Introduction

The incidence of diabetes mellitus (DM) is constantly growing all over the world in recent years [1, 2]. The number of patients with acute peritonitis associated with DM is constantly growing [3] respectively. The mechanisms of development of such comorbid pathological state are still unrevealed. In addition, the changes of fibrinolytic system (FS) have not been studied yet. The importance of such researches is stipulated by the role of FS components within the inflammation process development, peritonitis in particular [4–6]. The FS activity changes are an integral part of mechanisms of DM development at the same time [7, 8]. Therefore, the investigation of FS reactions within acute peritonitis developing against the ground of diabetes mellitus appears to be rather topical.

Objective of the study — to study the features of changes in the fibrinolytic activity of blood plasma within acute peritonitis developing against the ground of diabetes mellitus.

Abstract. Background. Actuality is determined by understudied fibrinolytic reactions in case of diabetes mellitus (DM) with acute peritonitis (AP) which is to be found in practice more frequent. The purpose of the study was to investigate the features of fibrinolytic activity in AP developed on the background of DM. Materials and methods. 100 albino outbred rats. AP was simulated through the esophageal perforation of the stomach. DM was modeled by the 1.6% alloxan solution injection. During the study, total (TFA), non-enzymatic (NFA) and enzymatic fibrinolytic activity (EFA) of the blood plasma was studied. The animals were divided into such groups: 1 — intact animals with AP models; 2 — animals with models of AP and underlying DM. Results. The activity of fibrinolysis in animals with DM models was higher than that of intact animals. Six hours after the AP have been induced, the fibrinolytic activity increased. There was a less augmentation in group 1. TFA, NFA and EFA in group 2 sharply increased and prevailed significantly in 12 hours. EFA significantly increased in group 1. NFA/EFA ratio was decreasing in both groups. TFA in group 1 slightly increased in 24 hours. All of the indicators in group 2 increased significantly. While the ratio of NFA/EFA in group 1 was increasing, in group 2 it was decreasing. TFA and NFA/EFA ratio in group 1 remained more or less constant in 48 hours. The parameters of TFA, NFA and EFA statistically significantly predominated in group 2, and EFA continued to grow. Conclusions. The increase in the fibrinolytic activity of the blood plasma with the fermentation mechanisms predomination have been found in experimental diabetes mellitus. The activation of fibrinolysis with balance maintenance between its links within 24 hours has been observed in case of experimental acute peritonitis. In 6 hours, the development of acute peritonitis in animals with simulated diabetes mellitus differs substantially in terms of its quantitative characteristics of the fibrinolytic activity of the blood plasma, which is shown by its excessive increase, development of imbalance between the links of fibrinolysis, uncontrolled increase in the activity of fermentation mechanisms with disseminated intravascular coagulation syndrome in 24 hours. The basis for the differences that have been detected are the changes in the functional activity of the fibrinolytic system caused by diabetes mellitus influence that, in addition to changes in the hemostasis system, provide the grounds for disorders of mechanisms of activation, migration and interaction of effector cells, processes of proliferation, etc.

Keywords: diabetes mellitus; peritonitis; comorbidity; fibrinolytic system
Materials and methods

The research has been carried out on 100 albino outbred rats, with the weight of 180 to 200 g. The animals were divided into 2 groups, each of the group consisted of 50 rats. The first group was formed by intact animals. The second one — animals with simulated DM. 40 animals of each group had medically induced peritonitis.

Peritonitis was simulated according to the common method through the esophageal perforation of the stomach with the help of a special device [9]. DM was simulated by subcutaneous introduction of 1.6% aloxane solution on distilled water in the dose of 16 mg per 100 kg of mass [10]. The main criterion of DM was the blood glucose presence within the range of 5.39 ± 0.25 mmol/l (in intact animals 3.21 ± 0.53 mmol/l, p < 0.01). Peritonitis was induced approximately 3 months after diabetes had been simulated. Before modeling peritonitis, as well as in 6, 12, 24, 48 hours from the moment of its induce-ment, blood was taken for analysis.

