BLOOD COAGULATION PARAMETERS IN RATS WITH ACUTE RADIATION SYNDROME RECEIVING ACTIVATED CARBON AS A PREVENTIVE REMEDY

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Radiation-induced coagulopathy (RIC) is one of the major causes of death during acute radiation syndrome (ARS). The aim of this study was to characterize the responses of the hemostasis system to ARS of a moderate level on the 1st and 9th days after irradiation. We aimed to identify molecular markers of the blood coagulation system that are most affected by ARS and to estimate the enterosorption effect on the development of irradiation-induced changes. Platelet aggregation rate, activated partial thromboplastin time (APTT) and fibrinogen concentration were determined by standard methods. Level of protein C (PC) was measured using chromogenic substrate S2366 (p-Glu-Pro-Arg-pNa) and Agkistrodon halys halys snake venom activating enzyme. Functionally inactive forms of prothrombin (FIFPs) were determined using two activators in parallel – thromboplastin or prothrombin activator from Echis multisqumatis venom. Rats of both irradiated groups had a higher risk of intravascular clotting in comparison to both control groups. Statistically significant shortening of clotting time in the APTT test (24 ± 4 s vs. 33 ± 5 s) and increased fibrinogen concentration (4.2 ± 0.6 mg/ml vs. 3.2 ± 0.3 mg/ml) were detected. Both parameters were normalized on the 9th day after irradiation. However the platelet count was decreased (0.3·10⁶ ± 0.05·10⁶ 1/µl vs. 0.145·10⁶ ± 0.04·10⁶ 1/µl) due to the impaired megakaryocytic function. The level of PC was decreased after X-ray irradiation (70 ± 10%) and partly restored on the 9th day after irradiation (87 ± 10%). Administration of activated carbon (AC) inhibited the drop in the PC concentration after X-ray irradiation (86 ± 15%) and accelerated its restoration on the 9th day (103 ± 14%). The statistically significant accumulation of FIFPs was detected in blood plasma of irradiated rats at the 1st and 9th days after irradiation. No FIFPs were found in any irradiated rat treated with AC. Characterization of the hemostasis system of rats that were exposed to a semilethal dose of X-rays allowed us to select parameters that can be used for monitoring of ARS development. Apart of from basic coagulation tests (APTT) and the measurement of platelet aggregation, fibrinogen and protein C level we can recommend the determination of FIFPs as a useful tool for estimation of the hemostasis response after irradiation with X-rays. This test indicates the intravascular thrombin generation and can help predict thrombotic complication or disseminated intravascular coagulation. Determination of FIFPs in blood plasma of irradiated rats allowed us to study the enterosorption effect on the development of irradiation-induced changes. It was shown that enterosorption with AC prevented accumulation of FIFPs which appears to be a newly discovered antithrombotic effect of therapy with AC. ARS influenced hemostasis by inducing thrombin generation (indicated by FIFPs generation), low-grade inflammation (indicated by PC concentration decrease) and thrombocytopenia. Enterosorption with AC minimizes inflammation and pro-coagulant processes caused by a moderate dose of X-ray irradiation. Accumulation of FIFPs can be assumed to be one of the most sensitive markers of the blood coagulation response to X-ray irradiation.

K e y w o r d s: enterosorption, activated carbon, platelets, blood coagulation, acute radiation syndrome of moderate level.

Acute radiation syndrome (ARS) is a complex due to pathological outcomes after irradiation by 0.5 Gy and above [1]. The reason for death from ARS is believed to be radiation-induced multi-organ dysfunction including damage of endothelial cells and consequential systemic inflammation [2]. It is not surprising then that blood coagulation disorder is the main factor for death resulting from ARS in mammals, including humans [3]. These disorders are called radiation-induced coagulopathy (RIC) and consist mainly of microvascular thrombosis and consequent coagulopathy caused by a fatal drop in coagulation factors [4, 5]. This phenomenon of clotting and bleeding at the same time is known as disseminated intravascular coagulation (DIC) [6]. On the other hand, the blood coagulation system can be assumed to be one of the most sensitive systems to inflammatory processes in humans [7]. Inflammation directly leads to activation of coagulation, and coagulation also considerably affects inflammatory activity [8]. As far as inflammation being the crucial point of most of ARS-induced pathogenetic changes [9], blood coagulation parameters can indicate not only the inflammatory processes, but also the rate of radiation syndrome development. Due to our knowledge, blood coagulation substantially reacts to liver injury [10] and low-grade inflammation during diabetes [11].

