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Pre-Clinical Studies Of The Preparation "Cobafen" Lyophilizate For Preparation Of Solution For Injection With Solvent

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Abstract: ***Non-steroidal anti-inflammatory drugs (NSAIDs), which have more than 50 years of history, are the most demanded drugs in the modern world. With the general trend towards aging of human society, the number of patients requiring the use of NSAIDs is also growing [1]. The class of traditional NSAIDs currently includes more than 20 drugs that are similar in chemical structure, but differ from each other in the strength of the analgesic effect and the frequency of side effects. In accordance with WHO recommendations, pain should be assessed when prescribing pain medications. It is well known that NSAIDs are an effective pathogenetic therapy for acute or chronic pain, which is used by millions of patients around the world.***

According to WHO statistics, this figure is almost 30 million people, among them 40% are elderly people, and this figure is constantly growing [2].

Mecobalamin (a derivative of vitamin B12) is necessary for cell growth and reproduction, for the formation of deoxyribose and DNA, creatine, methionine - a donor of methyl groups, in the synthesis of a lipotropic factor - choline, for the conversion of methylmalonic acid into succinic acid, which is part of myelin, for the utilization of propionic acid ... S-adenosylmethionine from homocysteine due to the transfer of methyl and other one-carbon fragments.

Diclofenac sodium is an NSAID derivative of phenylacetic acid. It has a pronounced anti-inflammatory, analgesic and moderate antipyretic effect. The mechanism of action is associated with inhibition of the activity of COX, the main enzyme of the metabolism of arachidonic acid, which is a precursor of prostaglandins, which play a major role in the pathogenesis of inflammation, pain and fever. The analgesic effect is due to two mechanisms: peripheral (indirectly, through suppression of prostaglandin synthesis) and central (due to inhibition of prostaglandin synthesis in the central and peripheral nervous system).

Inhibits the synthesis of proteoglycan in cartilage. In rheumatic diseases, it reduces joint pain at rest and during movement, as well as morning stiffness and swelling of the joints, and helps to increase the range of motion. Reduces post-traumatic and postoperative pain and inflammatory edema.[1]

Suppresses platelet aggregation. With prolonged use, it has a desensitizing effect.

The drug has a pronounced anti-inflammatory, analgesic and moderate antipyretic effect. The mechanism of action is associated with inhibition of the activity of cyclooxygenase - the main enzyme of the metabolism of arachidonic acid, which is a precursor of prostaglandins, which play a major role in the pathogenesis of inflammation,

pain and fever.[2] The analgesic effect is due to two mechanisms: peripheral (indirectly, through suppression of prostaglandin synthesis) and central (due to inhibition of prostaglandin synthesis in the central and peripheral nervous system). Inhibits the synthesis of proteoglycan in cartilage. In rheumatic diseases, it reduces joint pain at rest and during movement, as well as morning stiffness and swelling of the joints, and helps to increase the range of motion. Reduces post-traumatic and postoperative pain and inflammatory edema. Suppresses platelet aggregation induced by adenosine diphosphate and collagen.

Key words: "COBAFEN", diclofenac sodium, mecobalamin, lyophilisate, acute toxicity, anti-inflammatory and analgesic activity.

1. INTRODUCTION

Purpose of the study: preclinical study of the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent, in terms of acute toxicity, anti-inflammatory and analgesic activity.

Study of acute toxicity of the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent

Materials and research methods. All studies used healthy animals quarantined for at least 10-14 days [3,4].

The study of acute toxicity was carried out according to the generally accepted method on white mice (both sexes) weighing 18-22 g, in a group of 6 animals, a total of 60 animals were used.

As a reference drug for comparative assessment of acute toxicity and specific activity, we have chosen the drug "Dicloberl" injection solution, "Berlin-Chemie AG" (Menarini Group) Germany, produced by: "A. Menarini Manufacturing Logistics and Services s.r.l." Italy.

The test drug was injected into experimental animals intramuscularly (into the femoral muscle), in the form of a 1% solution, in doses: 50 mg / kg (0.1 ml / 20 g), 150 mg / kg (0.3 ml / 20 g), 200 mg / kg (0.4 ml / 20 g), 250 mg / kg (0.5 ml / 20 g) and 300 mg / kg (0.6 ml / 20 g).