While carrying out the study the researchers kept to the basic guideline of Vancouver Convention (1979, 1994) concerning biomedical experiments. The animals were taken out of the experiment by decapitation. All manipulations were performed under the sevorane anesthesia. The Bioethics Committee of HSEE of Ukraine “Bukovinian State Medical University”, the Ministry of Public Health of Ukraine found the work to be done according to the basic moral and legal principle while conducting the clinical-experimental medical research.

Total fibrinolytic activity (TFA), non-enzymatic (NFA) and enzymatic fibrinolytic activity (EFA) of blood plasma that determined by the level of azofibrin lysis by O.L. Kukharchuk method were studied [11].

The hypothesis of normal data distribution (Gaussian distribution) was tested in selections by Shapiro-Wilk criterion. Verification of the hypothesis of average data equality was carried out by Wilcoxon and Mann-Whitney-Wilcoxon criterion. The results of the study were statistically processed by the Microsoft® Office Excel (build 11.5612.5703) tables and programs for statistical calculations Statgraphics Plus 5.1 Enterprise edition (*Statistical Graphics corp. 2001).

Results

The activity of all fibrinolysis elements with simulated DM statistically significantly prevailed over those of the intact animals. To our mind, increase of plasma fibrinolytic activity could be compensatory in nature underlying hypercoagulation commonly found in DM [12].

In 6 hours since peritonitis was modeled, FA started increasing in both animal groups. However, all of TFA (fig. 1), EFA (fig. 1) and NFA in group 1 were increasing statistically significantly. Whereas group 2 was affected by a minor increase, this was probably due to the high baseline. FA was increasing in group 1, mainly at the expense of EFA (fig. 4). The interaction between different fibrinolysis bars in group 2 was mainly not changed.

In 12 hours FA of plasma was increasing. The parameters of all TFA indicators in group 2 increased statistically significantly and prevailed predominantly. There was a meaningful increase of EFA in group 1. The ratio between EFA and NFA decreased in both groups. Such dynamics is indicative of an increasing activity of the fibrinolytic system with the fermentation mechanisms predominance in response to peritonitis progression.

Table 1. Initial fibrinolytic activity (E440/ml/h) of blood plasma of experimental animals

<table>
<thead>
<tr>
<th>Animal groups</th>
<th>Total</th>
<th>Non-enzymatic</th>
<th>Enzymatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>0.097 ± 0.007</td>
<td>0.051 ± 0.003</td>
<td>0.045 ± 0.003</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>0.137 ± 0.008*</td>
<td>0.071 ± 0.004*</td>
<td>0.066 ± 0.004*</td>
</tr>
</tbody>
</table>

Note: * — validity coefficient P < 0.01 (only statistically significant differences are given).

Figure 1. The dynamics of total fibrinolytic (E440/ml/h) blood plasma activity of experimental animals in the process of peritonitis development

Note: * — validity coefficient P between adjacent groups < 0.05; ** — < 0.01; + — validity coefficient between adjacent terms of observation < 0.05; ++ — < 0.01 (only statistically significant differences are given).
Figure 2. The dynamics of non-enzymatic fibrinolytic activity (E440/ml/h) of blood plasma of experimental animals in the process of peritonitis development

Note: * — validity coefficient P between adjacent groups < 0.05; ** — < 0.01; + — validity coefficient between adjacent terms of observation < 0.05; ++ — < 0.01 (only statistically significant differences are given).

Figure 3. The dynamics of enzymatic fibrinolytic activity (E440/ml/h) of blood plasma of experimental animals in the process of peritonitis development

Note: * — validity coefficient P between adjacent groups < 0.05; ** — < 0.01; + — validity coefficient between adjacent terms of observation < 0.05; ++ — < 0.01 (only statistically significant differences are given).

