Search of a solution correction of a lipidic metabolism at acute pancreatitis

S G Anaskin¹, A P Vlasov² and I D Korniletsky¹

¹National Research Nuclear University MEPhI, Moscow, Russia
²N.P.Ogarev Mordovia State University, Saransk, Russia

E-mail: asg72@list.ru

Abstract. Following the results of a pilot study on studying of influence of an emoksipin, verapamil and a reamberin at acute pancreatitis it is possible to say that under the influence of these drugs in fabric structures of the inflamed pancreas there is a decrease in intensity of free radical processes of a lipopereokisleniye, activity of phospholipases, hypoxia phenomena. Level of antioxidant protection of fabric of body increases. In the first three days of supervision the accurate tendency to normalization of the transformed lipidic structure of fabric structures of a pancreas is revealed. At the same time these positive effects are noted in all experienced groups. It demonstrates that though pharmacological drugs are used multidirectional action (antioxidant emoksipin, an antigipoksant reamberin, inhibitor of calcium channels verapamil), they in a varying degree influence on studied pathological (membranodestruktivny, hypoxemic) processes, leading finally to reduction of their expressiveness. So, emoksipin found big ability to increase stability of membranes of pankreatotsit to pathological influence of molecular products the FLOOR, verapamil – to stopping of the activated phospholipases, reamberin – to a hypoxia.

The question solution on the key (prevailing) mechanism in trigger processes of sharp pancreatitis of a definite answer has no. Undoubtedly only the fact that efficiency of antioxidant and inhibitor of calcic channels was rather higher. It suggests that free radical processes of a lipopereokisleniye and activity of fosfolipazny systems predetermine the level and nature of defeat of a cellular biomembrane of pankreatotsit already on the earliest terms of inflammatory process.

1. Introduction
The problem of acute pancreatitis remains to one of actual in modern medicine. Now on occurrence frequency acute pancreatitis takes a leading place among acute diseases of abdominal organs [2]. At the same time at 15–20% of patients inflammatory changes of a pancreas have difficult destructive character [1]. Relevance of this problem is caused by the considerable duration of treatment, high level of a postoperative lethality at destructive forms of pancreatitis [3, 4].

Considerable difficulties in the choice of optimum medical tactics are created by a variety of tactical approaches in treatment of acute pancreatitis. In recent years most of surgeons agree in an opinion that carrying out conservative therapy is shown to most of patients with an acute inflammation of a pancreas [5]. Therefore now optimization of the existing schemes of therapeutic influence at acute pancreatitis represents an important task.
The work purpose – to study influence of an emoksipin, reamberin and verapamil on a functional and metabolic condition of fabric structures of a pancreas and membranodestabiliziruyushchy process of pankreatotsit in the conditions of an acute inflammation.

2. Materials and methods
Basis of work were pilot studies on 40 adult not purebred dogs with an of both sexes weight from 7.3 to 12.8 kg divided into the following groups: the first (n = 8) – control; the second (n = 8) – experienced (with emoksipiny), the third (n = 8) – experienced (with reamberiny), the fourth (n = 8) – experienced (with verapamil). For data acquisition, taken for a norm, researches at 8 healthy animals are made.

Acute pancreatitis was modelled on V. M. Buyanov's way et al. (1989). To adult not purebred dogs carried out a median laparotomy, carried out a puncture of a gall bladder with an intake of bile and the subsequent alloying of the place of a puncture. Further bile was entered into a parenchyma of a vertical part of a pancreas on 0.5 ml in 8 points. Thus reproduced a destructive form of acute pancreatitis. In experienced groups on model of acute pancreatitis estimated a number of pharmakodinamichesky effects of an emoksipin (daily intravenous administrations of 1% of solution (10 mg/kg)) of a reamberin (daily intravenous administrations in a dose of 15 ml/kg), verapamil (daily intravenous administrations of 0.25% of solution of drug (0.2 mkg/kg)).

In control terms of research (the 1st and 3rd days) an animal made relaparotomy, estimated a pancreas condition, defined nature of its damages, and also made a biopsy of its fabrics, a fence of a venous blood.

