydrocortisone on AKATOL tumor cells, h/w 10 minutes. Coloring by - trypan blue. Ocular 10^x, lens 100^x.



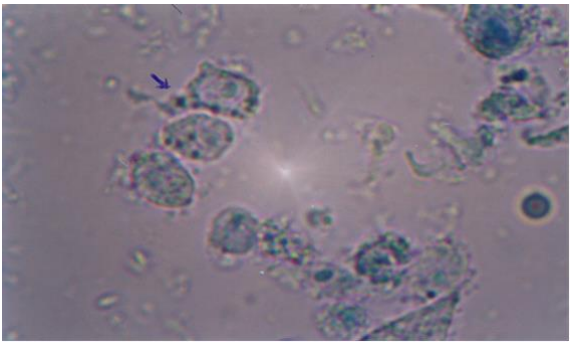
Picture 3.11. Group II. The effect of hydrocortisone on AKATOL tumor cells, h/w 30 minutes. Coloring by - trypan blue. Ocular 10^x, lens 100^x.



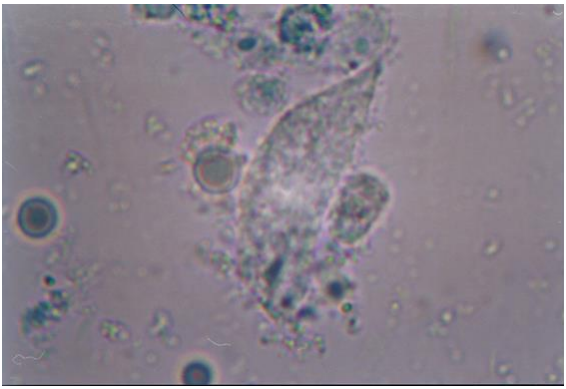
Picture 3.12. Group III. The effect of hydrocortisone on AKATOL tumor cells. Initial phase. Coloring by - trypan blue. Ocular 10^x, lens 100^x.



Picture 3.13. Group III. The effect of hydrocortisone on AKATOL tumor cells, h/w 25 minutes. Coloring by - trypan blue. Ocular 10^x, lens 100^x.



Picture 3.14. Group III. The effect of hydrocortisone on AKATOL tumor cells, h/w 30 minutes. Coloring by - trypan blue. Ocular 10^x, lens 100^x.



Picture 3.15. Group III. The effect of hydrocortisone on AKATOL tumor cells, h/w 60 minutes. Coloring by - trypan blue. Ocular 10^x, lens 100^x.

In group II of the studied AKATOL tumor cells, hydrocortisone was added at a dose of 0.05 mg / 1.6 x 10 cells. In the field of view, we observed 3 tumor cells - A, B and C (Fig. 3.10).

10 min after the start of the experiment, chromatin margin was observed in cell A at the periphery of the nucleus. In cell B, chromatin condensation was less pronounced, but its condensation processes were also observed at the periphery with clarification of the karyoplasm in the nucleolus region. In cell C, a pronounced chromatin margin was observed in the form of a rim at the proximal end of the nucleus. At the distal end, cytoplasmic outgrowths were fixed, the nucleolus was dark, round. After 30 minutes,

further chromatin condensation and the formation of apoptotic bodies occurred in cell A (Fig. 3.11). After the same period, in the cell B, further chromatin margin was observed into larger granules and the nucleolus disintegrated into small parts, which subsequently led to nucleus pycnosis (Fig. 3.11). After 15 minutes from the start of the experiment in cell C, the chromatin rim at the proximal end of the nucleus disintegrated into large blocks of chromatin. Further, the cytoplasmic outgrowths became denser, after 30 minutes after the start of the experiment they contracted and then disintegrated into nuclear-free cytoplasmic formations. By the end of the experiment (1 h), cells A and C turned into cells with clusters of apoptotic bodies.

In group III of the studied AKATOL tumor cells, hydrocortisone was introduced at a dose of 0.1 mg / 1.6 x 10 cells. In the field of view, we observed a group of tumor cells (A, B, C), among them a giant multinucleated cell B (Fig. 3.12).

Cells A and B throughout the entire period of the experiment did not change their morphological characteristics, Fig. 3.14 and 3.15. In cell C, enlightenment in the nucleolus region and condensation of large blocks of chromatin at the periphery of the nucleus (marginalization) were observed. After 25 minutes after the introduction of hydrocortisone, the concentration of chromatin was recorded over the entire circumference of the nucleus. In this case, invagination, folding of the membrane was observed (Fig. 3.13). Then outgrowths of the cytoplasm were formed, which then became denser. In fig. 3.13, individual apoptotic bodies with the contents of the nucleus and strong enlightenment of the nucleoplasm formed at the end of the study are visible.

Tumor cell B is elongated, contains several (3-4) large nuclei. Throughout the entire period of the experiment, no pronounced condensation and condensation of chromatin was observed (Fig. 3.12). An amitotic type of division was noted with the formation of a constriction of the cytoplasm along the periphery.

Thus, a dose of 0,025 mg / $1,6x10^6$ cells of hydrocortisone exerted a cytotoxic effect on AKATOL tumor cells, which was manifested in the course of necrotic processes: proteolysis, impaired integrity of the surface membrane, and release of the contents of the cytoplasm into the extracellular space. The action of this hormone in a dose of 0,05 mg /$1,6•10^6$ cells led to the death of tumor cells by the type of apoptosis. At a dose of 0,1 mg /$1,6·10^6$, hydrocortisone caused apoptotic cell death only in isolated cases, and the effect of the hormone did not adversely affect the activity of the

multinucleated tumor cell. It should be noted that multinucleated transformed cells by their nature are highly resistant to toxic or other biological effects.