Study of blood coagulation parameters can indicate not only the inflammatory processes, but can also serve as biomarkers of ARS and reflect the efficacy of preventive treatment Activated carbon (AC) acts by removing exogenous and endogenous toxins and is a promising remedy for ARS [12]. It is known that AC possesses prominent anti-inflammatory action [13]. Based on these findings, we expected that the anti-inflammatory action of enterosorption would suppress the activation of blood coagulation.

The aim of this study was to characterize the responses of the hemostasis system to ARS of a moderate level on the 1st and 9th days after the irradiation. The effect of oral administration of mesoporous AC with a highly developed surface (S_{BET} = 2900 m^2/g) on coagulation parameters of rats exposed to a semilethal dose of X-rays was studied. We aimed to identify molecular markers of the blood coagulation system that are most affected by ARS and to estimate the enterosorption effect on the development of irradiation-induced changes.

Materials and Methods

Blood sampling. Samples of rat blood were collected by heart puncture. 3.8% sodium citrate was added to blood immediately after collection. Platelet-rich plasma (PRP) was prepared from citrated blood by centrifugation at 1000 rpm for 30 min. Platelet poor plasma (PPP) was obtained from PRP by centrifugation of PRP for 15 min at 1500 rpm.

Reagents. Chromogenic substrates S2238 (H-D-Phe-Pip-Arg-pNA), S2765 (Z-D-Arg-Gly-Arg-pNA) and S2236 (p-Glu-Pro-Arg-pNa) were purchased from BIOPHEN (Neuville-sur-Oise, France). APTT-reagent, thromboplastin and protein C (PC) activator were purchased from Renam (Moscow, Russia). Ecamulin was purified from *Echis multisquamatis* venom according to the method of Solov'yev et al [14]. Factor X activator from *Daboia russellii* venom (RVV) and adenosine diphosphate (ADP) were purchased from Sigma-Aldrich (St. Louis, USA).

Activated carbon. AC was prepared by RE Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv, Ukraine and had the following properties: enterosorbent (ES) – granulated with diameter < 0.25 mm, and highly activated with bulk density of 0.12 g/cm^3, BET surface of 2 918 m^2/g and BJH volume of 4.5 cm^3/g (measured by nitrogen porosimetry).

Animal model. Male white Wistar rats (200 ± 20 g, *n* = 40) from the Institute of Experimental Pathology, Oncology and Radiobiology vivarium were divided into 4 groups of 10 animals each 2 weeks before the experiment for adaptation: 1st group – rats as irradiated control exposed to external X-ray irradiation at a dose of 6 Gy (self- recuperation group without ES) 2nd group – rats with 6 Gy irradiation exposure + AC per oral administration

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Abbreviations: RIC – Radiation-induced coagulopathy; AC - activated carbon; ARS – acute radiation syndrome; ES – enterosorbent; APTT – activated partial thromboplastin time; PC – protein C; aPC – activated protein C; DIC – disseminated intravascular coagulation; PRP – platelet rich blood plasma; PPP – platelet poor blood plasma; EI – ecamulin index; PI – prothrombin index; FIFPs – functionally inactive forms of prothrombin; NIH – National Institutes of Health units; ADP – adenosine diphosphate; BET – Brunauer, Emmett and Teller, the specific surface area of a sample is measured – including the pore size distribution; BJH – Barrett-Joyner-Halenda, parameter of the pore-size distribution; WMW – Wilcoxon-Mann-Whitney.