Experiments were made under an intravenous anesthesia with use of Thiopentalum-natrium at the rate of 0.04 mg/kg of body weight of an animal. After carrying out researches of animals brought out of experiment by introduction of a lethal dose of Thiopentalum-natrium. Researches are executed according to moral requirements to work with experimental animals, approved by local ethical committee.

Lipids from tissue of a pancreas extracted hloroformmetanolovy mix (Higgins J. A., 1990). Lipids fractioned by method of a thin-layer chromatography (Higgins J. A., 1990; Vaskovsky V.E. et al., 1975). The molecular analysis was carried out on the densitometer Model GS-670 (BIO-RAD, the USA) with the corresponding software (Phosphor Analyst/PS Sowtware).

Indicators of intensity of the peroxide oxidation of lipids (POL): the diene conjugates (DC) determined by a spectrophotometric method at the wavelength of 232-233 nanometers (Ganston F. D., 1986); level of low-new dial – a spectrophotometric method in reaction with thiobarbituric acid (TBK) (Sigma), activity of a superoxide scavenger (SOD) – in reaction with nitroblue tetrazoloy (Gurevich V. S., etc., 1990). Activity of a phospholipase of A2 150 mmol a triton of H-100 investigated in the environment containing 10 mmol the tris-HCL-buffer (pH 8,0), 10 mmol of CaCl2 and 1,2 mmol of substrate as which used phosphatidylsincalines of an egg yolk (Trofimov V. A., 1999).

Measurement of content of pyruvic acid (PVK) was performed when carrying out reaction with 2,4 dinitrophenylhydrazine (Kushmanova O. D., Ivchenko G. M., 1983). Content of the lactic acid (LA) was determined by reaction with paraoxodiphenyl (Menshikov V. V., 1987).

Statistical processing of the obtained data was made by the standard methods of statistics with determination of reliability of distinctions between data in experienced and control groups on the basis of calculation of a criterion of Styyudent. The revealed patterns and bonds of the studied parameters between groups and signs were significant at a probability of the faultless forecast r = 95% and more.

3. Results and discussion
Experiences showed that the chosen model of pancreatitis was quite adequate for the solution of the purpose and research problems. In all cases animals had an acute biliary pancreatitis of a destructive form that was confirmed macro - and is microscopic.

The data obtained in control group of experienced animals demonstrate that at acute experimental pancreatitis the forming of disturbances of a lipidic metabolism which is shown increase in tissues of a
pancreas of the recreation center level and TBK-aktivnykh products, and also fosfolipazny activity takes place. At the same time the antioxidant potential of the studied fabrics considerably decreased that was expressed by falling of activity of SOD in comparison with an outcome for 44,4–63,8% (p <0,05) in 1–3 days of research.

**Table 1.** Influence of some drugs on structure of lipids (% of the general maintenance of lipids) in pancreas tissue at acute pancreatitis (M ± m, n = 8 in each group)