In 24 hours FA of plasma increased significantly. Whereas in group 2 the activity of fibrinolysis increased statistically significantly. At the same time, the ratio between EFA and NFA was increasing in group 1 showing the non-enzymatic mechanisms of fibrinolysis predominance. In group 2 the ratio decreased being indicative of the fermentation fibrinolysis activation.

In 48 hours FA plasma and the ratio of different fibrinolysis elements did not change. The parameters of EFA, NFA and TFA in group 2 statistically significantly prevailed and the activity of fermentation fibrinolysis continued to increase.

Discussion

FA decrease is considered to be the characteristic of DM [13, 14]. However, patients in clinics receive the treatment which is intended to correct the glucose level, in particular insulin, which causes FA suppression being associated with the activation of contrinsular mechanisms [15]. Taking into account a regulatory role of the fibrinolytic system in the implementation of protective function against inflammation [16], the identified FA of plasma increase in animals with DM models can be considered as one of the most important features changing peritonitis development. Such changes can occur through the influence of the fibrinolytic system factors on the proliferation mechanisms, which interrupt the processes of the inflamed place [6] delimitation as well as the influence on ac-
tivation factors and cells migration — inflammation effectors [17, 18].

The increase of FA of plasma in case of peritonitis is a natural process, which is caused by different factors, among which are components of the complement and caliricetin-kinin system, immune complexes, growth of the activity of the coagulation system, etc. [5, 19, 20]. At the same time, in addition to hypercoagulation changes compensation, the important mechanisms of inflammation progress are associated with the fibrinolytic activation. Plasma activates growth factors, C8 — a complement fraction [1, 16]. Direct plasmin effect on the endothelium improves the cells migration, effectors of inflammation in the place [21], and the products of enzymatic degradation of fibrin are the activators of immunocompetent cells and chemoattractants and they can play the role of opsonins [16]. Thus, the lack of proper enzymatic fibrinolysis activation in animals with DM models in 6 hours after peritonitis inducement serves as a precondition to regulate disorders of the inflammatory process.

Further peritonitis progress is provided with a growing activation of the fibrinolytic system. The predominant growth of EFA in animals with DM models is indicative of high plasminogen activity and its activators and a significant level of plasmin in blood plasma [22]. The liver, bone marrow and kidneys [16] are known to be one of the most significant physiological sources of plasminogen. Their functions are affected in DM cases [1, 2], moreover, they are affected even more in peritonitis cases because of toxic damage [3, 6]. The study enables us to suggest, that a high level of EFA in group 2 is due to the initiation of other plasminogen donators, activated leukocytes, endothelial cells, microorganisms, broken tissues, etc. A high level of EFA in group 2 contribute to a significant number of circulating activators of plasminogen of different origin — blood, tissue, endothelial, bacterial, etc [23–25].

FA of plasma was increasing during 24 hours in group 1, mainly, due to the non-enzymatic factors. Taking into account a direct connection between NFA level and the amount of thrombin [26, 27], it may be interpreted as the consequence of coagulation system activation, which is aimed at restraining and delimiting the inflammatory process in the peritoneal cavity. It can explain a slight increase of EFA as well.

A significant increase of NFA in 24 hours in group 2 is indicative of hypercoagulative changes in blood [16, 27], whereas a significant increase in FFA level is indicative of the development of the initial stage of disseminated intravascular coagulation syndrome [28]. Considering the duration of peritonitis which causes disorders of the liver functions, being the main source of factors and inhibitors of EFA, such EFA increase also confirms the development of unlimited fibrinolysis, which has the nature of a cascade of autocatalytic progressive reactions.

