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PATHOMORPHOSIS OF INTRAVASCULAR COAGULATION IN EXPERIMENTAL ENDOTOXIC SHOCK IN RABBITS TREATED WITH PAMBA AND URBASON

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Intravascular coagulation (IC) is a pathological process with a complex pathogenesis which course is significantly influenced by the participation of numerous protecting or damaging factors [1,3,6,9,12]. These complicated interrelationships are emphasized by H. Selye in 1967 [14]. He suggested a concept of pluricausality of thrombo-haemorrhagic phenomena and of the role of the so-called by him "conditioning factors" in their origin and course.

In human pathology, IC not seldom originates on the background of pre-existing diseases which lead to fibrinolysis suppression, to enhanced functional activity of the adrenal cortex [3,7] or in the treatment of which antifibrinolytics [8] and glycocorticoids [2,4] are used. However, there are rather contradictory data about the role of fibrinolysis suppression and of glycocorticoids for the course and morphological manifestations of IC [7,10,11,13,15].

The purpose of the present work is to study the effect of fibrinolysis inhibition and of glycocorticoids on the morphological picture and course of IC in endotoxic shock in rabbits.

MATERIAL AND METHODS

Our study covered 63 rabbits of "New Zealand" breed of a body weight of approximately 2500 g. There were 48 experimental and 15 control animals. Experimental animals were divided into 3 groups: group one - classical model of endotoxic shock - 19 animals. Shock was caused by twofold (with an interval of 24 hours) intravenous application of a lipopolysaccharide from Escherichia coli 0_{111} in a dose of 100 mkg/kg b.w. for the first and of 2000 mkg/kg b.w. for the second injection. Group two - endotoxic shock with fibrinolysis suppression - 14 animals. In these cases 50 mg of PAMBA were i. m. applied simultaneously to the second lipopolysaccharide injection. Group three - endotoxic shock on the background of preliminary four-day Urbason premedication in a dose of 4 mg/kg b.w. i.m. daily (in 15 animals).

Saline solution was i. v. injected on control animals. Besides 50 mg of PAMBA or Urbason were i.m. injected according to the schedule designated for the third group to two animal groups of 4 rabbits each.

Animals were killed by means of the application of a lethal thiopental dose by i. v. injections. Both kinds of animals (deceased due to shock and killed by thiopental) were divided into 5 groups according to the duration of shock prior to death: from 15 min till 1 hour; till 2 hours; between 4 and 8 hours; between 18 and 24 hours, and between 48 and 72 hours. Autopsy was carried out immediately after death. Paraffin sections were stained with HE, PAS, phosphotungitic acid hematoxylin (PTAH), picromallory-Caarstears.

Density of microthrombi was estimated per 1 cm^2 of tissue section by means of direct planimetry by using planimetric ruler. Digital data are processed by the methods of alternative and variation analysis at a confidence threshold of $p < 0,05$.

RESULTS AND DISCUSSION

We establish morphological data of a disseminated intravascular coagulation (DIC) in all animals from the three groups. It is manifested mainly by fibrin microthrombi in two or more organs. No microthrombi can be detected in organs of control animals.

Differences between these three groups concern mainly DIC severity, organic predilection of microthrombi and the incidence rate of appearance of late organic lesions. It strikes that DIC distribution rate in various organs is considerably higher when PAMBA or Urbason conditioning has been used (table 1).

Table 1

DIC distribution in organs after endotoxic shock with or without additional influences

Experimental groups	Total number of examined organs	Of them with microthrombi	%	Sx	P
Group one endotoxic shock	171	68	39,77	+/- 7,34	-
Group two endotoxic shock with PAMBA	126	67	53,17	+/- 8,71	0,05
Group three endotoxic shock with Urbason	135	70	51,85	+/- 8,43	0,05

It is noteworthy that while in the first group with a classical model of endotoxic shock one can establish microthrombi in three or in more organs in 84,21 per cent of the rabbits in the rest two groups (with fibrinolysis suppression and Urbason premedication) there are microthrombi in three or more organs in 100 per cent of the animals ($p < 0,05$). The comparison of the mean microthrombus density in the examined organs reveals similar differences, too. Although density of microthrombi (357+/-276 microthrmbi/cm^2) is higher in the second than in the first group (223+/-186 microthrombi/cm^2) this difference is statistically insignificant ($p > 0,05$). However, Urbason premedication causes an almost twofold higher density of microthrombi (405+/-317 microthrombi/cm^2) than that in the first group ($p < 0,05$).

