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## THE USE OF THE SYNTHETIC ANALOGUE OF LEUCIN-ENCEPHALINE IN COMPLEMENTARY THERAPY OF DOGS WITH CHRONIC PANCREATITIS

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**Abstract.** A review of the problem of treating dogs for pancreatitis with peptide drugs is presented. The main attention is paid to the analysis of the use of somatostatin, octreotide and the synthetic analogue of leucine-enkephalin – dalargin. Despite numerous studies of the therapeutic effects of these agents for acute pancreatitis and pancreatic necrosis in humane medicine, there is no definitive answer about their efficacy in dogs due to the lack of work performed in accordance with the criteria of evidence-based medicine.

The use of dalargin in the complex therapy of dogs with chronic pancreatitis, eliminates or reduces pain and dyspeptic phenomena, contributes to the onset of positive changes at the same time a number of positive changes in the functional state of the pancreas manifested by decreased serum amylase activity 3 times, pancreatic lipase in the 1,9 times.

Studies carried out on dogs, patients with chronic pancreatitis, it is determined that against the background of treatment of synthetic analogue Leukin-enkefalin – Dalagin. The intensity of pain decreased (up to 5 days in 90% of patients), decreased amylase and pancreatic lipase activity and TBK-active product (malonic dialdehyde, MDA) in their blood serum. Ultrasound showed data on the reduction of pancreatic edema, in 7 sick dogs, its sizes were restored to indicators in healthy animals.

**Keywords:** chronic pancreatitis, acute pancreatitis, dogs, dalargin, serum amylase, pancreatic lipase

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### Introduction

At the beginning of this century, a group of scientists from the University of Zurich published an article entitled “Treatment of acute pancreatitis from the standpoint of evidence-based medicine. A look at the established paradigm” (Heinrich et al., 2006). It analyzed data on the results of treatment of patients with pancreatitis, ob-

tained by various scientific groups and in a large number of clinical trials, performed in accordance with the rules adopted in evidence-based medicine. An evaluation of the effectiveness of the most commonly used agents for the treatment of patients with acute pancreatitis was presented: mesexylate gabexylate, lexipaphant, aprotinin (contrical, trasilol), octreotide (somatostatin analogue). The findings of the meta-analyses

turned out to be quite unexpected: none of these agents can be regarded as an effective pancreatitis drug due to their insufficiently proven efficacy.

### ***Analysis of recent researches and publications***

The findings of this work forced scientists to review the importance of antisecretory and antiprotease drugs in the treatment of patients with acute pancreatitis. And if in the countries of Western Europe and the USA practically have refused to use these means, in the countries of the Eastern Europe till now the doctors appoint them quite often. Among them, sandostatin (octreotide) is most commonly used. Octreotide is a synthetic analogue of the somatostatin octapeptide. Somatostatin was isolated from the sheep hypothalamus in 1973, and was later found in the central and peripheral nervous systems, as well as in D-cells of the pancreas, in the stomach, and in the duodenum. Two molecular forms of natural somatostatin – S-14 and S-28 have been identified. The first form is treated as a neuropeptide, the second – as a circulating hormone. Somatostatin inhibits secretion but not synthesis of growth hormone. In the digestive canal of animals and humans, it inhibits the secretion of most intestinal hormones, along with it markedly reduces the secretion of glands of the stomach, pancreatic enzymes and bicarbonates, slows blood flow in the abdominal organs (Gelfand et al., 1998; Choi et al., 1989).

In the 80–90s of the XX century, one of the leading factors in the development of acute pancreatitis was considered activation of pancreatic enzymes, which leads to “self-digestion” of the gland. Inhibition of pancreatic secretion and enzymes by somatostatin or octreotide administration was promising. This was facilitated by the first positive results of clinical observations of the effect of somatostatin on pan-

creas in patients with acute pancreatitis (Makhija & Kingsnorth, 2002). Later in numerous clinical studies to confirm the effectiveness of somatostatin in the treatment of patients with acute pancreatitis failed (Li et al., 2011; Uhl et al., 2002).