It should be noted that the results obtained during this study generally correlate with the previously described data (section 3.2). There is also a tendency towards the optimal effect of hydrocortisone in physiologically moderate concentrations (0,05 mg/$1{,}6 \cdot 10^6$), while the effect of this hormone on the metabolic processes of tumor cells is dose-dependent and, as in the case of studying its effect on transformed breast cells in vitro, in low doses, it has a pronounced cytotoxic effect.

3.4. The study of the antitumor activity of hydrocortisone

An important step in testing antiproliferative effects in the study of biologically active substances is the establishment of their antitumor activity. To date, many methods for conducting such studies are known, but so far the experiments on experimental tumor strains implanted by laboratory animals are the most adequate and informative. There are a large number of experimental strains used in scientific and laboratory studies, and the choice of the most optimal one is the greatest difficulty for a modern researcher.

Our choice of a tumor strain for determining the antitumor activity of hydrocortisone in vivo was primarily determined by the resistance of the colon adenocarcinoma tumor (AKATOL) to the chemotherapeutic effect of the most effective drugs to date. The establishment of the antiproliferative effects of hydrocortisone on the AKATOL tumor strain seemed to us the most appropriate from a scientific point of view, since this tumor, like breast cancer, is hormone-dependent, and the treatment of solid tumors is the most time-consuming task for modern oncology, while for growth inhibition of such tumors is most often used hormone replacement therapy. The antitumor activity of hydrocortisone was studied in vivo on the transplantable tumor strain of the colon adenocarcinoma (AKATOL), tumor implantation was performed in mice of the BALB / s line.

As can be seen from the data table. 3.3, in all studied doses, hydrocortisone showed good antitumor activity, it suppressed tumor growth by more than 50%, judging by the change in tumor mass in the experimental groups and control.

At the lowest dose (1,25 mg / kg body weight), hydrocortisone showed the lowest antitumor activity, determined by the mass of the tumor (58,19%), while the decrease in tumor volume was the largest (74,9% in relation to the control). when compared with other experimental groups. The

explanation for such a significant decrease in the volume of tumor tissue can be as follows. Both the mass and volume of the tumor, which are critical signs in determining antitumor activity, serve as factors that, although they reflect the regressive processes occurring in the tumor tissue, they themselves depend on many circumstances that shift the quantitative indicators of these signs to one or another side. And first of all, this concerns the change in the volume of the tumor, which depends, firstly, on the inflammatory processes that occur in the tissue (neutrophilia, exudation, etc.), which leads to flooding and, accordingly, an increase in volume. Secondly, there is a reverse order dependence when the destructive effect of the drug on tumor cells is not so reactive and the body manages to utilize necrotic cell fragments in a short period, which leads to a decrease in the volume of tumor tissue with a relatively high mass. Based on such critical instability of the tumor volumetric parameters, only tumor tissue mass indices are taken into account when determining antitumor activity. Thus, in the case of the use of hydrocortisone at a dose of 1,25 mg / kg, we are dealing with compacted tumor tissue due to the low reactivity of the cytotoxic effect of the hormone, which led to the greatest decrease in its volume.

Dose of hydrocortisone (mg / kg body weight)	Number of animals	Tumor mass (g)	Tumor volume (cm3)	% inhibition of tumor growth	% reduction in tumor volume (compared with control)
1,25	**10**	**2,575±0,16***	**1,34±0,14***	**58,19**	**74,9**
2,5	**10**	**1,06±0,1***	**1,73±0,09***	**82,79**	**67,6**
5	**10**	**1,31±0,14***	**1,62±0,1***	**73,7**	**69,66**
control		**16±0,21***	**5,34±0,2***	**-**	**-**

Table 3.3. The results of a study of the antitumor activity of hydrocortisone on a transplanted tumor strain * - p <0,05 AKATOL

At a dose of 2,5 mg / kg of animal body weight, hydrocortisone has the largest (82,79%) of all experimental groups antitumor activity. The decrease in tumor volume is also significant (67,6%), but it is the lowest similar indicator of all used concentrations of the hormone. This is due to the high reactivity of the action of hydrocortisone in this dose, which invariably leads to the occurrence of inflammatory processes in the tumor tissue and, accordingly, to its watering.

At its highest dose (5 mg / kg), hydrocortisone also showed high antitumor activity (73,7%), which, although less than the same indicator in

group II, was statistically significantly different from the control. A decrease in the volume of tumor tissue (69,66%) also correlates with the data obtained in group II, inflammatory processes in the tumor tissue also occur and, accordingly, exudation is present.

Thus, a study of the antitumor activity of hydrocortisone showed that this hormone has high antiproliferative activity and is able to suppress the growth of cells of the experimental tumor strain AKATOL in all doses studied. In this case, the most effective antitumor effect of hydrocortisone is observed if it is used in doses of 2,5 / kg and 5 mg / kg of body weight.

3.5. Study of the morphological features of a colon adenocarcinoma tumor (AKATOL) under the influence of hydrocortisone

In order to characterize the mechanism of the antitumor effect of hydrocortisone at the tissue level, a morphological analysis of tumor tissue was carried out, which includes the study of signs of severity of tissue and cellular atypism, the nature of growth in relation to surrounding tissues.

We conducted histological studies of tumor tissue obtained from samples of colon adenocarcinoma (AKATOL) when exposed to it in different doses of hydrocortisone, as well as control samples of tumor tissue without hormone exposure.

