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Development and Research of a New Soluble Dosage Form of Adrenal Cortex Function Inhibitor - Mitotane (O,P' Ddd (Chlodytan))

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Abstract

For the first time, a soluble injectable dosage form of an inhibitor of the function of the adrenal cortex - o,p'-DDD - 1-,2-Chlorophenyl-1-(4'-Chlorophenyl)-2,2-Dichloroethane) (mitotane, chloditan), namely - a 5% solution of o,p'-DDD in a mixture of solvents of propylene glycol, ethyl alcohol, N,N-dimethylacetamide in a ratio of 7:2:1 for intravenous administration, was created. In vitro experiments on adrenal homogenates established the inhibitory effect of the drug on the synthesis of corticosteroids, and in vivo the influence of the specified dosage form on the morphofunctional state of the adrenal cortex was studied. The effect of o,p-DDD (mitotane, chloditan) on the cultured human adrenal cortex, inhibition of the synthesis of corticosteroid, has been established. It has been shown that when administered intravenously, the solution of chloditan inhibits the function of the adrenal cortex in experimental animals (dogs). Preclinical studies of the new dosage form of the drug o,p'-DDD were conducted and its acute and chronic toxicity were determined. According to the classification of compounds by toxicity, the solution of chloditan for injection belongs to class IV of low- toxic compounds, and the solvent to class VI of non-toxic substances. The chemical and pharmaceutical properties of the 5% solution for injection of o,p' DDD were studied, which include the following indicators regulated by the State Pharmaceutical Federal Service for the Control of Pharmaceuticals and Biotechnology: description, identification, transparency, color, mechanical inclusions, accompanying impurities, extractable volume, sterility, bacterial endotoxins, abnormal toxicity, quantitative determination, storage conditions and shelf life. The results obtained indicate that, according to its parameters, the tested chloditan solution can be used for further research in the treatment of adrenal tumors and Cushing's disease.

***Keywords**: Drug Form; Solution For Injection; Chloditan; Mitotane; Toxicity; Adrenal Cortex; Adrenocortical Activity*

Introduction

For the treatment of rare but extremely dangerous diseases of adrenocortical cancer (ACC), and Cushing's syndrome, o,p'-DDD was recommended at the end of the 20th century. Among hundreds of studied compounds, o,p'-DDD turned out to be one of the most effective blockers of corticosteroid synthesis in the cortex of adrenal (CAG). Under its influence, the main biochemical reactions are disrupted and the activity of a number of steroid biosynthesis enzymes in the CAG changes [1,2]. The drug o,p'-DDD, which was called mitotane (USA), lysodren (Italy, France), chloditan (Ukraine), remains the most active CAG inhibitor to this day [3-5]. The main intracellular target of the drug is the mitochondrial membranes and the outer membranes of adrenocorticocytes [6]. The only treatment for ACC is surgical resection of the adrenal gland along with lymph nodes and adjacent large veins. Despite a properly performed adrenalectomy, regional recurrence or distant metastases are detected in 91% of patients [7], therefore, adjuvant therapy with mitotane should be started 12 weeks after

surgery and continued for 2-5 years, provided it is well tolerated [8,9]. Existing data indicate the effectiveness of mitotane in selected patients with Cushing's syndrome [10]. To achieve a positive effect, high doses of the drug (maximum daily dose of 8 - 10g) and long-term (several months) treatment are used. The use of high doses of the drug is explained by its poor solubility in water, lipophilicity of the active substance and low bioavailability for tumor tissues when administered orally [11]. Only 15 - 17% of chloditan accumulates in the adrenal cortex, the rest is deposited in adipose tissue. Some patients with oral use of mitotane experience side effects - nausea, vomiting, itching of the skin, diarrhea, ataxia, leukopenia, gynecomastia, etc. [12,13]. In order to increase the therapeutic efficacy of mitotane, and accordingly, reduce its dosage and reduce side effects, we investigated the possibility of the action of the soluble form of the drug *in vitro* and *in vivo*. The new injectable dosage form of the drug (5% solution of o,p'-DDD in a mixture of solvents of propylene glycol, ethyl alcohol, N,N-dimethylacetamide in the ratio 7:2:1) is designed for intravenous administration, it has no world analogues. To implement it in practice, a complex of chemical-pharmaceutical and pharmacological studies was conducted, to which this material is devoted.