Dynamic follow-up of DIC distribution and of mean density of microthrombi in the organs demonstrates that till the end of the first hour thrombus formation influenced by PAMBA or Urbason is less outlined than that in the classical model of endotoxic shock. On the second hour, DIC distribution is almost equal in the three groups. It remains significantly greater in all subsequent termins when PAMBA or Urbason premedication is concerned. Density of microthrombi in the 2nd and 3rd group increases rapidly on the second hour and reaches its maximal level between the 4th and 8th hour. Comparison of values between these three groups reveals significant differences. In the first group, during the first 24 hours density remains over

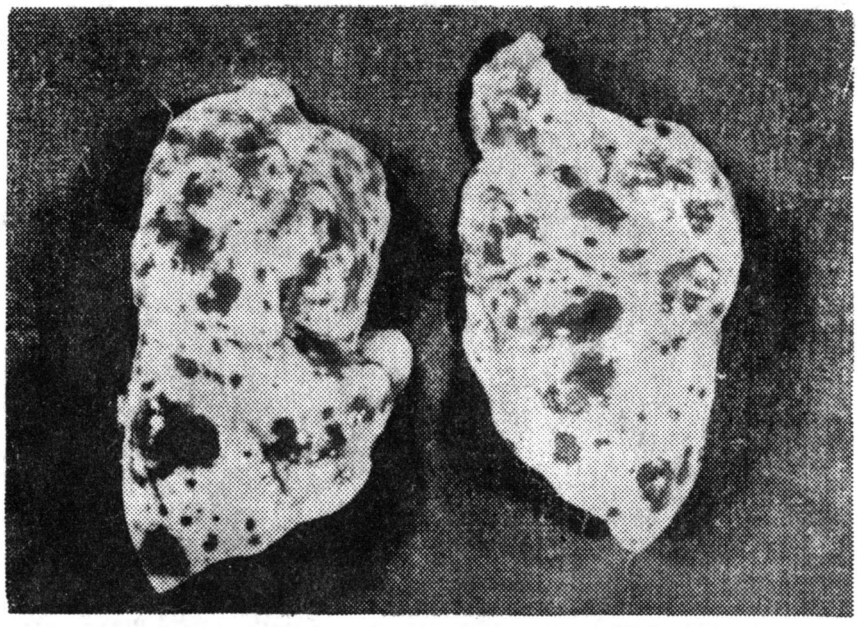

*Fig. 1. **Multiple haemorrhages in the lungs in endotoxic shock with fibrinolysis suppression***

200 microthrombi/cm^2 with maximal value (of 309 microthrombi/cm^2) on the 2nd hour and sharp diminution between the 48th and 72nd hour (down to 30 microthrombi/cm^2). Both distribution

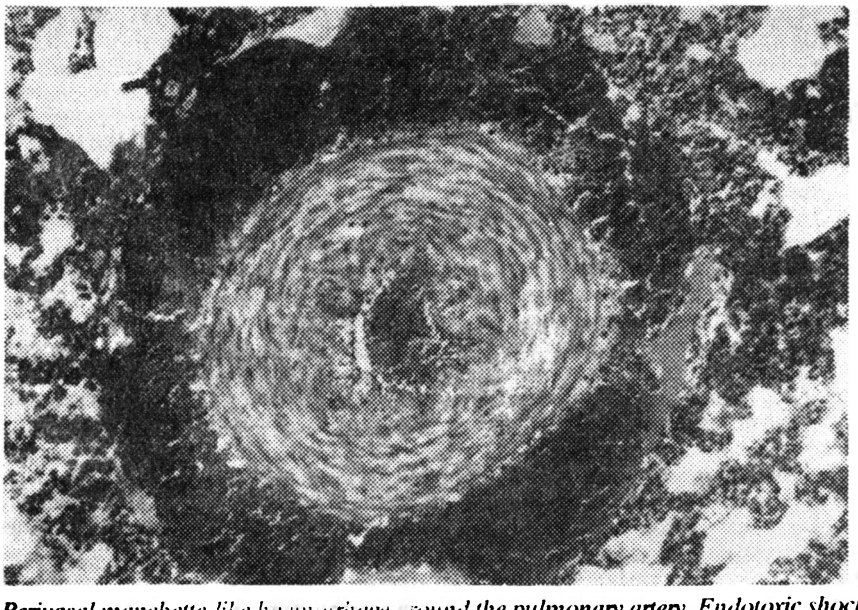

*Fig. 2. **Perivasal manchette-like haemorrhage around the pulmonary artery. Endotoxic shock on the background of preliminary Urbason treatment. Staining with HE, Magn. 10x6,3***

and microthrombus density increase gradually and parallelly in animals of the 2nd group. Besides they are significantly higher than these in the first group. The maximal value of 729 microthrombi/cm^2 is reached between the 4th and 8th hour ($p < 0,02$). Microthrombus density and distribution remain between the 48th and 72nd hour at a 10 times higher level as compared with these of the first group ($p < 0,05$). Urbason treatment demonstrates a similar effect. The only difference consists in the fact that between the 48th and 72nd hour density of microthrombi reduces sharply and comes near to that in the first group.