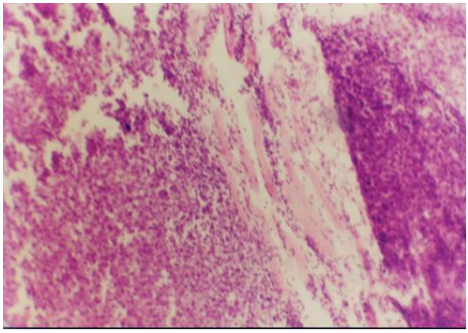
Hydrocortisone was administered according to protocol 3.4. Tumor tissue samples were taken on day 16 after tumor implantation and on day 7 after exposure.

In the control group of animals the following morphological characteristic of tumor tissue was observed. In the section, the tumor is light pink in color, homogeneous, lobed. Outside, it was surrounded by a capsule of muscle fibers (Fig. 3.16) and, in large numbers, with blood vessels (1-2 in the field of view), closer to the center of the tumor the number of vessels increases to 3-4. The cellular composition is polymorphic, represented by small, medium and large cells. Rounded, irregularly shaped, polygonal cells with normo- and hyperchromic nuclei containing single nucleols. Chromatin in most nuclei is coarse-grained, fine-mesh, cells with coarse-grained chromatin are found (Fig. 3.18). Condensed chromatin cells are insignificant. Multinucleated cells, often large ones, are found on the preparations. The number of nuclei in them is different, but cells with 3-4-5 nuclei predominate (Fig. 3.19). The cytoplasm is homogeneous, basophilic. Most tumor cells are actively dividing, mainly with a pathological course of mitosis (Fig. 3.20, 3.21). Necrotic areas of small size are rare (Fig. 3.22). In general, the studied

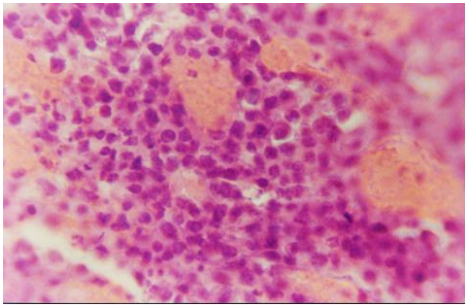
tissue is a low-differentiated cell array with high mitotic activity, with germination in muscle tissue, with a tendency to grow.

In the morphological picture of the tumor tissue of animals treated with hydrocortisone at a dose of 1,25 mg / kg body weight, it was found that the surface, encapsulated layer is also represented by muscle fibers. Closer to the center of the tumor, medium-sized necrotic areas are observed, often in the form of necrotic cords. In the layer of tumor tissue, blood vessels with blood filling (1-2 in the field of view) are found, around some there is a strong rarefaction of the tissue (Fig. 3.23). The cell composition, as well as in the control group, is polymorphic (Fig. 3.24), multinuclear cells of large sizes with different numbers of nuclei are found (Fig. 3.25). The content of the cytoplasm in most cells is small, the nuclear-plasma ratio shifts toward the nucleus, the structure of the cytoplasm is homogeneous, basophilic (Fig. 3.26). Mitotic activity decreases, compared with the control, alveolar structures formed from atypical epithelial-like cells are detected. Thus, the tissue under study is a medium-differentiated cell composition with low mitotic activity, the presence of large areas of necrosis, and an increase in tissue degradation is observed.

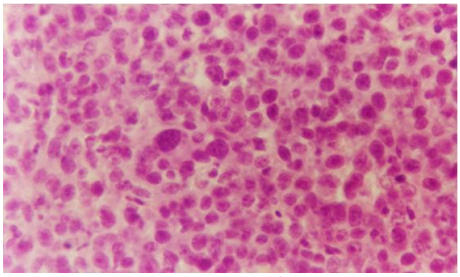
In the tumor tissue of animals obtained with the introduction of hydrocortisone at a dose of 2,5 mg / kg, unstructured detritus was found in most of the tumor, among which there are islands of cells with a preserved structure, as well as with a small number of blood vessels. Over the entire volume of tumor tissue, large foci of necrosis are observed (Fig. 3.27). The cell composition is highly differentiated, represented by small, medium and, rarely, large cells. The cells are round, irregular in shape, with normal and hyperchromic nuclei, also round, oval and irregular in shape, with coarse granular chromatin, which is most often located on the periphery of the nucleus. Detected multinucleated cells have a different number of nuclei. The nuclear-plasma ratio, as in the previous groups, is shifted towards the nucleus, but in a smaller number of cells. The mitotic activity of the tissue is low, the phases of mitosis are predominantly pathological, K-mitoses occur. The analysis showed that the studied tissue was a highly differentiated system, the cell composition with low mitotic activity, with high cell death by the type of necrosis, which indicates the cytotoxic effect of this dose of hydrocortisone, resulting in a process of tissue degradation.



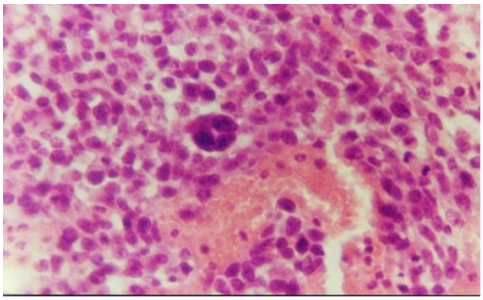
Picture 3.16. Tumor histostructure AKATOL. Control. Germination of muscle fibers in tumor tissue. Hemotoxylin and eosin stained. Ocular 10^x, lens 20^x.