| Indicator                | Norm     | Group | Supervision stages |
|--------------------------|----------|-------|--------------------|
|                          |          |       | 1st days           | 3rd days |
| Total phospholipids      | 28,88 ± 1,01 | I     | 16,71 ± 0,53 *     | 18,87 ± 0,49 * |
|                          |          | II    | 20,79 ± 0,52 *     | 24,85 ± 0,57 * |
|                          |          |       | P1 <0,05           | P1 <0,05 |
|                          |          |       | P2 > 0,05          | P2 < 0,05 |
|                          |          | III   | 18,42 ± 0,38 *     | 21,46 ± 0,21 * |
|                          |          |       | P3 < 0,05          | P3 > 0,05 |
|                          |          | IV    | 19,69 ± 0,34 *     | 22,34 ± 0,69 * |
| Cholesterol              | 28,41 ± 1,09 | I     | 27,16 ± 0,77       | 13,29 * + 0,43 * |
|                          |          | II    | 28,94 ± 0,74       | 17,09 + 0,64 * |
|                          |          |       | P1 <0,05           | P1 <0,05 |
|                          |          |       | P2 > 0,05          | P2 < 0,05 |
|                          |          | III   | 26,27 ± 0,59       | 15,07 + 0,31 * |
|                          |          |       | P3 > 0,05          | P3 > 0,05 |
|                          |          | IV    | 15,89 ± 0,80       | 15,58 + 0,58 * |
| Free fatty acids         | 5,97 ± 0,44 | I     | 14,27 ± 0,45 *     | 16,38 + 0,54 * |
|                          |          | II    | 11,43 ± 0,36 *     | 12,44 + 0,42 * |
|                          |          |       | P1 <0,05           | P1 <0,05 |
|                          |          |       | P2 > 0,05          | P2 < 0,05 |
|                          |          | III   | 13,62 ± 0,22 *     | 14,57 + 0,25 * |
|                          |          |       | P3 > 0,05          | P3 > 0,05 |
|                          |          | IV    | 12,58 ± 0,31 *     | 13,75 + 0,31 * |
| Triacylglycerols         | 18,34 ± 0,78 | I     | 28,06 ± 0,76 *     | 10,07 + 0,47 * |
|                          |          | II    | 24,42 ± 0,62 *     | 13,35 + 0,19 * |
|                          |          |       | P1 > 0,05          | P1 <0,05 |
|                          |          |       | P2 > 0,05          | P2 > 0,05 |
|                          |          | III   | 26,49 ± 0,77 *     | 11,62 + 0,63 * |
|                          |          |       | P3 > 0,05          | P3 > 0,05 |
|                          |          | IV    | 25,15 ± 0,47 *     | 12,84 + 0,26 * |
| Cholesterol ethers       | 14,31 ± 0,89 | I     | 7,43 ± 0,38 *      | 18,91 + 0,41 * |
|                          |          | II    | 9,62 ± 0,36 *      | 16,26 + 0,13 |
|                          |          |       | P1 <0,05           | P1 <0,05 |
|                          |          |       | P2 > 0,05          | P2 > 0,05 |
|                          |          | III   | 8,47 ± 0,20 *      | 17,37 + 0,34 * |
|                          |          |       | P3 > 0,05          | P3 > 0,05 |
|                          |          | IV    | 8,97 ± 0,30 *      | 16,79 + 0,35 * |

Notes: hereinafter: I – control group, II – experienced group with emoksipiny, III – experienced group with verapamili; IV – experienced group with reamberiny; * – reliability of changes of indicators in relation to a norm at p <0,05; a bold print – reliability of a difference between data of control and experienced group at p <0,05; P1 <0,05 – reliability of a difference between data II and III groups; P2 <0,05 – reliability of a difference between data II and IV groups; P3 <0,05 – reliability of a difference between data III and IV groups.
Qualitative and quantitative changes of structure of lipids of tissues of pancreas were characterized by decrease in level of total phospholipids, cholesterol at increase in a quantity monoacylglycerofishing (MAGICIAN), diacylglycerols (DAG) and the free fatty acids (FFA). Content of triacylglycerols (TAG) changed ambiguously: in the first days growth of an indicator, to third day – its reliable decrease took place. The amount of ethers of cholesterol changed exactly the opposite (table 1).

The composition of phospholipids when modeling acute pancreatitis differed in higher rates of lizofosfolipid (LFL), sphingomyelin (CM), phosphatidylsincaline (FH) and smaller values of phosphatidylserine (FS), a phospha-tidylinositol (FI) and phosphatidylethanolamine (FEA) in comparison with a norm (table 2).

### Table 2. Influence of some drugs on composition of phospholipids (% of the general maintenance of lipids) in pancreas tissue at acute pancreatitis (M ± m, n = 8 in each group)