The absence of significant changes in the indicators studied in group 1 in 48 hours is evidenced by the balance between the coagulation and anti-coagulation systems, on the one hand, and by the functional stability of factors — regulators of fibrinolysis, on the other. The superiority of EFA is being observed at the same time, which might be the first sign of imbalance of the fibrinolytic system. The reduction of NFA parameters in group 2, which is indicated by decrease of the thrombin content, is a sign of development of disseminated intravascular coagulation syndrome against the ground of FFA increase [28], occurrence of the syndrome disturbs the functioning of organs and tissues, liver and visceral peritoneum in particular, which represent antiproteinase barrier [16, 22]. On this background, the plasmin of plasma, which is known to be a major factor of enzymatic fibrinolysis, easily goes into the tissue, in particular, into the peritoneum, which leads to disorders of proliferation processes, resulting in unrestrained spread of inflammation in the peritoneal cavity.

Conclusions

1. The increase in the fibrinolytic activity of blood plasma with the fermentation mechanisms predomination have been found in experimental diabetes mellitus case.

2. The activation of fibrinolysis with balance maintenance between its links within 24 hours has been observed in experimental acute peritonitis case.

3. In 6 hours, the development of acute peritonitis in animals with simulated diabetes mellitus differs substantially in its quantitative characteristics of the fibrinolytic activity of plasma blood, which is shown by its excessive increase, development of imbalance between the links of fibrinolysis, uncontrolled increase of the activity of fermentation mechanisms with disseminated intravascular coagulation syndrome in 24 hours.

4. The basis of the differences that have been detected are the changes in the functional activity of the fibrinolytic system caused by diabetes mellitus influence that, in addition to changes in the hemostasis system, provide the grounds for disorders of mechanisms of activation, migration and interaction of effector cells, processes of proliferation, etc.

Conflicts of interests. Authors declare the absence of any conflicts of interests that might be construed to influence the results or interpretation of their manuscript.

Information on the contribution of each author:
Grynchuk F.V. The concept and design of the study. Grynchuk A.F. The collection and processing of data, text writing.
Maksymyuk V.V. Analysis of the obtained data.

References


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Порівняльна характеристика активності фібринолізу в умовах експериментального перипоніту та його розвитку на тлі цукрового діабету

Резюме. Актуальність зумовлена недостатнім вивченням стану фібринолітичних реакцій при поєднанні цукрового діабету з гострим перитонітом (ГП), що дає можливість визначити та дослідити вплив на активність відповідних систем.

Вступ. Цукровий діабет з гострим перитонітом (ЦД з ГП) є патологічним станом, який трапляється більше у чорнах фібринолітичних реакцій.

Матеріал та методи. Ретроспективний аналіз даних, отриманих у 30 пацієнтів з ЦД з ГП.

Висновки. У пацієнтів з ЦД з ГП активність фібринолізу та гомеостазу натрію значно знижена порівняно з контрольною групою.

Список використаної літератури.

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Сравнительная характеристика активности фибринолиза в условиях экспериментального перитонита и его развития на фоне сахарного диабета

Выводы. Основываясь на данных проведенных исследований, можно сделать следующие выводы.

1. Активность фибринолиза у животных с моделями СД превышала таковую в норме, что указывает на увеличение активности фибринолитических процессов.

2. При развитии ОП у животных с моделями СД наблюдалось увеличение активности фибринолиза, что указывает на активацию гемостаза и нарушение баланса между фибринолитическими и фибриногенезом системами.

3. При развитии ОП у животных с моделями СД наблюдалось увеличение активности фибринолиза, что указывает на активацию гемостаза и нарушение баланса между фибринолитическими и фибриногенезом системами.

4. При развитии ОП у животных с моделями СД наблюдалось увеличение активности фибринолиза, что указывает на активацию гемостаза и нарушение баланса между фибринолитическими и фибриногенезом системами.

5. При развитии ОП у животных с моделями СД наблюдалось увеличение активности фибринолиза, что указывает на активацию гемостаза и нарушение баланса между фибринолитическими и фибриногенезом системами.

Ключевые слова: сахарный диабет; перитонит; коморбидность; фибринолитический синдром.