(ES), 3rd group – healthy rats + AC to determine effect of ES on healthy rats, 4th group – healthy rats as intact control. 1st group & 4th group of rats received only food (oat) and water and 2nd group and 3rd group of rats received 2 ml of AC with food (oat) per each rat and water. 2nd and 3rd groups of rats began taking AC admixed with food 3 days before irradiation for preventive effect, on the day of irradiation and after irradiation (for radio-mitigation and treatment) until the end of the experiment. 20 animals were sacrificed under chloroform anesthesia on the 1st day after irradiation, and the other 20 rats – on the 9th day after irradiation.

Ionizing radiation was delivered using X-Rays from the RUM-17 calibrated irradiator (Dnipropetrovsk, URSS) with working current of 10.5 mA, 0.5-mm Cu filter system; dose rate – 63 R per min, time – 11 min.

Animals were kept in standard cages with free access to food and water in accordance with the General Ethical Principles of Experiments on Animals (Ukraine, 2001), which are consistent with the provisions of "The European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). This study was carried out with the approval of the Animal Care and Use Committee of the IEPOR of the NASU (Protocol No 4 from 16/04/2015).

Activated partial prothrombin time. Activated partial thromboplastin time (APTT) was measured according to the following procedure: 0.1 ml of blood plasma was mixed with an equal volume of APTT-reagent and incubated for 3 minutes at 37 °C. Then the coagulation was initiated by adding 0.1 ml of a 0.025 M solution of $CaCl_2$. Clotting time was monitored by the Coagulometer Solar CGL-2410 (Solar, Minsk, Belarus).

Fibrinogen concentration. Fibrinogen concentration in the blood plasma was determined by the modified spectrophotometric method. Blood plasma (0.2 ml) and PBS (1.7 ml) were mixed in glass tubes. Coagulation was initiated by the addition of 0.1 ml of thrombin (2 NIH/ml). To avoid fibrin cross-linking 0.1 ml of 40 mM monoiodacetic acid was added to the sample. The mixture was incubated for 30 min at 37 °C. The fibrin clot was removed and redissolved in 5 ml of 1.5% acetic acid. The concentration of protein was measured using a spectrophotometer Optizen POP (Mecacys, Seongnam, South Korea) at 280 nm ($\varepsilon = 1.5$).

Protein C level. PC level was determined using the activator of PC from *Agkistrodon halys halys*

venom, as previously described [15]. The generation of activated PC was measured by chromogenic substrate assay using specific chromogenic substrate S2236 (p-Glu-Pro-Arg-pNa). The analysis was done in 0.05M Tris-HCl buffer pH 7.4, at 37 °C. Chromogenic substrate concentration was 30 mM. The generation of *para*-nitroaniline was measured at 405 nm on a microtiter plate reader Multiscan EX (Thermo Fisher Scientific, Waltham, USA).

Platelet aggregation. Platelet aggregation measurements were based on changes in the turbidity of human PRP [16]. Aggregation was registered for 10 min using an Aggregometer Solar AP2110 (Minsk, Belarus). Platelet count was estimated using the same device. The initial rate and final level of aggregation were measured at 37 °C in the prepared mixture: 250 µl of PRP incubated with $CaCl_2$ (10 µM) and activated by platelet agonist ADP (12.5 µM) at 37 °C.

Functionally inactive forms of prothrombin (FIFPs). The level of functionally inactive forms of prothrombin (FIFPs) in blood was measured using thromboplastin as well as ecamulin activators of prothrombin. Ecamulin (prothrombin activator from Echis multisqumatis venom) is an enzyme that activates prothrombin, descarboxy-prothrombin and prethrombin 1. It makes it possible to determine the total prothrombin level [17]. Thromboplastin acts through the tissue factor pathway of coagulation and activates only functionally active carboxylated and uncleaved forms of prothrombin as it is in the "prothrombin index" test. The difference in prothrombin level found by the ecamulin index (EI) and prothrombin index (PI) tests corresponds to the level of FIFPs.

Results of prothrombin and ecamulin tests were presented as the PI and EI as calculated by the formulas: EI = An/Ap; PI = An/Ap, where An is the blood plasma thrombin activity of a healthy control animal; Ap is the blood plasma thrombin activity of exposed animal. The difference between PI and EI that was more than 10% indicated the accumulation of FIFPs above 12 µg/ml, that was preliminarily assumed as evidence of thrombotic complications [18].