Materials and Methods

The study was conducted *in vitro* on the adrenal glands of dogs, rats, chickens. 2% homogenates in 0.25M sucrose solution were prepared from the adrenal glands of these species, introduced into an incubation system containing 50 μg of the corticosteroid precursor progesterone or deoxycorticosterone and different amounts of o, p!-DDD dissolved in propylene glycol and blood plasma were added, incubation was carried out for 1 hour and the content of 11-hydroxycorticosteroids (11-GOCS) in the incubation medium was determined. Details of the studies have been published previously [14]. In further studies, a special 5% solution of o, p'-DDD (chloditan) was created in a mixture of solvents of propylene glycol, ethyl alcohol, N, N-dimethylacetamide in a ratio of 7:2:1. 1-,2-Chlorophenyl-1-(4'-Chlorophenyl)-2,2-Dichloroethane "Merck" (Germany) was used as the medicinal substance. The solvents from Merck were EMPARTA® qualified. All solvents are approved for use in injectables. For *in vitro* experiments, conditionally normal human adrenal gland tissue was obtained in the Department of Endocrine Surgery of the V. P. Komissarenko Institute of Endocrinology and Metabolism of the National Academy of Medical Sciences of Ukraine during adrenalectomy for pheochromocytoma or adenomatous hyperplasia of the adrenal gland, washed several times with sterile 0.9% sodium chloride solution with antibiotics (100U of benzylpenicillin sodium salt and 100 μg of streptomycin sulfate per 1 ml of solution), cleaned of fat and connective tissue, then cut into pieces of approximately 1 mm and washed again several times with the specified solution. The obtained tissue was cultured in glass vials with 1 ml of RPMI-1640 medium ("Sigma", USA) containing 10% fetal calf serum ("Sigma", USA) and antibiotics at a temperature of 37°C. The culture medium was changed once every two days. Within 24 hours, part of the samples also contained o,p'-DDD in final concentrations of 0.005; 0.05; 0.5 and 5.0 mg/ml. On the second day of cultivation, adrenocortical tissue was selected for histological examination, and on the 3rd, 4th, 8th, 12th, 16th, 20th, 24th and 28th days, aliquots of the medium were selected and frozen at −20°C for subsequent determination of the level of 11-GOCS. Two series of experiments were conducted to study the functional activity of human CAG tissue in the dynamics of long-term cultivation and the effect of o,p'-DDD on the level of 11-GOCS in the cultivation medium (in each series, 3 experiments with groups in duplicates and triplicates). Experimental determination of chronic toxicity of the drug and solvent mixture was carried out on 15 white sexually mature male Wistar rats by intraperitoneal route of administration for 90 days with daily administration of 0.2 ml (therapeutic dose) of solvent mixture or o,p'-DDD solution to rats. The control group of rats was administered 0.2 ml of saline solution, respectively. The animals were kept in controlled conditions of a vivarium at 20 - 25°C, humidity 60 - 65%, on a standard food diet. Before the start of the experiment and after 14, 30 and 90 days, the rats were placed in exchange cages for urine collection. The content of protein, glucose, acetone, leukocytes and erythrocytes was determined in the urine. At the same time, blood was taken from a vein to determine the number of erythrocytes, leukocytes and platelets.

Histological studies of the adrenal glands, kidneys, liver and heart were performed after the administration of a mixture of solvents and a solution of o,p'-DDD for 90 days. One day after the last injection, the rats were euthanized by decapitation under light ether anesthesia.