PAMBA or Urbason premedication induces certain changes of organic localization of

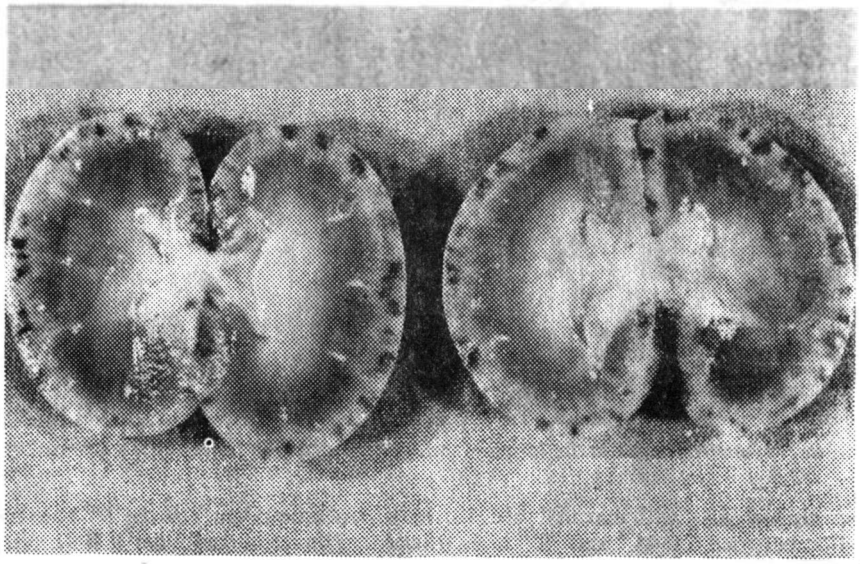

Fig. 3. Massive symmetrical cortical necroses in the kidneys in endotoxic shock on the background of preliminary Urbason treatment

microthrombi. Fibrinolysis suppression (in 50,00 per cent of the cases) and Urbason premedication (in 66,67 per cent of the cases) induce a tenfold increasing of the incidence rate of affection by microthrombi of adrenal glands in comparison with the classical model (in 5,26 percent of the cases) ($p < 0,01$). There is an essential thrombus formation increase in the brain, too (from 15,59 per cent up to 42,86 percent) in the second group ($p < 0,05$) as well as in the kidneys (from 63,16 per cent up to 86,67 per cent) in the third group ($p < 0,05$).

Lungs, liver, spleen and kidneys present the main target organs showing an incidence rate of microthrombus formation over the mean one when all three groups are concerned. Urbason premedication transforms adrenal glands into a fifth target organ for DIC.

There are certain differences between experimental groups concerning other organic lesions, too. For instance, after fibrinolysis inhibition one finds out massive haemorrhages in the lungs (fig. 1), which is more often than that in the other groups. Urbason treatment results mainly in perivascular manchette-like haemorrhages in the lungs which can be only microscopically observed (fig. 2).

There is a much more severe renal damage. Glomeruli are affected in 100 per cent in animals from the second and third group by microthrombi. While in the classical model of endotoxic shock symmetrical cortical necroses develop in 21,05 per cent of the animals they are two times more frequent after treatment with PAMBA (in 42,86 per cent of the cases) and with Urbason (in 40,00 per cent of the cases). The early appearance (between 5-6 and 18 hours after the second injection) and the broader dissemination of symmetrical cortical necroses (fig. 3 and fig. 4) are typical of