Thrombin generation was measured by chromogenic substrate assay [19] using thrombin-specific S2238 (H-D-Phe-Pip-Arg-pNA).

Statistics. Statistical data analysis was performed using the Wilcoxon-Mann-Whitney (WMW) test and Student's *t*-test. All blood coagulation assays were replicated thrice. Results are presented as

means ± standard deviation. Data were considered significant when $P < 0.05$.

Results and Discussion

Coagulation system proteins. The APTT test is basic clotting test that indicates the ability of blood plasma to form a clot via the intrinsic pathway of the coagulation cascade. Despite the fact that the APTT was shown to be insufficient for accurate diagnostics [20], it can be used as a screening test to study biological models, with the obligatory application of more precise testing. Time of clotting in the APTT test was shortened on the 1st day after irradiation independently of ES to 24 ± 4 vs. 33 ± 5 s in control animals (Fig. 1). After 9 days after irradiation animals showed overall normalization of clotting time in the APTT test, two of eight self-recuperative rats animals after irradiation had slightly prolonged clotting time (Fig. 1).

Shortened APTT is a quite unusual condition [21], however it can be clearly associated with a hypercoagulability state [22] or can be caused by an increased level of fibrinogen [23]. Fibrinogen is the main protein of the coagulation system. Under the action of thrombin it converts to the monomeric fibrin that forms the core of a thrombus. On the other hand, fibrinogen is the protein of the acute phase of inflammation and an increase in its concentration is a known phenomenon of the inflammatory response [24].

We did not observe any significant irradiation-stimulated changes in fibrinogen concentration. We can suggest that there is a tendency toward an increase in fibrinogen concentration in blood plasma of irradiated rats in both groups (4.2 ± 0.6 mg/ml vs. 3.2 ± 0.3 mg/ml in control) that indicates the development of the inflammatory process. Administration of ES slightly prevented the increase in fibrinogen concentration after the irradiation (3.8 ± 0.6 mg/ml). Changes in the fibrinogen level may be evidence of the inflammatory process, but this finding needs to be confirmed more precisely.

To confirm this finding, we studied the effect of irradiation and ES administration on the level of PC. PC is the major anticoagulant proenzyme of the coagulation system. Being activated by the
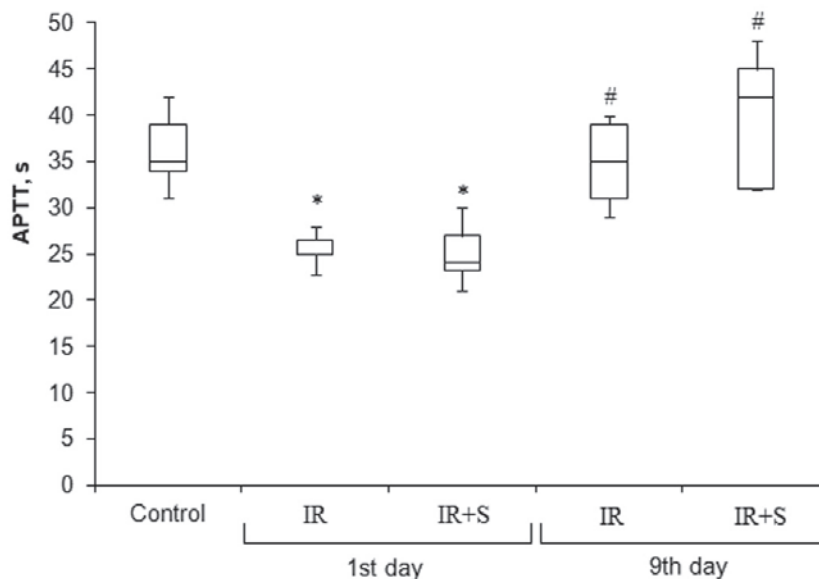