Acute toxicity studies were conducted on sexually mature male Wistar rats by the intraperitoneal route of administration in several stages: in the first, the range of average lethal doses, and in the following stages more accurate values of lethality indices were obtained. *In vivo* experiments were conducted on dogs. Male dogs weighing (10.0 ± 0.3) kg were kept in controlled conditions of a vivarium at 20 - 25°C, humidity 50 - 55%, on a standard diet. Ten dogs were divided into two groups. Five dogs of the first group served as controls, they were intravenously injected with 10 ml of o,p'-DDD solvent. Five experimental animals were injected with a 5% o,p'-DDD solution. Before the first intravenous injections, 5 ml of blood was taken from a vein to determine 11-GOCS and a test for functional reserves of the adrenal cortex was performed by stimulating with synthetic 1-24-corticotropin (synacten-depot, Novartis, Germany) intravenously at a dose equivalent to 25 IU of pituitary ACTH. After 1.5h, 5 ml of blood was taken for repeated determination of 11-GOCS content and o,p'-DDD solution or solvent was injected. The administration of the drug or solvent was repeated in the next two days (a total of three injections per course), immediately after which a repeated test with synacten was performed. Two weeks after the end of the experiment, the basal level of 11-GOCS in plasma was determined again. Quantitative determination of the level of 11-GOCS, i.e. the total content of cortisol and corticosterone, in aliquots of the medium for cultivating human CAG and in dog blood plasma was carried out by the fluorometric micromethod on a "Hitachi MPF-4" spectrofluorimeter (Japan) using crystalline cortisol ("Reanal", Hungary) as a standard. Before the start of the study, a positive decision was obtained from the Bioethics Commission of the Institute, as well as informed consent from each patient from the surgical department for use in the experiment of their removed adrenal tumor. All manipulations with animals were carried out in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986) and national bioethics standards (I National Congress on Bioethics, Kyiv, 2001). The results obtained were processed by the Student method using the t criterion. The main physical and chemical-pharmaceutical indicators were determined according to the requirements of the State Federal University of Science and Technology [15].

Results and Discussion

The solubility of o, p'-DDD in the aqueous phase is very low. This circumstance probably did not allow other researchers to create the optimal effective concentration of the inhibitor when studying the action of the inhibitor *in vitro*. To overcome this obstacle, the drug was dissolved in propylene glycol and the solution was combined with plasma, imitating its protein solution. The synthesis of 11-GOCS after the introduction of the precursor of biosynthesis-progesterone into the incubation medium increased by 4-5 times. Against this background, the introduction of o, p'-DDD into the incubation medium at a dose of 3 μmol inhibited the synthesis of 11-GOCS by rat adrenal homogenates. Increasing the dose of the drug to 6 μmol and above inhibited the synthesis of 11-GOCS by the adrenal glands in all studied species (Table 1), and at a dose of 18 μmol there was almost complete inhibition of corticosterone synthesis.

Species	n	Control	o,p!-DDD in μmol			
			3	6	12	18
Dogs	8	19,8 ± 1,01	-	14,1 ± 1,2*	12,1 ± 1,3*	6,3 ± 06*
Chickens	10	33,7 ± 2,11	37,8 ± 1,5	20,0 ± 1,4*	13,3 ± 0,62*	2,1 ± 0,35*
Rats	10	76,3 ± 4620	27,5 ± 2.6 *	13,1 ± 1,2*	3,9 ± 0,5*	0,0

Table 1: *Effect of o, p'-DDD on the formation of corticosteroids by adrenal homogenates (μg/100 mg tissue per hour).*

*Note: * P < 0.05 relative to control.*

It is known that some animal species are not sensitive to the corticolytic action of the drug, including rats. Our studies have shown that in these animals, corticosterone synthesis is significantly inhibited under the influence of o, p'-DDD. This probably indicates the need for the drug to enter the adrenal glands for its action. The dependence of the drug's action on its concentration in the blood confirms this assumption [16,17]. Based on this and the need to reduce the toxic effect of the drug, we developed a soluble form of o, p'-DDD and investigated its effectiveness.

According to the results of the conducted studies, the presence of hormonal activity of human CAG tissue was established in the dynamics of long-term cultivation: the basal level of 11-GOCS in the culture medium underwent certain fluctuations and was on the 4th day (44.5 ± 3.5) μg/ml, on the 8th - (48.0 ± 7.0) μg/ml, on the 12th - (44.5 ± 7.5) μg/ml, on the 16th - (34.5 ± 6.5) μg/ml, on the 20th - (42.8 ± 9.2) μg/ml, on the 24th - (31.8 ± 3.2) μg/ml, on the 28th - (48.2 ± 1.2) μg/ml.