Similar was the situation with the use of octreotide in the treatment of patients with GP. Initially there were encouraging reports about the effectiveness of octreotide in stopping pancreatitis attacks, but as evidence from studies of evidence-based medicine accumulated, the number of negative responses to octreotide as an effective agent increased. An example is the work of S. Heinrich et al. (Heinrich et al., 2006). This meta-analysis combined four clinical studies. Its results showed that octreotide does not reduce the number of surgical interventions in people with GP (23,3 % vs. 16,3 %;  $P = 0,09$ ), does not reduce the rate of sepsis, does not reduce the number of fatal cases, and overall number of complications (70,6 % vs. 63,2 %;  $P = 0,2$ ). In addition, no route of administration (subcutaneously or intravenously) was established. There are known guidelines for the use of octreotide in small pets (Bulgakov, 2015), the overall conclusion of which is the fact of safety and the absence of undesirable effects of the drug, however, there is no meta-analysis of its effectiveness for pancreatitis. As a result, the authors do not recommend that patients with severe acute pancreatitis prescribe octreotide. In other works of the same period, the number of negative responses about the possibility of using the peptide drug “Octreotide” for acute pancreatitis steadily increased, which led to the situation when the medical standards of the UK, the International Association of Pancreatology did not recommend the use of octreotide and somatostatin for this pathology (Vasilev, 2010; Zohirov et al., 2016). Thus, the idea of using powerful antisecretory and anti-enzymatic agents in the treatment of patients with acute

pancreatitis has not been confirmed in clinical practice. However, octreotide and somatostatin are used in modern surgery. They are effective for stopping bleeding from varicose and varicose veins of the esophagus and stomach in patients with cirrhosis, used for the prevention of recurrence, secreting endocrine tumors of the pancreas (glucagon, carcinoid tumors, gastrinoma).

In this situation in the late 80's and early 90's in our country for the treatment of people with acute pancreatitis began to widely use peptide drug dalargin. Dalargin is a synthetic analogue of leucine-enkephalin (Tyr-D-Ala-Gly-Phe-Ley-Arg). Being a synthetic opioid neuropeptide, it almost does not penetrate the blood-brain barrier, does not cause addiction, physical dependence, and tolerance (Choi, 1989; Chaturvedi, 2003). The pharmacological action of opioids is predominantly caused by interaction with delta receptors and, to a lesser extent, with mu-receptors. Dalargin, in the first place, is an active regulator of homeostasis of the body, has the ability to affect various biological systems. In experimental studies using a model of cystamine duodenal ulcer in rats, it was shown that dalargin has a strong reparative activity for ulcerative lesions (Lyashev, 2002). The established activity was confirmed by stimulation of the marker enzyme of the processes of regeneration – ornithine decarboxylase (ODC) in the mucous membrane of the duodenum. The expressed ability of hexapeptide to activate the processes of tissue regeneration and growth, along with the revealed moderate antisecretory effects on gastric and pancreatic secretion, improvement of blood microcirculation in the area of damage (Clayton, 2013) identified the possibility of treatment in patients with a variety of gastroenterologists. As a drug dalargin is currently used for the treatment of patients with peptic ulcer, pancreatitis, including acute pancreatitis, pancreatic necrosis.

Already in the first works of surgeons, convincing data were obtained about the ability of dalargin to limit or stop the progressive course of destruction of the exocrine parenchyma of the software (Oruc, 2004; Zheng, 2013). Dalargin was highly effective in the combined diseases of the pancreatoduodenal zone. According to the results of the research, it was suggested to prescribe hexapeptide therapy to all patients at the pre- and postoperative stage of treatment, which led to accelerated healing of complicated gastroduodenal ulcers, prevention of the transition of edema to pancreatic necrosis, inhibition of enzymatic excretory function. Compared to the therapeutic activity of dalargin and other agents, dalargin in its activity exceeded all other antiulcer drugs. In the treatment of malignancies, hexapeptide was superior in activity to cytostatics, protease inhibitors, ribonuclease, pantriptyne, somatostatin, calcitonin, glucagon. The therapeutic efficacy of dalargin was considered by the authors as a result of the systemic effect of the peptide on the digestive system.

