THE STUDY OF HEPATOPROTECTIVE ACTION OF THE DRUG USED FOR THE TREATMENT OF TOXIC HEPATITIS IN PIGLETS

OLGA A. GRACHEVA*, ALIZADE S. GASANOV, BULAT F. TAMIMDAROV, DINA M.
MUKHUTDINOVA, ILSUR G. GALIMZYANOV, NAZHIYA V. SHAMSUTDINOVA, DAMIR R.
AMIROV, ALFIYA R. SHAGEEVA

Kazan State Academy of Veterinary Medicine by N.E. Bauman, Sibirsky tract 35, Kazan city, 420029, Russia

ABSTRACT

Nowadays, there is a large number of ways to prevent and treat toxic hepatitis. Taking into account the fact that tissue hypoxia plays a significant role in the pathogenesis of hepatocyte damage leading to disruption of mitochondrial functions, depletion of ATP reserves with the activation of free radical processes, the use of drugs containing mitochondrial substrates such as succinic acid (succinate) is effective to have an additional influence on the course of ischemic processes in the hepatocyte, moreover, it has been proved that succinic acid and its derivatives have an anti-oxidant action. The aim of the research was to determine etiological factors, to study the effectiveness of the new drug that contains succinic acid and organic phosphorus compound and its effect on the clinical and physiological status, some hematological factors, as well as the productivity of piglets with toxic hepatitis. It has been found that the etiological factors that cause toxic hepatitis in the weaned piglets on the experimental farm are exogenous intoxication, lack of selenium, methionine and Vitamin E in feeds. The administration of the studied drug at doses of 0.3 and 0.5 ml/kg intramuscularly three times a day in addition to the adopted scheme of treatment had a stimulating effect on haematopoiesis of the piglets - at the end of the experiment, the levels of hemoglobin and erythrocytes increased compared to the control group, protein and carbohydrates metabolism in piglets improved, the function of the liver recovered. There was also a stimulating effect on the productivity and liveability of the piglets, but a more pronounced effect was achieved in the piglets treated by the studied drug at a dose of 0.5 ml/kg of live weight.

KEYWORDS: Piglets, Toxic Hepatitis, Liver, Etiology, Succinic Acid, Treatment, Selenium, Blood, Symptoms, Productivity

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INTRODUCTION

These days pig breeding plays a major role in providing population with food, as pigs are one of the most precocious animals. Meanwhile, one of the problems on pig farms is diseases of young animals. These diseases are of mixed nature, mass character and cause great economic damage to farms. Toxic hepatitis occurs on large pig farms all year round and is often combined with the pathology of other organs that leads to mortality of piglets [1]. Nowadays, there is a large number of ways to prevent and treat toxic hepatitis. Taking into account the fact that tissue hypoxia plays a significant role in the pathogenesis of hepatocyte damage leading to disruption of mitochondrial functions, depletion of ATP reserves with the activation of free radical processes, the use of drugs containing mitochondrial substrates such as succinic acid (succinate) is effective to have an additional influence on the course of ischemic processes in the hepatocyte, moreover, it has been proved that succinic acid and its

derivatives have an anti-oxidant action [2,3,4]. Thus, the development of new methods for prevention and treatment of toxic hepatitis in piglets is very relevant.

The aim of the research was to determine etiological factors, to study the effectiveness of the new product that contains succinic acid and organic phosphorus compound and its effect on the clinical and physiological status, some hematological factors, as well as the productivity of piglets with toxic hepatitis.

MATERIALS AND METHODS

Experimental studies on the effect of the Yantovet drug on the clinical and hematological status and productivity of weaned piglets were carried out on the pig farm in the Baltasinsky district in the Republic of Tatarstan and at the Department of Therapy and X-ray Clinical Diagnostics in Kazan State Academy of Veterinary Medicine named after N.E. Bauman.

The object of the study was a new drug containing succinic acid and organic phosphorus compound that was developed at the Department of Therapy and X-ray Clinical Diagnostics [5, 6].

To study the effect of the drug, the Large White piglets of 2 months old with toxic hepatitis were selected, three groups of 15 animals in each were formed according to the principle of pair analogues.

The diagnosis was established on the basis of anamnestic, clinical and hematological factors. Clinical examination was carried out daily, hematology test was performed before the beginning of the experiment and every ten days. Blood was extracted from the vena cava cranialis and the orbital venous sinus early in the morning.

In order to determine the etiological factors, the soil samples were taken from the pig farm, the average samples of the used feed compound were selected to determine the content of some trace elements, in addition, the presence and concentration of mycotoxins in the feed were evaluated. The determination of trace elements in the soil was performed by the atomic absorption method on Anylist 400 atomic absorption spectrometer (the USA).