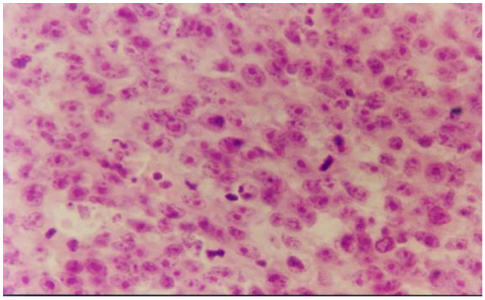
Picture 3.17. Tumor histostructure AKATOL. Control. An increase in the number of vessels in the central part of the tumor.
Hemotoxylin and eosin stained. Ocular 10^x, lens 40^x.



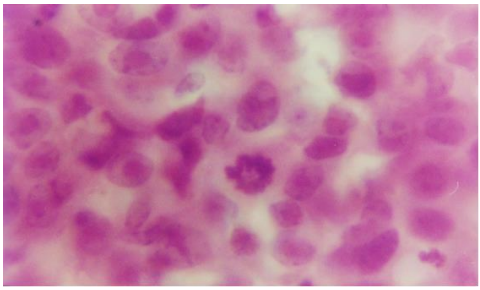
Picture 3.18. Histological characteristics of tumor tissue AKATOL. Control. Cell polymorphism, coarse chromatin. Hemotoxylin and eosin stained.
Ocular 10^x, lens 40^x.



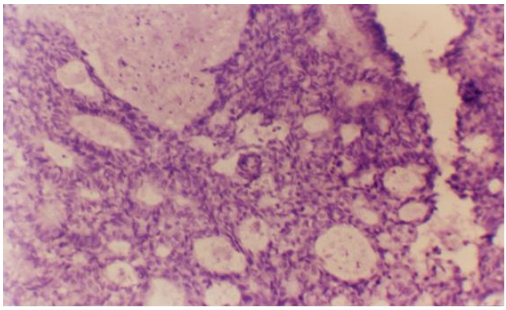
Picture 3.19. Histological characteristics of tumor tissue AKATOL. Control. A multinucleated cell in the center, to the right of the mitosis figure. Hemotoxylin and eosin stained. Ocular 10^x, lens 40^x.



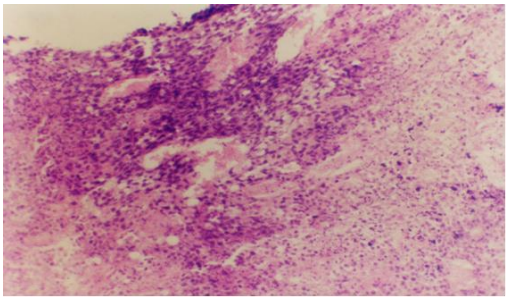
Picture 3.20. Histological characteristics of tumor tissue AKATOL. Control. High proliferative activity of cells. Pathological and normal phases of mitosis. Hemotoxylin and eosin stained. Ocular 10^x, lens 40^x.



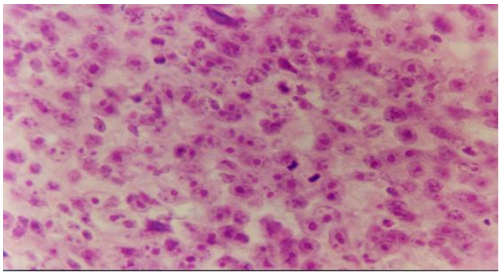
Picture 3.21. Tumor histostructure AKATOL. Control. Round, pathological metaphase. Hemotoxylin and eosin stained. Ocular 10^x, lens 100^x.



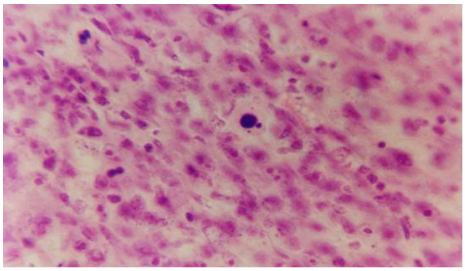
Picture 3.22. Tumor histostructure AKATOL. Control. Necrotic sites are located in small groups in the center of the tumor. Hemotoxylin and eosin stained. Ocular 10^x, lens 20^x.



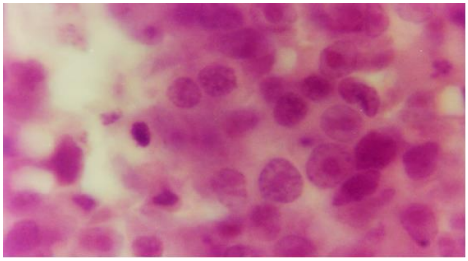
Picture 3.23. Histology of AKATOL tumor after administration of hydrocortisone at a dose of 1,25 mg / kg. Large necrotic areas, rarefaction of tumor tissue. Hemotoxylin and eosin stained. Ocular 10^x, lens 20^x.



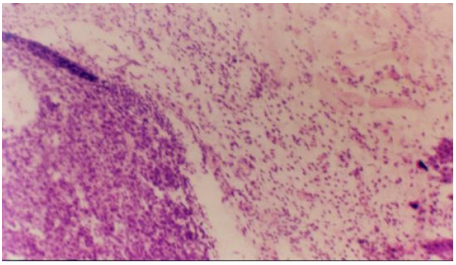
Picture 3.24. Histology of AKATOL tumor after administration of hydrocortisone at a dose of 1,25 mg / kg. Polymorphism of tumor cells and their nuclei, the presence of figures of mitosis. Hemotoxylin and eosin stained. Ocular 10^x, lens 40^x.