The comparison drug was injected into experimental animals intramuscularly (into the thigh muscle), at doses: 50 mg / kg (0.1 ml / 20 g), 150 mg / kg (0.3 ml / 20 g), 200 mg / kg (0, 4 ml / 20 g), 250 mg / kg (0.5 ml / 20 g) and 300 mg / kg (0.6 ml / 20 g).

Since, according to the literature, the maximum volume for intramuscular injection is 0.5 ml / 20 g, we used the fractional method to inject a larger volume. With the introduction of a volume of 0.6 ml / 20 g, 0.3 ml / 20 g was first injected, then after 10 minutes 0.3 ml / 20 g was injected.

Then the animals were placed in separate cages in groups, and were continuously monitored for the first hour, then were monitored hourly during the first day, and once a day, in the next 13 days of the experiment (total observation period was 14 days). At the same time, the clinical picture of intoxication and the lethality of animals were recorded [1, 2]. The average lethal dose (LD50) was calculated by the Litchfield and Wilcoxon method using probit analysis [3].

During the experiment, all animals were kept in standard vivarium conditions, and were on a complete food and water diet.[3]

Research results. After the administration of the drugs, a number of symptoms of intoxication, changes in the general condition and other effects characterizing the toxic effect were observed (Tables 1, 2).

Table 1
The results of the toxic effect of the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent

Dose	**Result**
50 mg / kg	In 10-15 minutes after the administration of the drug, a decrease in motor activity and crowding were observed within 5-6 hours. Subsequently, the condition of the animals returned to normal, while no death of animals was observed during the entire period of the experiment.
150 mg / kg	In 10-15 minutes after the administration of the drug, a decrease in motor activity, paralysis and bradypnea were observed within 48 hours. On the first day, death of one mouse was observed, and on the second day, the death of one more mouse was observed.
200 mg / kg	10 minutes after the administration of the drug, a decrease in motor activity, paralysis, convulsions and bradypnea were observed for 3-4 days. At the same time, against the background of these symptoms, after 1 hour, the death of one mouse was observed, after 3 hours, the death of another mouse was observed, after 6 hours, the death of another mouse was observed.
250 mg / kg	5-10 minutes after administration of the drug, a decrease in motor activity, paralysis, convulsions and bradypnea were observed for 3-4 days. At the same time, against the background of these symptoms, after 1 hour, the death of two mice was observed, after 3 hours, the death of another mouse was observed, on the second day, the death of two more mice was observed.
300 mg / kg	5-10 minutes after the administration of the drug, a decrease in motor activity, paralysis, convulsions and bradypnea were observed. At the same time, against the background of these symptoms, an hour later, death of one mouse was observed, after 2-3 hours, the death of three more mice was observed, and after 4 hours, the death of two more mice was observed.

Table 2
The results of the toxic effect of the drug "Dicloberl" solution for injection, "Berlin-Chemie AG" (Menarini Group) Germany, produced by: "A. Menarini Manufacturing Logistics and Services s.r.l." Italy

Dose	**Result**
50 mg / kg	In 20-25 minutes after the administration of the drug, a decrease in motor activity and crowding were observed for 3-4 hours. Subsequently, the condition of the animals returned to normal, while no death of animals was observed during the entire period of the experiment.

100 mg / kg	In 10-15 minutes after the administration of the drug, a decrease in motor activity and bradypnea were observed within 48 days. On the second day, death of one mouse was observed.
150 mg / kg	In 10-15 minutes after the administration of the drug, a decrease in motor activity, paralysis, convulsions and bradypnea were observed within 2-3 days. At the same time, against the background of these symptoms, the death of one mouse was observed after 3 hours, the death of one more mouse was observed after 6 hours, the death of two more mice was observed on the second day.
200 mg / kg	5-10 minutes after administration of the drug, a decrease in motor activity, paralysis, convulsions and bradypnea were observed for 3-4 days. At the same time, against the background of these symptoms, the death of two mice was observed after 2-3 hours, on the second day, the death of two more mice was observed, on the third day, the death of one more mouse was observed.
300 mg / kg	5-10 minutes after the administration of the drug, a decrease in motor activity, paralysis, convulsions and bradypnea were observed. At the same time, against the background of these symptoms, the death of one mouse was observed after 2 hours, after 3-4 hours, the death of two mice was observed, on the second day, the death of two more mice was observed, on the third day, the death of another mouse was observed.