In order to evaluate the treatment efficacy of the studied drug that was given in addition to the adopted scheme of treatment of toxic hepatitis which includes the use of E-selenium at standard doses, the test drug was administered at the dose of 0.3 ml/kg intramuscularly into the neck of piglets three times during the experiment with an interval of 7 days in the first experimental group, 0.5 ml/kg - in the second experimental group, the third group served as a control. The experiment lasted 30 days.

In whole blood, the hemoglobin content was determined by the hemoglobin-cyanide method, the total number of erythrocytes, leukocytes and the leukocyte formula was determined by standard methods. Biochemical studies included the diagnosis of toxic hepatitis and an assessment of the effectiveness of the studied drug according to some biochemical parameters: total protein, albumin, globulin, glucose, cholesterol and triglyceride concentrations, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), glutamyltransferase (GGT) activity, total bilirubin level, which were carried out on the "BiochemSA" biochemistry analyzer.

Weighing was performed before and after the experiment to determine the average daily weight gain of the animals. Liveability of the piglets was taken into account.

The data obtained as a result of the research were subjected to variation-statistical processing using the Student's

t-test on a personal computer with Microsoft Excel.

RESULTS AND DISCUSSIONS

The main cause of toxic hepatitis is food intoxication caused by feeding animals with spoiled and affected by mold fungi feed. The weaned piglets on the farm were fed with dry feed of own production. Mycotoxicological analysis of animal feeds, in which mycotoxins were determined (T-2 toxin, aflatoxin, DON, Zearalenone, Ochratoxin A), was carried out. High toxicity in those feeds was not identified, the concentration of mycotoxins corresponded to the MPC. However, it can be assumed that the total effect of mycotoxins could have a pathological effect on the liver and contribute to the development of the pathological process [7].

Also, this disease is often registered in areas with deficiency of digestible forms of selenium in soils [8,9]. The northern regions of the Republic of Tatarstan are a selenium-deficient biogeochemical area, including the Baltasinsky district. Our research has shown that selenium levels are insufficient both in the soil and in the feeds made from local raw materials, which is one of the reasons for toxic hepatitis on the farm. The content of selenium in the soil was 0.05 mg/kg, in feed - 0.01 mg/kg, which is below the normal values.

It is known that the cause of toxic hepatitis can be a violation of protein-carbohydrate ratio and a deficiency of Vitamin E, cystine, methionine and choline in the diet [10,11]. In this case, a large number of insufficiently oxidized products that are toxic to the body are formed.

The analysis of the diet used for feeding piglets showed that the provision with cystine and methionine was 63%, with Vitamin E was 85%. Thus, according to the results of the diagnostic stage, the conclusion about the causes of spreading toxic hepatitis among the weaned piglets on the farm was made.

Clinical studies have established that animal health was assessed as satisfactory. However, in that age group, about 8% of animals were lagging behind in growth and development and had reduced food excitability. There was no yellowness of the mucous membranes and skin or it was very slight. At the same time, the animals were sluggish, depressed, food excitability decreased, vomiting was rarely observed. There was a decrease in motion activity in sick piglets. Body temperature was within normal limits. The disease was accompanied by tachypnea and tachycardia. The live weight of piglets with toxic hepatitis was lower by 8%-10% compared to healthy peers.

When observing experimental animals with toxic hepatitis that were treated according to the test scheme, it was established that the clinical manifestation of the disease in them was smooth. Then, the clinical state of the animals returned to normal according to clinical status indicators. As a result of the use of the studied drug, the clinical features were eliminated faster in the piglets in the experimental groups compared to the control one, and the hematological factors that indicate the development of toxic hepatitis were normalized that proves effectiveness of the drug in the treatment of the disease.

At the baseline stage and after the use of the drug, piglets' blood was tested to determine morphological parameters. The data obtained showed that the level of erythrocytes, hemoglobin did not have statistically significant differences in the groups and fluctuated within lower values of physiological range. Significant changes in all morphological parameters were seen only on the 30th day of the experiment, when the differences between the experimental and control groups were significant. Thus, the hemoglobin level in the experimental groups increased by 23.5% and 31.3% and was higher by 16.8% and 26% than in the control group at the end of the experiment, respectively. A

similar tendency was with respect to the number of erythrocytes, the increase in the number of which was 15.3% and 20.1% on the 30th day of the experiment, respectively, compared to the baseline study, while the level of that group of cells remained almost unchanged in the control animals.