Fig. 1. Effect of irradiation at the dose of 6 Gy on clotting time in the activated partial thromboplastin time (APTT) test of rat blood plasma (n = 40). Control – clotting time of blood plasma of intact healthy animals including clotting time of blood plasma of healthy animals that received activated carbon (AC) admixed with food 3 days before irradiation and until the end of the experiment. IR – clotting time of blood plasma of irradiated rats on the 1st and 9th day after the irradiation. IR+S – clotting time of blood plasma of irradiated rats on the 1st and 9th day after irradiation in rats that received AC (denoted by "S" for sorbent) admixed with food 3 days before irradiation for a preventive effect, on the day of irradiation and after irradiation until the end of the experiment. *According to Wilcoxon-Mann-Whitney (WMW) test the result is significant at P < 0.05 (in comparison to control); #According to WMW test the result between columns is significant at P < 0.05 (in comparison to data from 1st day)

thrombin-thrombomodulin complex, aPC (activated protein C) inactivates coagulation factors VIIIa and Va, thereby reducing thrombin generation. On the other hand, aPC cleaves RAR-1 receptors of cells providing an anti-inflammatory and anti-apoptotic action. A decrease of the PC concentration is often a consequence of over-use of this enzyme caused by sporadic thrombin generation or the inflammation response [25].

The level of PC was decreased after irradiation (70 ± 10%) and partly restored on the 9th day after irradiation (87 ± 10%) (Fig. 2). Administration of AC inhibited the drop in the PC concentration after irradiation (86 ± 15%) and accelerated its restoration on the 9th day (103 ± 14%) (Fig. 2). The drop in the concentration of PC in the blood plasma of irradiated rats demonstrates both the inflammatory response and low-grade generation of thrombin. Administration of ES allowed us to prevent such a dramatic decrease of PC and propagation of anti-inflammatory and anti-thrombotic effects.

Platelets. The platelet count demonstrated typical behavior without significant changes on the 1st day after irradiation but shut down on the 9th day after the irradiation ($0.145 \cdot 10^6 \pm 0.04 \cdot 10^6$ 1/µl against $0.3 \cdot 10^6 \pm 0.05 \cdot 10^6$ 1/µl) independently from enterosorption. This irradiation-induced thrombocytopenia is assumed to be one of the main reasons for post-irradiative death [26]. It is caused by impaired thrombocytopoiesis – the process of platelets releasing from megakaryocytes including proliferation, differentiation, maturation, and fragmentation of the latter [27]. Megakaryocytes can be affected by irradiation or application of some drugs [28].

Another consequence of irradiation is irradiation-induced vascular injury. It consists in apoptosis of endothelial cells and disruption of the endothelial surface and is known to influence platelet production [29]. At the same time, endothelial disruptions can contribute to overall irradiation-induced procoagulant actions as well as hemorrhages [30]. They are certainly one of the reasons that causes increased
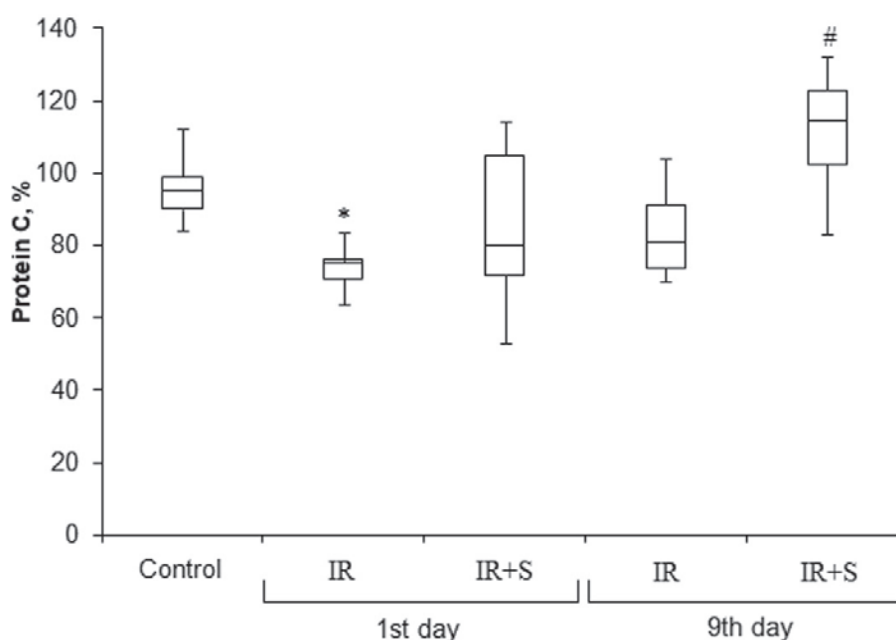