Human CAG tissue culture with o,p'-DDD resulted in a dose-dependent decrease in the level of 11-GOCS in the medium: at a drug concentration of 0.005 mg/ml - by 11.0%, at 0.05 mg/ml - by 39.5%, at 0.5 mg/ml - by 54.3% ($P < 0.05$) and at 5 mg/ml - by 69.8% ($P < 0.02$). The results obtained by us indicate that o,p'-DDD in the composition of the proposed solution causes, at comparable concentrations, a similar effect in adult CAG tissue cultures. The presence of the organic solvents used does not affect the ability of o,p'-DDD to inhibit corticosteroid secretion *in vitro*. The next stage was the study of safety, in particular the acute and chronic toxicity of the o,p'-DDD solution. The acute toxicity of the soluble form of o,p'-DDD and solvents was studied in male Wistar rats with the intraperitoneal route of administration, which is the closest to its intravenous administration. The study of acute toxicity was carried out in several stages: in the first, the range of median lethal doses was determined, and in the subsequent stages, more accurate values of lethality indicators were obtained [18]. The o,p'-DDD solution was administered in the following doses: 1, 10, 100, 1000 mg/kg, i.e. each subsequent dose was 10 times greater than the previous one, and the solvent was administered in doses of 0.1, 10, 25 ml/kg. The dose of 25 ml/kg of the solvent was the maximum permissible, which could be administered to rats intraperitoneally (at the rate of 5 ml per rat). The condition of the rats was observed for 2 weeks. Results of the first stage of studies: death of rats was observed after administration of the o,p'-DDD solution in a dose of 1000 mg/kg. At a dose of 100 mg/kg, the o,p'-DDD solution did not cause death of the animals. That is, the value of the median lethal doses was in the range from 100 mg/kg to 1000 mg/kg. After the introduction of the solvent, the death of rats was observed when it was administered in doses of 10 and 25 ml/kg. At a dose of 1 ml/kg, the solvent did not cause the death of rats. Thus, the value of the median lethal doses of the solvent was in the range from 1 to 10 ml/kg. In acute poisoning with the solvent administered in large doses, general depression, weakness were initially observed, and later - convulsions, after which the animal died. The death of rats occurred within 30 - 60 minutes after the introduction of the drug. To determine the LD_{50} of the o,p'-DDD solution and the solvent in the first approximation, each tested dose of both the drug and the solvent was administered intraperitoneally to one animal. In the series of doses studied, each subsequent one was 1.5 times greater than the previous one. The lowest dose that caused the death of the animals was taken as the LD_{50} (approximate). The lowest dose of the solvent that caused the death of rats after intraperitoneal administration was a dose of 10 ml/kg, which can be taken as the LD_{50} for the solvent. The lowest dose of the o,p'-DDD solution that caused the death of rats after intraperitoneal administration was a dose of 510 mg/kg, which can be taken as the LD_{50} for the o,p'-DDD solution. The median lethal doses of the o,p'-DDD solution significantly exceed the usual therapeutic doses of the drug. According to the classification of substances by toxicity, the o,p'-DDD injection solution belongs to the IV class of low-toxic substances, which includes compounds whose LD_{50} when administered intraperitoneally is in the range from 101 to 1000 mg/kg of body weight. The solvent can be classified as class VI of relatively harmless substances, which includes compounds whose LD_{50} when administered intraperitoneally is more than 3000 mg/kg of body weight. The chronic toxicity of the drug and solvent was also studied in male Wistar rats by intraperitoneal route of administration under the above conditions [19]. When determining the chronic toxicity of the drug, no experimental animal died, which indicates good tolerance of the injectable form of the drug o,p'-DDD. During the experiment, isolated cases of diarrhea were observed in animals. Also, at the beginning of each stage of the experiment, an increase in aggressive behavior was observed in rats (which is apparently associated with pain from the action of the solvent). A few days later, aggression stopped and normal behavior was restored. As a result of the drug's action, small induration formed under the skin of the animals at the injection site on the 90th day. All experimental animals had normal fur, were active and ate normally. The administration of solvents, as well as the o,p'-DDD solution, did not cause changes in the body weight of the rats. At the beginning of the experiment, no significant difference was observed in any of the blood parameters between the control and experimental groups of rats ($P>0.1$). The table summarizes the results of blood tests in rats that were intraperitoneally administered 0.2 ml of saline, a mixture of solvents, and a solution of o,p'-DDD. According to the data obtained, significant changes in