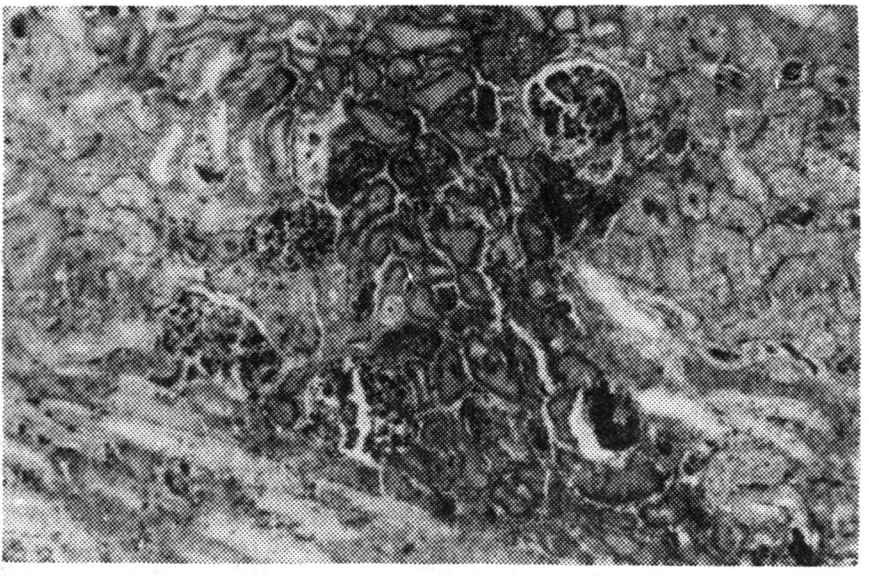

Fig. 4. Symmetrical cortical necroses with numerous fibrin microthrombi in glomerular capillaries and interstitial veins. Endotoxic shock on the background of preliminary Urbason treatment. Staining PTAH, Magn. 10x6,3

the third group. The latter indicates that Urbason potentiates thrombus formation in the kidneys already after the preparatory dose of lipopolysaccharide.

Our results demonstrate the possibilities of drug-induced pathomorphosis of DIC in endotoxic shock in rabbits. The role of inhibited fibrinolysis in IC is known from human and experimental pathology [3,11]. Its influence upon the morphological manifestations and dynamics of thrombus formation in the microcirculatory bed is not investigated enough yet. Much higher density of microthrombi established in case of suppressed fibrinolysis than that in case of classical endotoxic shock model indicates rather markedly the role of fibrinolysis in elimination of microthrombi. At the same time, it suggests that, usually, only a small part of really formed microthrombi are established by the morphologist.

Glycocorticoids are administered in the course of therapy of numerous diseases and shock states, incorporated. DIC occurs in the course of these diseases not seldom, too. Data about glycocorticoid influence upon IC process are contradictory. It is supposed that hydrocortison exerts a protective effect in endotoxic shock by means of enhancement of receptor susceptibility to catecholamines and of improvement of cellular metabolism [13,15]. Their effect is favourable when applied during the shock stage. However, their premedication possesses an unfavourable influence. Preliminary application of ACTH or glycocorticoids results in a more severe IC in experimental endotoxic shock [10,11]. In some cases, there are more severe renal lesions [11] while in other ones [10] adrenal glands are more severely affected. However, the mechanism of these differences in the effect of glycocorticoids in dependence on the time of application remains unclarified yet.

Our present investigation shows that Urbason premedication leads to a catastrophic course of endotoxic shock with an extraordinarily severe DIC selectively affecting adrenal glands and kidneys. The causes for the origin of this state of defencelessness against hypercoagulation disturbances of the haemostatic system after preliminary glycocorticoid treatment are unclear. Recently, J. Machin [5] notes that corticosteroids induce disturbance of prostacycline synthesis and a relative predominance of thromboxane A_2 in the microcirculatory bed.

It could be concluded that our results demonstrate the possibility for DIC pathomorphosis in endotoxic shock after preliminary conditioning by means widely used in the therapy as well as in endogenically determined enhancement of glycocorticoid production or in inhibited fibrinolysis in different diseases.

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ПАТОМОРФОЗ ВНУТРИСОСУДИСТОГО СВЕРТЫВАНИЯ КРОВИ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ЭНДОТОКСИЧЕСКОМ ШОКЕ У КРОЛИКОВ ПОД ВЛИЯНИЕМ ПРЕПАРАТОВ ПАМБА И УРБАЗОН

А. Ангелов

РЕЗЮМЕ

Исследование проведено на 63 кроликах (48 опитных и 15 контрольных). Животные были распределены на три группы: первая группа - классическая модель эндотоксического шока; вторая группа - эндотоксический шок с подавлением фибринолиза посредством применения препарата ПАМБА; третья группа - эндотоксический шок на фоне предварительной подготовки препаратом Урбазон.

Устанавливается значительное изменение в протекании и морфологической картине внутрисосудистого свертывания крови под влиянием кондиционирования при помощи препаратов ПАМБА или Урбазон. Отмечается нарастание как распространенности органов, так и средней частоты микротромбов. В первой группе максимальная интенсивность внутрисосудистого свертывания крови наступает в течение второго часа, в то время как во второй и третьей группах свертывание внутри сосудов наступает в течение четвертого - восьмого часов. Во второй и третьей группах частота образования микротромбов в почках и надпочечниках на много раз выше. Она значительно возрастает также в мозгу (II группа). Подавление фибринолиза препаратом ПАМБА связано с более массивными и более частыми кровоизлияниями. Кондиционирование препаратом Урбазон приводит к более ранним и более распространенным симетрическим кортикальным некрозам в почках. Выявляется возможность патоморфоза внутрисосудистого свертывания при эндотоксическом шоке посредством предварительного кондиционирования препаратами, широко применяющимися в терапевтической практике.