Dalargin has been successfully used in the treatment of patients with chronic pancreatitis (Habtezion, 2011). The results of the study showed that dalargin reduced the intensity of pain in the epigastrium faster and in a greater number of cases (84 %) and improved overall health than patients in the control group (cessation of pain in 72 % of patients). There are other works in which dalargin has been widely used with a positive result for the treatment of patients with postoperative pancreatitis and for its prevention (Vasilev, 2011).

### ***Materials and methods of research***

The studies were performed on 20 dogs with chronic pancreatitis. Animals were divided into two groups – I control, II experimental 10 dogs each with age

(from 3 to 11 years), severity and duration of the disease, the severity of pain.

The diagnosis was made on the basis of anamnestic data, clinical features, laboratory tests, biochemical studies of blood and serum, ultrasonography data.

The exocrine function of the pancreas was evaluated by the activity of amylase and lipase in serum. The activity of amylase in serum was determined by the kinetic method, and lipase by the kinetic colorimetric method. The activity of lipid peroxidation was evaluated by the level of TBK-active product (malonic dialdehyde, MDA) in serum.

The main principles of treatment of animals of both groups were: elimination of pain syndrome, antisecretory therapy, normalization of exocrine function of pancreas.

For the treatment of animals in the (control) group, a 2% solution of drotaverine hydrochloride at a dose of 2 mg/kg body weight was administered intramuscularly twice daily; omeprazole lyophilisate for the preparation of a solution for infusion of 40 mg at a dose of 1,5 mg/kg body weight intravenously once a day, enzyme agents – 10000 units of lipase internally during each feed feeding.

Dogs of the II (experimental) group were treated with the same treatment as the animals in the control group, and additionally intravenously, a 0,1% solution of dalargin (1 mg/ml) was administered at a dose of 2 mg/kg body weight, which was dissolved in 100 ml of isotonic solution. sodium chloride, twice a day for 5 consecutive days.

The figures obtained during the studies were statistically processed using Microsoft Excel.

### ***Results of the research and their discussion***

Clinical signs of dogs suffering from CP were characterized by impaired appetite, frequent belching, signs of nausea,

vomiting, bloating, pain, dyspeptic phenomena. The body temperature of sick dogs was within the reference values and was  $38,3 \pm 0,28$  °C in dogs of group I and  $38,1 \pm 0,34$  °C in dogs of group II; the number of respiratory movements per minute in dogs of group I was  $24,9 \pm 2,3$ , and in dogs of group II –  $25,1 \pm 3,1$ ; the heart rate was  $86,3 \pm 9,7$  and  $88,6 \pm 5,9$  heart rate per minute, respectively.

The study of the serum of dogs with chronic pancreatitis showed an increase in amylase activity up to  $2136,8 \pm 23,6$  U/l in animals of control group I and up to  $2152,4 \pm 19,8$  U/l in animals of experimental group II. Serum pancreatic lipase activity was  $401,8 \pm 11,3$  U/l in animals of group I and  $392,6 \pm 10,9$  U/l in animals of group II. The content of malondialdehyde in the serum of dogs was  $7,68 \pm 0,47$  and  $7,73 \pm 0,41$   $\mu\text{mol/l}$  in animals of groups I and II, respectively.