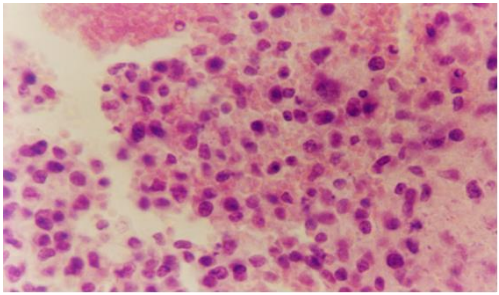
Picture 3.25. Histology of AKATOL tumor after administration of hydrocortisone at a dose of 1,25 mg / kg. A large tumor cell with 4 nuclei of various sizes. Pathological figures of mitosis, cell polymorphism. Hemotoxylin and eosin stained. Ocular 10x, lens 40x.



Picture 3.26. Histology of AKATOL tumor after administration of hydrocortisone at a dose of 1,25 mg / kg. The nuclear-plasma ratio is shifted towards the nucleus, coarse-grained chromatin with a concentration on the periphery. Hyperchromic nuclei can befound. Hemotoxylin and eosin stained. Ocular 10^x, lens 40^x.



Picture 3.27. Histology of AKATOL tumor after administration of hydrocortisone at a dose of 2,5 mg / kg. A large array of tissue with necrosis, areas of tumor tissue with dilution. Hemotoxylin and eosin stained. Ocular 10^x, lens 20^x.



Picture 3.28. Histology of AKATOL tumor after administration of hydrocortisone at a dose of 5 mg / kg. Vacuum of the tumor tissue, most of the nuclei are hyperchromic, small.
Hemotoxylin and eosin stained. Ocular 10^x, lens 40^x.

The morphological picture of the tumor tissue of animals treated with hydrocortisone at a dose of 5 mg / kg body weight is mostly similar to that observed in the experimental group II (exposure to hydrocortisone at a dose of 2,5 mg / kg body weight), but it also has some differences. This applies, first of all, to the encapsulated layer, which although it has the same composition as in group II, is much thinner and with a large number of degraded areas. On the periphery and closer to the center of the tumor, extensive necrotic areas and necrotic cords are also observed. Blood vessels are found in the layer of tumor tissue (1-2 in the field of view), around some there is a strong rarefaction of the tissue. Cells are highly differentiated and are found both small and medium, rarely - large, their shape is round, irregular. The nuclei are normal and hyperchromic, also rounded oval and irregular in shape, with coarse granular chromatin (Fig. 3.28). Multinucleated cells with a low cytoplasm content are found in the preparations; its structure is homogeneous, basophilic. Mitotic tissue activity is low.

Thus, as in the experimental group II, the test tissue has a highly differentiated cell composition with low mitotic activity and increased antiangiogenic effect. The process of tissue degradation is pronounced. However, the main difference between the morphological pictures of this group, compared with group II, is that the face has a strong cytotoxic effect of the test substance. This is expressed, firstly, in the more extensive and widespread areas of necrosis, and, secondly, the destruction of the outer capsular layer, caused, apparently, by the cytotoxic effect of large doses of hydrocortisone.

Summing up the morphological analysis of AKATOL tumor tissue when exposed to various doses of hydrocortisone, we can draw the following conclusions. The effect of the hormone on the tumor has a pronounced cytotoxic effect, which consists of extensive areas of necrotic tissue destruction with structureless detritus, a high degree of exudation, and neutrophilic invasion. The presence of this pathology suggests that high doses of hydrocortisone have a negative effect on the metabolism and vital activity of tumor cells. At the same time, low doses of this hormone have a more gentle and, at the same time, pronounced physiological effect on tumor tissue cells.

In general, the obtained morphological picture corresponds to those characteristics that were obtained by studying the effects of hydrocortisone on proliferation and apoptosis in in vitro experiments (sections 3.1-3.3). It should also be noted that the effect of hydrocortisone on tumor tissue, although it causes serious violations in its integrity and physiological life, is not of a cardinal and final nature. The action of both small and large doses of the hormone studied does not lead to full-scale degradation of tumor tissue, does not completely inhibit the processes of mitotic division of tumor cells and, therefore, cannot pretend to be a monotherapeutic drug during hormonal therapy of malignant neoplasms. And, at the same time, the high antitumor activity shown allows us to hope for its use as an agent in combined hormone replacement therapy.

3.6. Inhibition of proliferation and induction of apoptosis by hydrocortisone in experimental tumor tissue of colon adenocarcinoma (AKATOL)

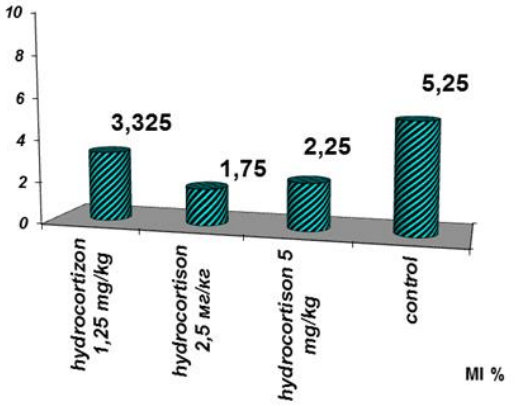
The antitumor activity of the studied biologically active compounds is determined primarily by the ability of anti-cancer agents to suppress the proliferation of tumor tissue and cause apoptosis in transformed cells. This is explained by the fact that these parameters of the activity of cancer cells determine the processes of tissue degradation, answer the question of the further development of neoplasms after pharmacological intervention, and, along with morphological analysis, allow us to adequately assess the results of the antitumor effects of the tested compounds.