| Indicator           | Norm         | Group | Supervision stages |
|---------------------|--------------|-------|--------------------|
|                     |              |       | 1st days | 3rd days |
| Lizofosfolipida     | 1,55 ± 0,04  | I     | 7,65 ± 0,32 *   | 14,67 ± 0,46 * |
|                     |              | II    | 4,97 ± 0,22 *   | 11,91 ± 0,30 * |
|                     |              |       | P1 > 0,05      | P1 > 0,05    |
|                     |              |       | P2 < 0,05      | P2 < 0,05    |
|                     |              | III   | 5,42 ± 0,13 *   | 12,73 ± 0,40 * |
|                     |              |       | P3 < 0,05      | P3 > 0,05    |
|                     |              | IV    | 5,83 ± 0,10 *   | 13,02 ± 0,30 * |
| Sphingomyelin       | 4,84 ± 0,17  | I     | 8,51 ± 0,18 *   | 7,46 ± 0,08 * |
|                     |              | II    | 6,83 ± 0,22 *   | 5,74 ± 0,25 * |
|                     |              |       | P1 < 0,05      | P1 > 0,05    |
|                     |              |       | P2 < 0,05      | P2 < 0,05    |
|                     |              | III   | 7,57 ± 0,22 *   | 6,38 ± 0,18 * |
|                     |              |       | P3 > 0,05      | P3 < 0,05    |
|                     |              | IV    | 7,82 ± 0,23 *   | 7,05 ± 0,21 * |
| Fosfatidilinozit    | 19,06 ± 0,52 | I     | 16,55 ± 0,30 *  | 18,06 ± 0,52 |
|                     |              | II    | 17,92 ± 0,21    | 18,96 ± 0,45 |
|                     |              |       | P1 > 0,05      | P1 > 0,05    |
|                     |              |       | P2 < 0,05      | P2 < 0,05    |
|                     |              | III   | 17,42 ± 0,22 *  | 18,63 ± 0,77 |
|                     |              |       | P3 > 0,05      | P3 > 0,05    |
|                     |              | IV    | 16,99 ± 0,27 *  | 18,27 ± 0,35 |
| Fosfatidiletanolamin| 41,73 ± 1,03 | I     | 33,88 ± 0,78 *  | 29,12 ± 1,08 *|
|                     |              | II    | 36,07 ± 0,44 *  | 33,90 ± 0,68 *|
|                     |              |       | P1 < 0,05      | P1 < 0,05    |
|                     |              |       | P2 > 0,05      | P2 > 0,05    |
|                     |              | III   | 34,66 ± 0,64 *  | 34,11 ± 0,47 *|
|                     |              |       | P3 > 0,05      | P3 < 0,05    |
|                     |              | IV    | 34,85 ± 0,79 *  | 32,65 ± 0,42 *|

Destabilization of lipidic structure of a pancreas was combined with development of the hypoxemic phenomena in it that was shown by growth of the MK, PVK level and coefficient of a hypoxia in control group.

Thus, the results received in control group of animals demonstrate that at acute experimental pancreatitis in fabric structures of a pancreas there is an essential modification of a lipidic metabolism
and the hypoxia of body forms that demonstrates development of the membranodestructivny phenomena in it.

In view of the fact of the major role of frustration of a lipidic exchange in a pathogeny of acute pancreatitis, research of a pharmacodynamics of drugs of metabolic type of action is represented reasonable, one of objects of influence of which are the lipids or processes regulating their exchange. As us are chosen from their numerous quantity emoksipin – drug of antioxidant type of action, reamberin – an antigipoksant and verapamil – inhibitor of calcium channels.

Researches showed that use of the studied drugs allowed to influence therapeutic intensity the FLOOR in pancreas tissues at experimental pancreatitis. Under the influence of the studied drugs the quantity of a recreation center and TBK-aktivnykh products in the 1st and 3 days after modeling of biliary pancreatitis in tissue of a pancreas was reliable less concerning control.

At comparative analysis of experienced groups it turned out that the FLOOR in tissue of a pancreas have the greatest influence on intensity emoksipin and verapamil, a smaller effect – reamberin.

Research of influence of the studied drugs on activity of a phospholipase of A2 in fabric structures of a pancreas at its acute inflammation showed that under the influence of an emoksipin activity of enzyme on the first – third day of experiment authentically decreased in comparison with control by 21,41–28,58%, verapamil – for 37,34–37,48%, a reamberin – for 18,07–19,59% (p <0,05).

Comparative analysis of impact of the studied drugs on fosfolipazny activity in fabric structures of a pancreas at biliary pancreatitis showed that the greatest effect verapamil, smaller – emoksipin and the smallest – reamberin has.

Activity of SOD in fabric structures of the body inflamed experimentally under the influence of an emoksipin on the first and third day increased concerning control respectively by 15,31 and 40,92% (p <0,05). Reliable distinctions of indicators of activity of SOD in pancreas tissue in experienced groups with use of verapamil and a reamberin in comparison with control group appeared only for the 3rd days of a pilot study – activity of enzyme was respectively 25,25 higher and than 22,17% (p <0,05).