Ultrasonographic studies revealed an increase in the size of the pancreas in dogs of both groups, heterogeneity of structure, small echospositive inclusions in the parenchyma of the organ, a decrease in the rate of blood flow in the small arteries of the pancreas. In all patients of dogs, ultrasound revealed an increase in echogenicity of the structure of the organ, indicating the development of sclerotic processes in the gland tissue as a result of previously exacerbated diseases.

In complex therapy with the use of dalargin, the reduction of pain in dogs of the II (experimental) group was established at 3 days, and the complete disappearance at 5–6 days. In dogs I (control group) treated with traditional therapy, only 70% of patients managed to eliminate the pain syndrome, and the intensity of the pain syndrome decreased by 5–7 days. Appetite improvement in 80% of the animals in the experimental group occurred 3 days after the use of complex therapy, and

most recently disappeared vomiting and belching. Dyspeptic phenomena gradually disappeared, and at 6 days defecation returned to normal. In animals of the control group – nausea, bloating, dyspeptic phenomena were slower.

The use of dalargin eliminated pain during palpation of the abdominal wall in the area of pancreas projection of the software in 80 % of patients.

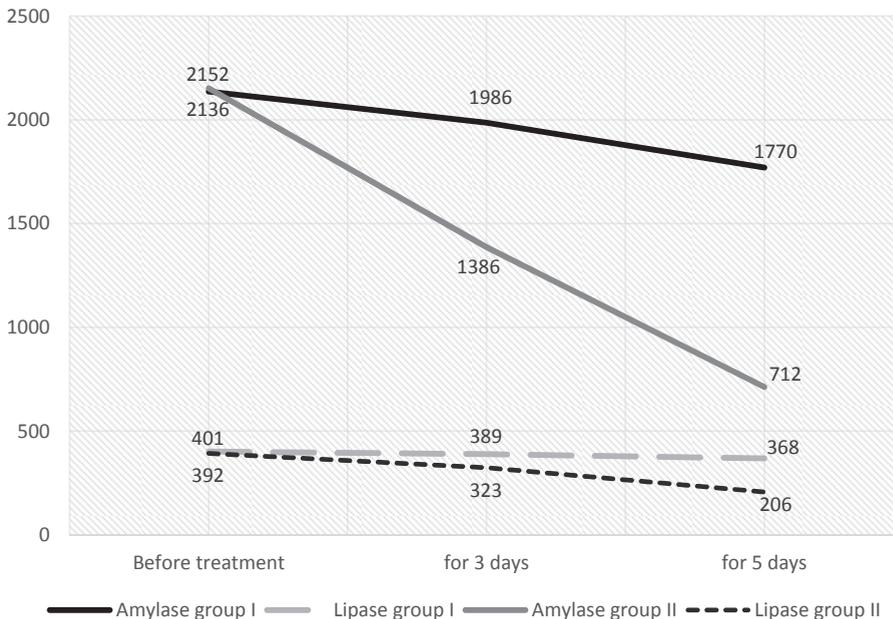
In the course of treatment, the activity of enzymes in the serum of dogs in both groups decreased. Thus, in the serum of dogs of group II (experimental) amylase activity decreased by almost 2,9 times ( $P \leq 0.05$ ) after 5 days from the beginning of treatment, whereas in traditional treatment (animals of group I), its activity decreased from the initial. The indicator was only 1,2 times ( $P \leq 0.05$ ) and was greater than the normative value (125–640 U / l) (Fig. 1).

The normalization of amylase activ-

ity can be explained by the stimulating effect of dalargin on software cells. Dalargin stimulates the processes of cell division and DNA synthesis, eliminates the development of erythrostasis and microthrombosis, improves microcirculation in the pancreas, prevents the development of inter-intracellular and intracellular edema in the intact gland segment (Zohirov et al., 2016).

The activity of pancreatic lipase in the serum of dogs of control group (I) after 5 days after the traditional treatment was at the level of the initial indicator, whereas in dogs of II (experimental group) decreased to 206,4 U / l, against 392,6±10,9 U / l initial ( $P \leq 0,05$ ).