hematological parameters were observed after 14 days of administration of a mixture of solvents and a solution of o,p'-DDD, especially leukocytes (2-2.5-fold increase) and platelets (2-3-fold increase). During this period of the study, there was no significant difference in the number of leukocytes between the two experimental groups, while the number of platelets in rats that were administered o,p'-DDD was significantly higher ($P < 0.01$). When the study period was extended to 30 days, compared with the group of rats that received solvents, the number of leukocytes ($P < 0.05$) and platelets ($P < 0.01$) significantly increased. This difference probably reflects the significant destruction of the adrenal cortex during this period, which caused a decrease in the level of glucocorticoids, which suppress the immune system by reducing leukocytes in the blood. At the same time, other indicators (the number of erythrocytes, hemoglobin and hematocrit) practically did not change throughout the experiment. On day 90, a layer of small cells of the fascicular zone was found in the adrenal glands under the glomerular zone, which had a normal structure. Their appearance may indicate the activation of the hypothalamic-pituitary-adrenal system in response to a significant decrease in the level of glucocorticoid hormones due to the destruction of the cells producing them by o,p'-DDD. It is noteworthy that the final indicators of the number of leukocytes and platelets when administering a mixture of solvents and a solution of o,p'-DDD do not differ significantly. This indicates the possibility of toxic effects of solvents when administered intraperitoneally. The results of the study of subchronic toxicity of the injectable form of the drug o,p'-DDD are presented in table 2.

Research periods, days	Physiological solution		Mixture of solvents		Injection drug o,p'-DDD	
	Leukocytes	Thrombocytes	Leukocytes	Thrombocytes	Leukocytes	Thrombocytes
1	8,54 ± 0,20	683,2 ± 99,5	8,64 ± 0,28	642,2 ± 72,3	7,88 ± 0,44	552,2 ± 35,0
14	8,90 ± 0,22 P1,2>0,25	665,2 ± 22,3 P1,2>0,5	13,24 ± 0,62 P1,2<0,001	905,8 ± 21,1 P1,2<0,01	13,66 ± 0,61 P1,2<0,001	1337,4 ± 81,3 P1,2<0,001
30	9,94 ± 0,29 P1,3 <0,01 P2,3 <0,05	549,6 ± 35,9 P1,3 >0,25 P2,3 >0,25	17,32 ± 0,37 P1,3<0,001 P2,3 <0,001	1071,8 ± 40,0 P1,3 <0,001 P2,3 <0,01	18,76 ± 0,41 P1,3 <0,001 P2,3 <0,001	1995,2 ± 96,9 P1,3<0,001 P2,3 <0,001

Table 2: *The effect of chronic use of the injectable form of the drug o,p'-DDD on the content of leukocytes and platelets in the blood of rats, ×100/l.*

Note: P1.2, P1.3, P1.4 - reliability of changes on 14, 30, 90 days compared to the first; P2.3, P3.4 - reliability compared to the previous one.

Analysis of rat urine did not reveal any significant changes in either the control or experimental groups, indicating a minor effect of solvents and o,p'-DDD on the urinary system when administered parenterally. Pathological examination of rats administered o,p'-DDD, in contrast to rats administered only the solvent, revealed adhesions between intestinal loops and attachment of individual loops to the peritoneum. Individual liver lobes also had adhesions, which led to their deformation.

Gravimetric analysis revealed that the weight of the heart and kidneys in experimental rats did not change, there was a tendency to increase the weight of the liver (in control rats 3.5 ± 0.1 g/100g of body weight versus 4.0 ± 0.2 g/100g of body weight in rats administered o,p'-DDD, $0.05<P<0.1$). Histological analysis of the adrenal glands was performed on median sections, on which the medulla was present and the glomerular, fascicular and reticular layers of the cortex were distinguished. In rats receiving o,p'-DDD, a small part of the cells of the glomerular zone had reduced hyperchromic nuclei and vacuolated cytoplasm. The structure of the fascicular zone was significantly disturbed due to severe swelling of the cells and a change in their shape from cubic or prismatic to rounded-polygonal. The cells of the fascicular zone underwent vacuolar degeneration. The nuclei of these cells were wrinkled, hyperchromic or pyknotic, the cytoplasm was coarse-grained, weakly oxyphilic. The capillaries of the fascicular zone were filled with erythrocytes, often with signs of blood stasis. Local accumulations of inflammatory cells were found in the fascicular zone. Leukocytes and erythrocyte slags were also found in the dilated vessels of the reticular zone. Unlike the fascicular zone, the cells in the reticular zone had a normal structure - spherical normochromic

nuclei with clear nucleoli and homogeneous oxyphilic cytoplasm. In the adrenal glands of animals that were injected with only the solvent, the nuclei of the cells of the fascicular zone retained their normal structure - they remained spherical normochromic, contained clear nucleoli, and the cells were less vacuolated (Figure).