Fig. 2. Effect of irradiation at the dose of 6 Gy on the level of protein C in the blood plasma of irradiated rats (n = 40). Control – the level of protein C in healthy animals including this parameter of blood plasma in healthy animals that received activated carbon (AC) admixed with food 3 days before irradiation and until the end of the experiment. IR – The level of protein C in the blood plasma of irradiated rats on the 1st and 9th day after the irradiation. IR+S – The level of protein C in the blood plasma of irradiated rats that received AC (denoted by "S" for sorbent) admixed with food 3 days before irradiation, on the day of irradiation and after irradiation until the end of the experiment. *According to Wilcoxon-Mann-Whitney (WMW) test the result is significant at P < 0.05 (in comparison to control); #According to WMW test the result between columns is significant at P < 0.05 (in comparison to data from 1st day IR group)
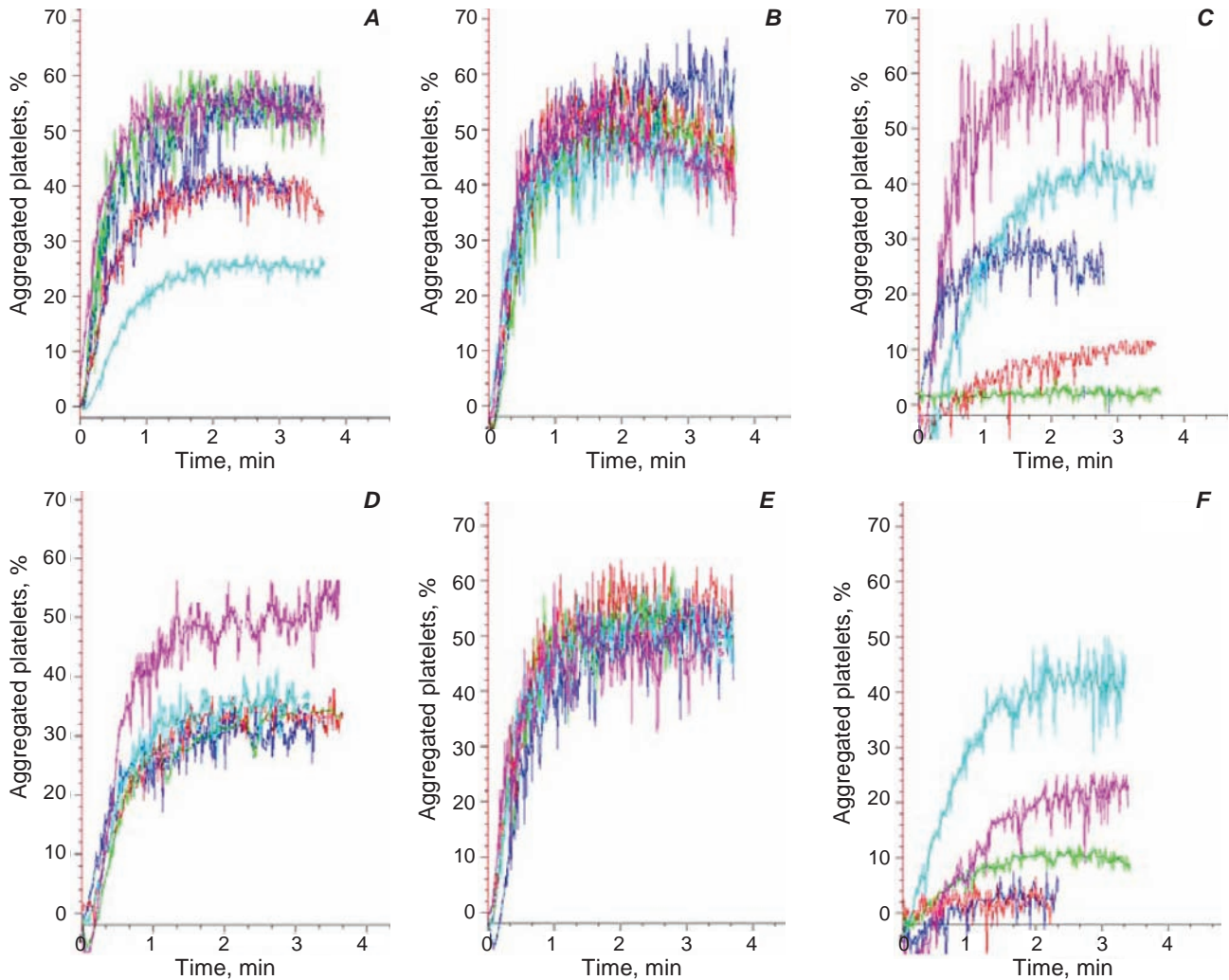
*Fig. 3. Typical aggregation curves of platelets derived from the blood of intact healthy animals (**A**); irradiated rats on the 1st (**B**) and 9th (**C**) days after the irradiation; healthy animals that received activated carbon (AC) (**D**); irradiated rats that received AC on the 1st (**E**) and 9th (**F**) days after the irradiation. Each group contained 5 animals*