We have studied the effect of hydrocortisone on the proliferation and induction of apoptosis in the cells of the colon adenocarcinoma. Tumor tissue proliferation was evaluated in the prepared morphological preparations by the

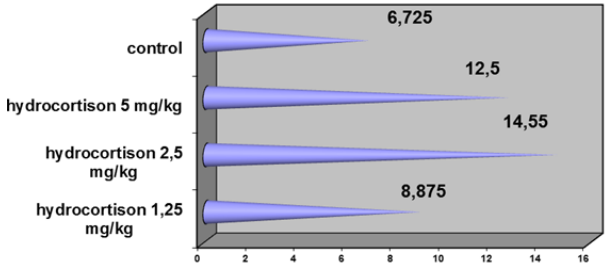
number of mitoses found, i.e. the mitotic index (MI) was calculated. The results of the study are presented in (Fig. 3.29).

The AKATOL tumor in experimental animals of the control group, as seen in (Fig. 3.9), actively proliferates. The mitotic index (MI) in the tumor tissue is quite high (5.25 ‰), which is typical for experimental tumor strains. The action of hydrocortisone at a dose of 1.25 mg / kg body weight led to a decrease in the rate of mitotic activity by about one and a half times. The effect of this hormone at higher doses (2.5 mg / kg and 5 mg / kg body weight) significantly reduced the mitotic activity of AKATOL tumor cells (1.75 2,2 and 2.25 ‰, respectively). It should be noted that the tendency of a more effective action of hydrocortisone at a dose of 2.5 mg / kg on tumor growth, shown in the previous sections, remains, however, a similar dependence in this case is visible only in absolute numbers, so the reliability of mitotic activity between these experimental groups is absent. Another parameter that characterizes the kinetic activity of tumor tissue is the apoptotic index.

The importance of this parameter for constructing an adequate picture of the kinetic processes of tumor tissue is explained by differences in the cell cycle of tumor cells. In particular, upon transformation, the cell acquires the ability to enter from the G0 phase (the state of the cell before the division stimulus is received) into G1 (preparation for DNA synthesis) and go through the entire division cycle without external or weak stimulation. The duration of the phases G1 + S + G2 + M in tumor cells does not decrease compared to the norm. The mechanism of intense unstimulated tumor division is associated in most cases with a mutation in the proto-oncogen encoding a receptor protein in the cell membrane and making this protein permanently activated, transmitting signals for chain division into the cell, or with mutations in the genes of one of the intermediate proteins, which also leads to to the constant activation of this protein and signal transmission to the following components of the chin.



Picture 3.29. Proliferative activity in experimental tumor tissue of the colon adenocarcinoma (AKATOL) when exposed



Picture 3.30. Programmed cell death of experimental colon adenocarcinoma (AKATOL) when exposed to hydrocortisone.

The effect of antitumor drugs on inhibiting the proliferation of pathological tissues should lead to an interruption in the chain of stimulation of division, by affecting the receptors or proteins responsible for mitosis, or to changes in the genome of the cell. In both cases, we can talk about the activation of the mechanisms of programmed cell death or apoptosis. Therefore, when conducting an analysis of the proliferative activity of tumor tissue, it is impossible to talk about the progressive or, conversely, regressing nature of this process only taking into account the number of mitoses, since morphological analysis cannot adequately assess absolutely all the number of dividing cells due to the transience of some phases of mitosis, and apoptotic

The index, together with the mitotic index, gives an objective assessment of the growth or death of the tumor. In fig. 3.30 presents data on the number of apoptotic cells in an experimental AKATOL tumor after exposure to various doses of hydrocortisone. Hydrocortisone at a dose of 1.25 mg / kg body weight slightly increased the number of apoptotic cells in the tumor tissue (8.875 ‰ compared to 6.725 ‰ in the control group). Exposure to this hormone at higher concentrations led to a significant increase in AI (14.55 ‰ and 12.5 ‰), when compared with the control (Fig. 3.30). As already noted, the ratio of mitotic and apoptotic activities suggests a regression or progression of tumor tissue. In the case of the use of hydrocortisone in doses of 2.5 and 5 mg / kg body weight, we observe a pattern of rapid regression of tumor tissue, since the number of dying cells exceeds more than 2 times the number of dividing cells. At the same time, it is noticeable that the number of apoptotic cells in the control group of experimental animals also exceeds, although not so significantly, the number of dividing cells. In addition, the regression of the tumor tissue in the experimental groups with high AI values is so high that, in the case of the death of the tumor at a similar speed, we would not be able to obtain tumor material on day 16 after its implantation. This leads to the fact that the rate of regression of tumor tissue, as, for example, in the experimental groups, can be overestimated in relation to the real processes in the tumor. However, the picture of proliferative processes occurring in the tumor, reflected by the indices of mitotic and apoptotic activity is absolutely adequate, and the tumor regression rate shown by the ratio of these indices is comparable and indicative.