At the beginning of the experiment, the number of leukocytes in all the piglets was below regulatory values which may indicate dyslymphopoiesis, in the experimental groups during the experiment the number of leukocytes slightly increased and reached the lower values of the physiological range, which can be regarded as positive changes. In the control group, these changes were less significant, on the 20th and 30th days of the experiment the difference between the experimental and control groups was 11.8% and 7.5%, respectively.

When analyzing the leukocyte formula at the beginning of the experiment, eosinopenia and neutrophilia were established as the level of segmented neutrophils increased, similar changes occurred in all the groups during the experiment: the level of eosinophils and lymphocytes increased, segmented neutrophils decreased within the age physiological range.

For laboratory confirmation of toxic hepatitis and further control of the effectiveness of the drug, biochemical blood tests were performed.

A baseline study has shown that the total protein content was at the lower limit of the physiological range, the level of albumin significantly reduced, and the level of glucose in the blood was also low that indicated a decrease in the synthetic function of the liver.

The determined functional liver markers in the blood of experimental animals indicated the pathology in this organ, the total bilirubin content, the activity of the AST, ALT, GGT enzymes were increased. All biochemical values in the piglets from both the experimental and control groups were similar and the difference was not significant.

Thus, biochemical blood test confirmed the results of the clinical study and blood test, that allowed us to make a diagnosis – toxic hepatitis. Then, the similar tests were performed every 10 days.

The use of the studied drug to treat toxic hepatitis in the piglets contributed to the improvement of blood values which characterize the state of protein metabolism in animals. Thus, a fairly low level of total protein increased in all the groups, but in the experimental groups the increase was more dynamic and after the 20th day of the experiment there was a statistically significant difference compared to the control group, which at the end of the experiment was 10.12%-19.65%. Similar changes have been established for albumin levels. So, the difference of its quantity in the first experimental group with respect to the control group was the highest on the 20th day of the research - 25.4%, in the second experimental group - 25.8% on the 30th day of the experiment, whereas in the animals of the control group, the level was below the physiological standard at all the stages of the research.

Glucose level at the beginning of the experiment was below the physiological standard and its increase was observed in all the groups, but more pronounced changes occurred in the groups where the studied drug was additionally used. Glucose values were higher by 38.1%; 24.89%; 29.15% in the first experimental group and by 44.4%, 33.9% and 30.5% in the second group compared to the control group at certain stages of the experiment. At the end of the experiment, the glucose level was within the reference range in all the animals.

Hypocholesterolemia in the case of exogenous intoxications may be due to a decrease in cholesterol synthesis and, probably, cholesterol malabsorption connected with diarrhea. A low level of cholesterol was observed in all the

experimental piglets at the stage of a baseline study, but the values were growing in the experimental groups during the entire research period. At the end of the experiment, its concentration grew within the normal range values and was significantly higher than in the control group.

The hepatoprotective effect of the treatment and the physiologically determined compensatory properties of the liver parenchyma led to the normalization of pigment metabolism. Increased total bilirubin level by 20-25% in the sick animals at the beginning of the study has reduced by 1.2-8.8; 5.33-19.85; 32.2 - 33.6% compared to the control group at certain stages of the experiment under the influence of the studied drug, reaching the upper values of the physiological range in the experimental groups, whereas in the control group it changed slightly and was higher than the standards throughout the experiment.

While assessing the biochemical blood test in the sick with hepatic pathology, cytolysis syndrome is identified, caused by disruption of membrane permeability, disintegration of membrane structures or necrosis of hepatocytes with enzymes entering into plasma (ALT, AST, GGT, LDH, etc.). The first three enzymes are more often tested in clinical laboratory practice. The changes in the activity of the enzymes in the blood serum of the experimental piglets are presented in Table.

Table 1: Enzyme Activity - Liver Markers in the Blood of the Piglets Suffering from Toxic Hepatitis