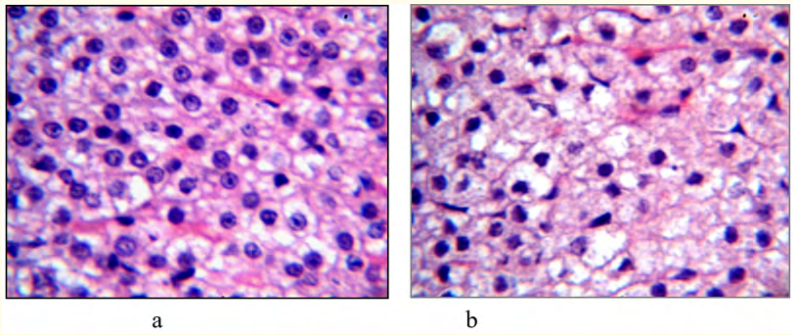

Figure: *Micrographs of the fascicular zone of the adrenal cortex of rats injected with solvent or o,p'-DDD solution: a - Moderately vacuolated cells with normochromic nuclei in the fascicular zone of the adrenal cortex of a rat injected with solvent; b - Hyperchromic nuclei without nucleoli and shrunken nuclei in strongly vacuolated cells of the fascicular zone of rats injected with o,p'-DDD solution. Hematoxylin-eosin, ×400.*

This indicates a selective damaging effect of the drug o,p'-DDD upon parenteral administration on the cells of the fasciculus zone, the increased proliferation of which causes the development of ACC and Cushing's disease. In animals injected with o,p'-DDD solution, the ventrally located liver lobes, which may have been in contact with the injected solution, had a thick edge and a thickened capsule. The organ was hyperemic much more severely. Hepatocytes located on the periphery were swollen, often had vacuolated cytoplasm and pyknotic nuclei. In these areas, the structure of the hepatic beams was disrupted, and the cells contained less glycogen. Small areas of destroyed hepatocytes were found. Disturbances in the structure of the peripheral parts of the liver under the action of o,p'-DDD were more pronounced than in animals injected with only the solvent. In the central areas of the sections, the liver structure remained normal. In the dorsally located lobes of the liver, no structural abnormalities were noted in the peripheral parts. No structural abnormalities of the heart were detected under the influence of the o,p'-DDD solution or one solvent. The structure of the kidneys was also not significantly damaged. An important stage was the experimental determination of the main chemical and pharmaceutical indicators that are necessary for the creation of analytical and regulatory documentation for the 50 mg/ml injection solution according to the requirements of the State Federal University [15]. Based on the tests conducted, analytical regulatory documentation was created for the o,p'-DDD solution for injection 50 mg/ml. The main characteristics of the solution are given in table 3.

Points	Indicator name	Permissible limits	Control methods relatively SPU*
1	Description	Transparent colorless liquid with a faint specific odor	P.1.SPU organoleptically
2	Identification	UV spectrum of 0.02% solution in the range from 220 to 350 nm has absorption maxima at 261 +/- 1 nm. 268 +/- 1 nm, 275 +/- 1 nm. Reaction to chlorides	P.2. SPU 2.2.2.5 P.2. SPU 2.3.1
3	Transparency	Should be transparent	P.3. SPU 2.2.1

4	Color	Should be colorless or the color should not be more intense than the VU standard	P.4. SPU 2.2.2 method II
5	Mechanical inclusion	Must meet the requirements	P.5. SPU 2.9.20
6	Accompanying impurities	A spot is observed on the chromatograph of the test solution, which is located at the level of the reference spot.	P.6. SPU 2.2.27 method TLC **
7	Extracted volume	Must meet the requirements	P.7. SPU 2.2.17
8	Sterility	The drug must be sterile	P.8. SPU 2.6.1
9	Bacterial endotoxins	The limiting concentration of endotoxins is 3.5 IU per 1 ml	P.9. SPU 2.6.14 method A.
10	Abnormal toxicity	Test dose of 50 µg o,p'-DDD in 1 ml for 1 mouse	P.10. SPU 2.6.14 method A.
11	Quantitative determination of o,p'-DDD	From 0.049 to 0.051g per ml of the drug	P.11. SPU 2.6.14 method GC.
12	Storage conditions	In a tightly closed container, protected from light, at a temperature of 15 to 25°C.	