3.7. The effect of hydrocortisone on bone marrow cell proliferation

In the study of the antitumor properties of biologically active substances, the main attention is paid to the greatest efficiency of their inhibition of the growth of transformed cells. Moreover, with an increase in the cytotoxic properties of new antiproliferative drugs in relation to tumor cells, their toxic effect on non-transformed, but also highly proliferating, cells of the body, and primarily on hematopoietic bone marrow cells, also increases. Insufficient attention paid to this problem has led to the fact that the vast majority of modern antitumor drugs have such strong side effects that it prevents them from being used in optimal high doses and that, in turn, increases the resistance of tumor cells to pharmacological effects. Hormonal drugs are no exception. Moreover, their side effect, when used in high concentrations, is often even stronger than the chemotherapeutic effect, since

the range of the effect of hormones on the physiological systems of the body is wide.

Our task in this monograph was to determine the possible inhibitory effect of various doses of hydrocortisone on the proliferation of non-transformed highly proliferating bone marrow cells in experimental animals in vivo.

Bone marrow cells were isolated from tumor-bearing animals with an implanted tumor strain of the colon adenacarcinoma (AKATOL), which were injected with hydrocortisone in physiological saline on days 3, 5, 7, and 9 after tumor inoculation at doses: 1,25 mg / kg body weight - I group; 2,5 mg / kg body weight - group II; 5 mg / kg body weight - group III. Bone marrow cell proliferation was evaluated by the number of mitoses found, i.e. the mitotic index (MI) was calculated. The results of the study are presented in (Fig. 3.31).

As can be seen from the data presented in (Fig. 3.31), all studied doses of hydrocortisone to one degree or another reduce the proliferation of bone marrow cells. The most significant decrease in MI indicators occurred with the use of the hormone in the highest concentrations (5 mg / kg body weight) - up to 2.73 ‰, with a similar indicator in the control of 4.2 ‰. At lower doses (1,25 and 2,5 mg / kg body weight), hydrocortisone had a more sparing effect on normal cells, while at a dose of 1.25 mg / kg body weight, the inhibitory effect of the hormone was minimal and the MI did not statistically significantly differ from the control group. ‰

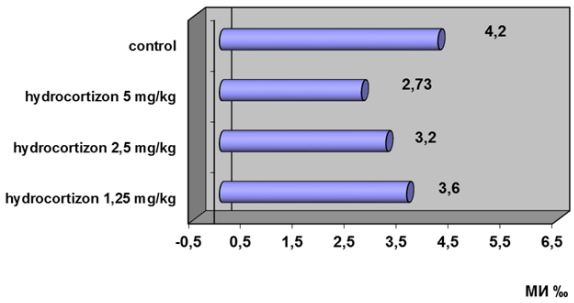


Figure 3.31. The effect of hydrocortisone on the proliferation of untransformed bone marrow cells.

In general, hydrocortisone, when compared with the inhibitory effect of popular modern chemotherapy drugs, slightly reduces the

proliferating activity of bone marrow cells. However, there is a tendency that, with an increase in the dose of exposure to the hormone, its side effect against non-transformed cells also increases. Consequently, the use of hydrocortisone in hyperdoses is not necessary since. Reducing the dose of the drug does not reduce its antitumor activity. As noted in previous sections, an increase in the active concentration of hydrocortisone does not mean an automatic increase in its cytotoxic properties. The most optimal dose of the antitumor effect of hydrocortisone, to summarize all the studies, is 2.5 mg / kg body weight, and it has the least side effect at a concentration of 1.25 mg / kg body weight. But at the same time, the use of the hormone in ineffective, minimal doses can lead to an increase in the resistance of tumor cells to pharmacological effects and, accordingly, to the ineffectiveness of the therapy. Thus, we consider the use of hydrocortisone at a dose of 2.5 mg / kg body weight the most preferable, despite the fact that this can lead to a slight decrease in the proliferative activity of bone marrow cells. Carrying out immunocorrection will avoid the negative side effects of hydrocortisone therapeutic effect. And the studied mechanism of the antitumor effect of hydrocortisone will make it possible to scientifically substantiate the use of this hormone in clinical practice in complex therapy for hormone-dependent neoplasms.

CONCLUSION

Immunohistochemical analysis revealed transformed mammary cells with no estrogen receptors on their surface, which allowed us to study the inclusion of alternative mechanisms for inhibiting the proliferation of these cells when exposed to hydrocortisone. The marker for identifying the antiproliferative effect of hydrocortisone in transformed mammary cells was the СегвВ2 gene (HER / 2neu), the expression product of which is oncoprotein, which belongs to the epidermal growth factor receptor (EGFR) family. Overexpression of this gene is most often detected in the development of breast cancer (breast cancer), stomach cancer (cancer), colorectal cancer (PK). An increase in the amount of HER / 2neu oncogenic protein on the surface of transformed epithelial cells of the intestine, stomach, or breast indicates a worse prognosis of the disease and necessitates the use of higher doses of cytostatics, which negatively affects the quality of life of patients. The obtained results of experimental in vitro studies have established that when exposed to various doses of hydrocortisone (from 0.025 mg / 1.6 x 106 cells to 0.1 mg / 1.6 x 106 cells), the number of HER / 2neu receptors on the tumor membranes decreases estrogen - negative breast cells. The mechanism of this effect probably lies in the inhibition of tumor cell proliferation by hydrocortisone through the signaling system of hydrocortisone-mediated receptors.

An important aspect of these studies is that a reduction in the dose of hydrocortisone does not affect the inhibition of the expression of HER / 2neu oncoprotein on the surface of estrogen-negative breast tumor cells.