The studied drugs influenced also change of structure of lipids in pancreas tissue. So the amount of total phospholipids in a day of experiment authentically increased in comparison with control at inclusion in the scheme of treatment of all approved drugs. On third day of experiment similar dynamics remained. At the same time reduction of the MAGICIAN, DAG and SZhK level was observed. Dynamics of other indicators was less expressed (tab. 1).

Influence of the studied drugs is noted also on composition of phospholipids in pancreas tissue at biliary pancreatitis. As showed research results the most essential changes were observed concerning indicators of LFL and CM which level decreased from first days of experiment in comparison with control data. Reliable dynamics of indicators of FH, FS and FEA is noted mainly only on third day of research, and FI – for the first days of experiment (tab. 2).

When studying values of the indicators allowing to judge lipidic composition of tissue of pancreas at pancreatitis in a comparative aspect it is established that connection of an emoksipin in therapy of this disease rendered the greatest effect for normalization pathologically of modified structure of lipids and phospholipids in fabric structures of body. The smaller effect is gained when using verapamil and a reamberin.

Pilot studies revealed impact of the studied drugs on the arising hypoxemic processes in pancreas tissue at experimental pancreatitis that was shown by decrease in the MK, PVK level and coefficient of a hypoxia from first days of experiment.

Comparative analysis of indicators of the phenomena of a hypoxia in fabric structures of a pancreas at acute pancreatitis showed that most hypoxemic processes in fabrics of the inflamed body were influenced by an antigipoksant reamberin, to a lesser extent – emoksipin and verapamil.

4. Conclusion
Following the results of a pilot study on studying of influence of an emoksipin, verapamil and a reamberin at acute pancreatitis it is possible to say that under the influence of these drugs in fabric structures of the inflamed pancreas there is a decrease in intensity of free radical processes of a
lipopereokisleniye, activity of phospholipases, hypoxia phenomena. Level of antioxidant protection of fabric of body increases. In the first three days of supervision the accurate tendency to normalization of the transformed lipidic structure of fabric structures of a pancreas is revealed. At the same time these positive effects are noted in all experienced groups. It demonstrates that though pharmacological drugs are used multidirectional action (antioxidant emoksipin, an antigipoksant reamberin, inhibitor of calcium channels verapamil), they in a varying degree influence on studied pathological (membranodestruktivny, hypoxemic) processes, leading finally to reduction of their expressiveness. So, emoksipin found big ability to increase stability of membranes of pankreatotsit to pathological influence of molecular products the FLOOR, verapamil – to stopping of the activated phospholipases, reamberin – to a hypoxia.

The solution of a question of the key (prevailing) mechanism in trigger processes of acute pancreatitis of a definite answer has no. Undoubtedly only the fact that efficiency of antioxidant and inhibitor of calcium channels was rather higher. It suggests that free radical processes of a lipopereokisleniye and activity of fosfolipazny systems predetermine the level and nature of defeat of a cellular biomembrane of pankreatotsit already on the earliest terms of inflammatory process.

Acknowledgments
This work was supported by Competitiveness Program of National Research Nuclear University MEPhI.

References
[1] Beriaishvili Z. A., Parsadanyan A. M., Amiragyan D. M. 2001 Experience of treatment of destructive forms of pancreatitis//Materials of the Third congress of Association of surgeons of N. I. Pirogov (M, on October 15-17), 103-104.
[2] Vlasov A. P., Trofimov V. A., Krylov V. G. 2009 A system lipidic distress syndrome in surgery (M.: Science) 224 p.
[3] Gubergrits N. B., Hristich T.N. 2000 Clinical pankreatologiya (Donetsk: Swan) 416 p.
[4] Beger H. G., Rau B. 1995 Acute pancreatitis//Ann. Ital. Chir. vol. 66, p. 209–215.
[5] Benchimol D., Firtion O., Bereder J.M. 1996 Acute pancreatitis treated in a surgery ward. Apropos of 57 cases//J. Chir. vol. 133, No. 5, p. 208–213.