One of the important indicators that reflect the state of lipid peroxidation in animals is the content of MDA in the serum. The use of dogs of group II (experimental) complex therapy, which included dalargin, contributed to the reduction of MDA content in their serum from 7,73 ±



**Fig. 1. The activity of amylase and pancreatic lipase in the serum of dogs during treatment**

0,51 to 5,18 ± 0,44 mmol / l (P ≤ 0,05) . In control (I) dogs, MDA content in serum after 5 days of treatment was 6,74 ± 0,43 versus 7,68 ± 0,29 mmol / l initial. The decrease in MDA levels in the serum of dogs in the experimental group is explained by the antioxidant effect of dalargin.

After treatment, all test animals were recorded. Dogs were monitored for 6 months to evaluate the effectiveness of the therapy. Disease recurrence was observed in 9 dogs (90 %) of the control group, whereas in the experimental group exacerbation of the disease was observed in 6 (60 %) dogs.

Most animal owners have been linked to feeding disorders, physical activity and stress as the cause of disease recurrence.

### ***Conclusions and future perspectives***

The use of dalargin in the treatment of dogs with CP, eliminates or reduces pain and dyspeptic phenomena, contributes to the onset of positive changes at the same time noted a number of positive changes in the functional state of the software, which is manifested by a decrease in the activity of amylasin in syatase parol 1,9 times. A pronounced antioxidant activity of dalargin, which is manifested by a significant decrease in the level of MDA in the blood serum of dogs with chronic pancreatitis by 33 %, which is one of the pathogenetic mechanisms of its therapeutic action. The use of dalargin in dogs with chronic pancreatitis, according to close and long-term observations, was superior to traditional therapy in efficiency.

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**Міластняя А. Г. (2020). ВИКОРИСТАННЯ СИНТЕТИЧНОГО АНАЛОГУ ЛЕЙЦИН-ЕНКЕФАЛІНУ У КОМПЛЕКСНІЙ ТЕРАПІЇ СОБАК, ХВОРИХ НА ХРОНІЧНИЙ ПАНКРЕАТИТ.** *Ukrainian Journal of Veterinary Sciences*, 11(1): 52–58, <https://doi.org/10.31548/ujvs2020.01.006>

**Анотація.** Представлено огляд проблеми лікування собак за панкреатиту пептидними лікарськими засобами. Основну увагу приділено аналізу застосування соматостатину, октреотиду і синтетичного аналогу лейцин-енкефаліну – даларгіну. Незважаючи на численні дослідження терапевтичної дії цих засобів за гострого панкреатиту і панкреонекрозу у гуманній медицині, отримати однозначну відповідь про їх ефективність у собак не вдається через відсутність робіт, виконаних відповідно до критеріїв доказової медицини.

Застосування даларгіну у складі комплексної терапії собак, хворих на хронічний панкреатит, усуває або зменшує больовий синдром і диспепсичні явища, сприяє настанню позитивних змін, одночасно відзначається ряд позитивних змін функціонального стану підшлункової залози, що проявляється зниженням активності амілази у сироватці крові в 3 рази, панкреатичної ліпази – в 1,9 рази.

Дослідженням, проведеним на хворих на хронічний панкреатит собаках, визначено, що на тлі лікування синтетичним аналогом лейцин-енкефаліну – даларгіном інтенсивність болю зменшувалась (до 5 доби у 90 % хворих тварин), знижувала активність амілази та панкреатичної ліпази та рівень ТБК-активного продукту (маленового діальдегіду, МДА) у їх сироватці крові. Дані ультразвукового дослідження засвідчили зменшення набряку підшлункової залози, у 7 хворих собак її розміри відновлювалися до показників у здорових тварин.

**Ключові слова:** хронічний панкреатит, гострий панкреатит, собаки, даларгін, сироваткова амілаза, панкреатична ліпаза

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