A study of the cytotoxic (death) effect of hydrocortisone on tumor cells revealed a dose-response reaction. The higher the concentration of hydrocortisone molecules, the more pronounced the cytotoxic effect and the higher the death of tumor cells (up to 69%). The observed cell death is carried out by the type of necrosis and is associated with a change in their metabolism. The low apoptotic cell death detected in this experiment is explained by the fact that hydrocortisone at especially high doses leads to receptorocytosis. Overexpression of the hormone either causes the redistribution of receptors relative to the cytoplasmic and nuclear membranes (invasion of surface receptors deep into the cytoplasm), or disables the transmission of the apoptotic signal associated with the excess of the physiologically acceptable norm of the number of hydrocortisone molecules, as a result of which active mechanisms of antistress protection of the cell against the toxic effect of hormone hyperconcentration are activated. Another

possible way of the cytotoxic effect is the competitive interaction of a large number of hydrocortisone molecules with an insufficient number of receptors, which leads to the shutdown of apoptosis i.e. physiological cell death and vice versa the inclusion of a cytotoxic reaction.

In vivo experiments to study the effect of hydrocortisone on a BALB / c tumor transplanted into mice of the AKATOL tumor allowed the results to be correlated with the results obtained on tumor cell culture. The data of this experiment showed a high antitumor activity of hydrocortisone, capable of inhibiting the proliferation of tumor cells and inducing their death by the type of necrosis. The most effective dose in both cell culture and the body as a whole was the average dose (0.05 mg / 1.6 106 cells and 2.5 mg / kg body weight, respectively).

By analyzing the antitumor activity of hydrocortisone using morphological studies, we were able to characterize the mechanism of this effect.

Violation of the mitotic regime of tumor tissue comes to the fore. Inhibition of mitosis is observed at the metaphase stage, which are mainly pathological. K - mitoses are more common when exposed to high doses. The presence of large necrotic sites indicates the cytotoxic effect of hydrocortisone, with a dose-response response. Low doses of the hormone have a more gentle, physiological effect on the tumor, which is expressed in a delayed cytotoxic effect, in a lesser inhibition of proliferation, less pronounced necrosis, but pronounced induction of apoptosis with gradual degradation of the tumor tissue, which allows the body to cope with the toxins coming in with less negative effect with the death of a large number of cells. The ratio of mitotic and apoptotic activity suggests a regression or progression of tumors. The results obtained indicate a direct proportion of the inhibition of proliferation and the induction of apoptosis, this dependence is most pronounced when exposed to an average dose (2.5 mg / kg). The effect of hydrocortisone on the proliferation of non-transformed cells (normal bone marrow cells) caused a slight decrease, with the dose-response response, with an increase in the dose, the negative side effect leading to erythro- and leukopenia increases. This effect is probably the main factor in the development of immunosuppression when using hydrocortisone in high doses. Medium and low doses neutralize this side effect. Therefore, the use of immunocorrection in combination with hydrocortisone will reduce the side effect of this therapy and will effectively influence the tumor process in the absence of a response of transformed cells to traditional therapy, as well as in the formation of an individual body resistance.

RESULTS

🕮 1. Exposure to hydrocortisone at all concentrations (0,1; 0,05; 0,025 mg / 1,6 x 10^6 cells) led to a decrease in the average number of HER-2 / neu receptors on the membranes of estrogen-negative (ER-) transformed breast cells on average 27,25 ± 1,14%, p <0,001.

🕮 2. The reduction in the dose of the therapeutic effect of hydrocortisone does not affect the efficiency of inhibition of the expression of the HER-2 / neu protein on the surface of breast cancer ER cells.

🕮 3. Hydrocortisone in experiments both in vitro and in vivo showed high cytotoxic activity against various transformed (breast, AKATOL). The dose-dependent effect of hydrocortisone is observed: at a dose of 0,025 mg / 1,6 x 10^6 cells, the hormone inhibits the viability of breast cells by 42%, while apoptosis induction was not observed, while doses of 0,05 mg and 0,1 mg / 1,6 x 10^6 cells, suppressed cell viability by 69% and 60%, respectively, but the effect of these doses caused the induction of apoptosis.

🕮 4. Hydrocortisone has a high antiproliferative activity and is able to suppress the growth of cells of the experimental tumor strain AKATOL in all doses studied. MI at a dose of 1,25 mg / kg was 3,32 ‰, at a dose of 2,5 mg / kg – 1,75 ‰, at a dose of 5 mg / kg -2,25 ‰. Hydrocortisone in doses of 1,25 mg / kg, 2,5 mg / kg, 5 mg / kg causes inhibition of tumor growth of ACATOL by 58,2%, 82,8%, 73,7%, respectively.

🕮 5. Morphological changes in tumors were expressed in the form of extensive zones of necrotic tissue destruction with structureless detritus, a high degree of exudation, neutrophilic invasion, a pronounced decrease in the number of cells, and nuclear degradation.

🕮 6. Hormone in a high dosage (5 mg / kg of body weight causes a significant decrease in proliferation of bone marrow cells to 2,73 в, in the control of 4.2 B. At lower doses (1,25 and 2,5 mg / kg of body weight body) hydrocortisone has a more gentle effect on non-transformed (normal) cells of the body, but at the same time at the doses of 1,25 mg / kg and 2,5 mg / kg of body weight, the inhibitory effect of the hormone was minimal, MI did not statistically differ from the control group (MI – 3,6 ‰ and 3,2 ‰, respectively).

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MONOGRAPH

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Subscribe to print 24/06/2020. Format 60×90/16.
Edition of 300 copies.
Printed by "iScience" Sp. z o. o.
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ISBN 978-83-66216-38-9