Table 3: *Specification for the 50 mg/ml o,p'-DDD solution for injection.*

*Note: *SPU: State Pharmacopoeia of Ukraine; **TLC: Thin Layer Chromatography; ***GC: Gas Chromatography.*

The study of the intravenous action of the drug *in vivo* showed that three-day intravenous administration of 10 ml of the mixture of solvents o,p'-DDD per day did not affect the content of 11-GOCS in the blood plasma of dogs (P > 0.05) (Table 4).

Experimental conditions, indicator	Solvent	o,p'-DDD
Before the administration of the solvent or o,p'-DDD		
Basal level	481,8 ± 65,6	414,2 ± 17,3
1.5 hours after synacten administration	654,4 ± 93,1**	556,0 ± 38,6**
After 3 days of solvent or o,p'-DDD administration		
Basal level	465,0 ± 56,3	115,8 ± 7,8#
1.5 hours after synacten administration	712,0 ± 85,5*	166,0 ± 18,5*,#
In 2 weeks	-	269 ± 15,59***

Table 4: *Effect of 5% solution of o,p'-DDD on the concentration of 11-GOCS in the blood plasma of male dogs (nmol/l, n = 5).*

*Note: ***p < 0.001, **p < 0.01, *p < 0.02 relative to the corresponding basal level; # p<0.001 relative to the corresponding control.*

Similarly, under the influence of solvents, no significant changes in the response of the CAG to synacthen stimulation were detected (P > 0.05) (Table 4). With intravenous administration of a 5% solution of o,p'-DDD. The basal level of 11-GOCS decreased almost 3.5 times after three days. Against the background of synacthen administration, there was an almost threefold weakening of the CAG response, which was assessed both by the increase in the concentration of 11-GOCS in blood plasma (the increase in the control was 141.8 nmol/l, in the study - 50.2 nmol/l), and by their level achieved after hormonal stimulation. Thus, under the influence of the o,p'-DDD solution, not only was their basal production deeply suppressed, but also the functional reserves of the CAG were significantly reduced. At the same time, an increased level of leukocytes and platelets in the blood of the experimental dogs was not observed, which requires further research.

It should be emphasized that with intravenous administration of chloditan, the required effect is achieved when using 10-15 times reduced amounts of the inhibitor per course, which should significantly weaken the general toxic effect of o,p'-DDD.

Conclusion

In order to increase the effectiveness of chloditan, reduce its dosage and reduce side effects, we developed an injectable dosage form of the drug (5% solution of o,p'-DDD in a mixture of solvents of propylene glycol, ethyl alcohol, N,N-dimethylacetamide in a ratio of 7:2:1 for intravenous administration), which has no analogues in the world. It has been shown that o,p'-DDD (chloditan) in the form of a solution initiates a cytotoxic effect on the cultured human adrenal cortex and inhibits adrenocortical function both in human organ culture and in dogs when administered intravenously. With long-term continuous administration of the drug, the hypothalamic-pituitary-adrenal system is activated in response to a significant decrease in the level of glucocorticoid hormones due to the destruction of the cells that produce them. Studies of acute and chronic toxicity of the indicated dosage form were conducted. According to the classification of substances by toxicity, the o,p'-DDD injection solution belongs to the IV class of low-toxic substances, which includes compounds whose LD_{50} when administered intraperitoneally is in the range from 101 to 1000 mg/kg of body weight. The solvent can be attributed to the VI class of relatively harmless substances, which includes compounds whose LD_{50} when administered intraperitoneally is more than 3000 mg/kg of body weight. When determining the chronic toxicity of the drug, it was found that the toxic effects with a 90-day intraperitoneal administration of the solvent and the o,p'-DDD preparation are due to the action of the solvent. Histological studies have established that with peritoneal administration the solution has a specific damaging effect on the cells of the fascicular zone of the adrenal glands, and the o,p'-DDD solution also has a moderate effect on the liver of rats.

A full range of chemical and pharmaceutical properties of a 5% solution of o,p'-DDD was conducted in accordance with the requirements of the State Federal University of Ukraine and a specification for the drug was drawn up. The drug showed an effective inhibitory effect on the function of the adrenal cortex of dogs when administered intravenously.

Thus, the studied solution for injection of o,p'-DDD is safe for therapeutic use and can be recommended for further clinical studies.

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Conflict of Interest

There is no conflict of interest.

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