Based on the results of the death of the experimental animals, we calculated the LD50 of the preparations (Table 3). If we compare the LD50 of both drugs, it turns out that the difference between them is statistically significant.

Таблица 3
Results of studying the indicators of acute toxicity of drugs (p = 0.05)

"Kobafen" lyophilisate for preparation of solution for injection with solvent, JV LLC "Jurabek Laboratories" Uzbekistan		**"Dikloberl" injection solution, Berlin-Chemie AG (Menarini Group) Germany, produced by: A.Menarini Manufacturing Logistics and Services s.r.l. Italy**	
Dose	Number of animals killed / total	Dose	Number of animals killed / total
50 mg/kg	0/6	50 mg/kg	0/6
150 mg/kg	2/6	150 mg/kg	1/6
200 mg/kg	3/6	200 mg/kg	4/6
250 mg/kg	5/6	250 mg/kg	5/6
300 mg/kg	6/6	300 mg/kg	6/6
$ЛД_{50}$= 180 (141,7÷228,6) mg/kg		$ЛД_{50}$= 190 (159,7÷226,1) mg/kg	

2. DISCUSSION OF THE RESULTS OBTAINED.

Based on the data obtained on the average lethal dose, we determined the toxicity class according to the classifier (the classifier contains six levels of toxicity classification), described in the methodological manual for preclinical research of drugs, edited by A.V. Stefanov. [4]. According to this classifier, the drug when administered intravenously belongs

to the fourth class of toxicity (Low toxicity). Since in the classifier, among the injection routes of administration, only the intraperitoneal route of administration is given, the definition of the toxicity class was carried out according to the intraperitoneal route of administration, which is similar to the intramuscular route.

When studying the clinical picture of intoxication, it was found that the putative organs and systems targets are the nervous and respiratory systems.

The obtained data on the average lethal dose show that the tested drug has the same harmlessness as the reference drug.

Study of the anti-inflammatory activity of the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent

3. MATERIALS AND RESEARCH METHODS.

The study of the anti-inflammatory activity of the drugs was carried out by the method of formalin edema of the paw in animals [3,4]. The experiments were carried out on 18 white outbred rats weighing 180-200 g, followed by division into groups of 6 animals each.[4]

For this, one hour before the induction of inflammation, the animals were injected once, intramuscularly (into the femoral muscle):

1.control group (control) - animals with test modeling, but without exposure to the drug;

2.the test group - animals received the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent in the form of a 1.33% solution, at a dose of 26.6 mg / kg, in a volume of 0.4 ml / 200 g (the injected sample contains 20 mg / kg of diclofenac - the administered dose of diclofenac is equivalent to the dose of diclofenac of the reference drug);

3. comparison group - animals received the drug "Dikloberl" injection solution, "Berlin-Chemie AG" (Menarini Group) Germany, produced by: "A. Menarini Manufacturing Logistics and Services s.r.l." Italy, in the form of a 1% solution, at a dose of 20 mg / kg, in a volume of 0.4 ml / 200 g.

Then, subplant animals of each group were injected with 0.1 ml of 2% formalin solution in the form of an aqueous solution into the rat's left hind paw.

The amount of edema of the extremity was measured oncometrically, after 2 hours and 4 hours, and for anti-inflammatory activity, data were taken when the maximum inflammatory response was reached.

The criterion for evaluating the pharmacological activity of the drugs was a decrease in the edema of the paws of the experimental animals, compared with the control.

The results were processed by the method of variation statistics according to the Student's test at $p = 0.05$ [3,4]. The tables show the arithmetic mean values (M), the corresponding standard errors of the mean (m), Student's test (t), the number of samples (n), confidence limits (lower confidence limit ÷ upper confidence limit).[5]

4. RESEARCH RESULTS.

During the experiment, it was found that both drugs at 2 and 4 hours of observation (after induction of inflammation), statistically significantly reduced the volume of paw edema,

compared with the control (Table 4). If we compare the experimental data of both drugs, it turns out that they are comparable.