Parameter	Group	Stages of the Research, Days			
		Baseline	10	20	30
Total bilirubin, $\mu\text{mol/L}$	Group 1	12.18 $\pm$ 0.51	10.80 $\pm$ 0.68	8.72 $\pm$ 0.46**	7.66 $\pm$ 0.28**
	$\pm$ to control		-8.8	-19.85	-32.2
	Group 2	10.94 $\pm$ 0.43	11.98 $\pm$ 0.41	10.30 $\pm$ 0.59	7.5 $\pm$ 0.34**
	$\pm$ to control		1.2	-5.33	-33.63
	control	11.84 $\pm$ 0.65	11.84 $\pm$ 0.65	10.88 $\pm$ 0.53	11.30 $\pm$ 0.61
Alkaline phosphatase, U/L	Group 1	148.20 $\pm$ 21.74	168.00 $\pm$ 15.57	194.00 $\pm$ 6.29	173.40 $\pm$ 12.13
	$\pm$ to control		-10.83	-18.21	-0.72
	Group 2	184.20 $\pm$ 9.28	178.00 $\pm$ 10.88	192.40 $\pm$ 10.54	158.80 $\pm$ 1.19
	$\pm$ to control		-13.34	-1.54	-13.13
	control	166.20 $\pm$ 16.61	205.40 $\pm$ 9.52	195.40 $\pm$ 11.84	182.80 $\pm$ 9.23
ALT, U/L	Group 1	81.40 $\pm$ 2.71	64.20 $\pm$ 4.89	48.20 $\pm$ 1.92**	43.00 $\pm$ 2.76**
	$\pm$ to control		-12.06	-29.12	-15.7
	Group 2	62.40 $\pm$ 1.35	63.80 $\pm$ 3.42	47.80 $\pm$ 2.82	35.60 $\pm$ 1.72**
	$\pm$ to control		-12.6	-29.7	-30.2
	control	76.00 $\pm$ 3.26	73.00 $\pm$ 1.46	68.00 $\pm$ 2.69	51.00 $\pm$ 1.94
AST, U/L	Group 1	96.60 $\pm$ 3.46	82.80 $\pm$ 6.37	62.20 $\pm$ 2.95**	50.60 $\pm$ 1.35**
	$\pm$ to control		-14.11	-27.34	-39.33
	Group 2	85.40 $\pm$ 1.68	82.20 $\pm$ 2.53	69.40 $\pm$ 3.75**	49.2 $\pm$ 1.19**
	$\pm$ to control		-14.73	-18.93	-41.01
	control	97.80 $\pm$ 4.19	96.40 $\pm$ 2.71	85.60 $\pm$ 3.15	83.40 $\pm$ 4.78
GGT, U/L	Group 1	59.80 $\pm$ 2.43	53.20 $\pm$ 3.65	31.20 $\pm$ 2.46**	31.60 $\pm$ 3.33**
	$\pm$ to control		-22.68	-53.02	-53.12
	Group 2	68.60 $\pm$ 2.99	58.80 $\pm$ 1.52	36.00 $\pm$ 1.41**	29.00 $\pm$ 1.77**
	$\pm$ to control		-14.54	-45.78	-56.97
	control	64.00 $\pm$ 2.74	68.80 $\pm$ 3.44	66.40 $\pm$ 2.59	67.40 $\pm$ 4.16

During the experiment, a decrease in the activity of enzymes occurred in all the groups under the influence of the prescribed treatment, but more pronounced changes occurred in the experimental groups. So at the beginning of the study, the ALT activity exceeded the standard values almost twice in all experimental piglets, after treatment ALT activity decreased by 15.7%–30.2% in experimental piglets compared to the control group and reached the upper limits of the

reference range.

Similar changes were observed in relation to the activity of the AST enzyme, the increase in which was above the norm by 40%-50%. At the end of the study that parameter reached normal physiological values in the experimental groups, whereas in the control group changes were very insignificant. An increase in the activity of ALT and AST was a definite marker of liver damage. However, increased ALT activity is considered more common to liver damage. Almost all other organs besides liver contain AST, in particular, heart, as a result, the increase in this aminotransferase is not always specific to liver damage. Therefore, AST/ALT ratio (De Ritis ratio) has a clinical significance. At the beginning of the experiment, it was 1.16, on average, which is below the norm.

The activity of GGT was significantly increased at the beginning of the experiment, the parameter decreased by almost 2 times and returned to normal under the influence of the treatment in both experimental groups at the end of the experiment, whereas in the control group, it remained at a quite high level.

The use of the drug stimulated productive qualities, on the 30th day of the experiment, there was an increase in the average daily weight gain in the animals of the experimental groups, which was by 5.88% and 8.00% higher than in the control group. That tendency remained unchanged in subsequent month of observation, in which the average daily weight gain of the piglets was higher by 6.35% and 9.86% than in the control group, respectively.

CONCLUSIONS

Thus, it has been found that the etiological factors that cause toxic hepatitis in the weaned piglets on the experimental farm are exogenous intoxication caused by compound feeds that contain mycotoxins and lack of selenium, methionine and Vitamin E in feeds.

The administration of the studied drug in addition to the adopted scheme of treatment had a stimulating effect on hematopoiesis of the piglets - at the end of the experiment, the levels of hemoglobin and erythrocytes increased compared to the control group, protein and carbohydrates metabolism in the piglets improved, the function of the liver recovered. There was also a stimulating effect on the productivity and liveability of the piglets, but a more pronounced effect was achieved in the piglets treated by the studied drug at a dose of 0.5 ml/kg of live weight.