Таблица 4
Results of the study of anti-inflammatory activity (M□tm; n = 6; p = 0.05)

Group	**Swelling volume of the paws (ml) after**	
	2 h.	**4 h.**
Control	0,700 (0,585÷0,815)	1,017 (0,913÷1,120)
"Kobafen" lyophilisate for preparation of solution for injection with solvent, JV LLC "Jurabek Laboratories" Uzbekistan	0,433 (0,306÷0,560)	0,567 (0,440÷0,694)
"Dikloberl" injection solution, Berlin-Chemie AG (Menarini Group) Germany, produced by: A.Menarini Manufacturing Logistics and Services s.r.l. Italy	0,450 (0,340÷0,560)	0,550 (0,378÷0,722)

Discussion of the results obtained. The results obtained indicate that the tested drug has reliable anti-inflammatory activity, which is not inferior to the reference drug.

Study of the analgesic activity of the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent

Materials and research methods. The study of the analgesic activity of drugs was carried out on the model of thermal immersion of the tail when immersed in hot water [4]. The experiments were carried out on 30 white mice (both sexes) weighing 19-23 g, followed by division into three groups of 10 animals each.

For this, one hour before the experiment, the animals of the experimental groups were injected once, intramuscularly (into the femoral muscle):

1. control group (control) - without exposure to the drug;
2. test group - animals received the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent in the form of a 0.133% solution, at a dose of 26.6 mg / kg, in a volume of 0.4 ml / 20 g (the injected sample contains 20 mg / kg of diclofenac - the administered dose of diclofenac is equivalent to the dose of diclofenac of the reference drug);
3. comparison group - animals received the drug "Dikloberl" injection solution, "Berlin-Chemie AG" (Menarini Group) Germany, produced by: "A. Menarini Manufacturing Logistics and Services s.r.l." Italy, in the form of a 0.1% solution, at a dose of 20 mg / kg, in a volume of 0.4 ml / 20 g.

After that, the tails of the animals were immersed in hot water at a temperature of 55 ° C, and the time for flicking the tail was recorded. The criterion for evaluating the pharmacological activity was considered to be an increase in the reaction time compared to the control.

The results were processed by the method of variation statistics using the Student's test at p = 0.05 [4,5]. The tables show the arithmetic mean values (M), the corresponding standard errors of the mean (m), Student's test (t), the number of samples (n), confidence limits (lower confidence limit ÷ upper confidence limit).

Research results. As a result of studying the analgesic activity of the test drug, it was found that the test drug has a statistically significant analgesic effect, however, the analgesic effect of the test drug is significantly greater than the comparison drug (Table 5).

Table 5
Results of studying the analgesic activity of drugs (M□tm; n = 8; p = 0.05)

Group	**Reaction time (sec)**
Control	4,90 (4,37÷5,43)
"Kobafen" lyophilisate for preparation of solution for injection with solvent, JV LLC "Jurabek Laboratories" Uzbekistan	14,00 (13,25÷14,75)
"Dikloberl" injection solution, Berlin-Chemie AG (Menarini Group) Germany, produced by: A.Menarini Manufacturing Logistics and Services s.r.l. Italy	12,10 (11,47÷12,73)

Discussion of the results obtained. The results obtained indicate that the test drug has reliable analgesic activity, which is superior to the reference drug, which indicates the synergism of the combination of active substances of the test drug.

5. CONCLUSION

Preclinical studies of the drug "Kobafen" lyophilisate for the preparation of a solution for injection with a solvent were carried out according to indicators of acute toxicity, anti-inflammatory and analgesic activity. As a result of the study of acute toxicity, it was found that the tested drug belongs to the fourth class of toxicity "Low toxicity", and it was also found that the tested drug has the same harmlessness as the reference drug.

When studying the anti-inflammatory and analgesic activity of the test drug, it was found that the test drug has reliable anti-inflammatory activity, which is not inferior in anti-inflammatory activity to the reference drug, but surpasses the reference drug in analgesic activity. The latter indicates the synergism of the combination of active ingredients of the test drug, which makes the test drug promising for clinical use.

6. REFERENCES

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