pro-coagulant activity of blood after irradiation. Activated or disrupted endothelium can also activate platelets promoting their aggregation [31].

In addition to a slight increase in platelet count during the 1st day after the irradiation (Fig. 3), we detected the propagation of the platelet aggregation rate in both irradiated groups (Fig. 3, panels *B* and *E*). The aggregation rate of control animals was not higher than 50% and unfortunately differed greatly between individual animals within a control group (mean was 43 ± 10%). However, all animals after irradiation had a platelet aggregation rate above 50% (mean was 54 ± 4%). Panels *A-B*, *D-E* in Fig. 3 clearly demonstrate this remarkable tendency.

On the 9th day after irradiation we detected the visible result of thrombocytopenia (Fig. 3, panels *C*

and *F*). The rate of platelet aggregation in control groups was 40 ± 11%, most of the animals in both irradiated groups demonstrated greatly impaired platelet aggregation – mean rate was 24 ± 20%.

Thus platelet irradiation caused the increase of platelet reactivity on the 1st day, increased the total pro-coagulant consequences, and resulted in thrombocytopenia in the later stages of ARS. Surprisingly, we did not observe any effects of administration of ES on the irradiation-induced number or rate of aggregation of platelets.

Functionally inactive forms of prothrombin. Observing the evidence of disseminated intravascular coagulation (changes of platelet reactivity and blood clotting time) and inflammatory process (higher fibrinogen concentrations, drop of PC level)

we could not avoid the characterization of thrombin as the enzyme that is located at the crossroads between coagulation and inflammation [32, 33]. Thrombin is formed from the inactive precursor prothrombin as a result of a chain of hydrolytic reactions of the coagulation cascade [34].

Apart from its multiple functions thrombin can also act directly on prothrombin forming prethrombin [35]. The generation of this autolytical form is strongly correlated with the amount of active thrombin that appeared (Fig. 4). Previously its accumulation in blood plasma during pathologies was demonstrated [36].

Another functionally inactive form of prothrombin is uncarboxylated prothrombin that is formed in the case of liver disease that affects the process of carboxylation of prothrombin among other vitamin K-dependant proteins [37].

Previously we described the effective and useful method of prethrombin and uncarboxylated prothrombin detection in blood plasma. It consists in simultaneous application of the commonly used prothrombin test based on activation of the full-length molecule by exogenous thromboplastin and the ecamulin test based on application of prothrombin activator from the venom of *Echis multisquamatis*. Results are presented as the PI for prothrombin activation by thromboplastin, and as the EI for pro-

thrombin activation by ecamulin. The difference between PI and EI indicates the accumulation of FIFPs.

Analysis on the 1st day after irradiation showed the appearance of FIFPs in the blood plasma of self-recuperative rats without ES (Fig. 5).

As far as the half-life of prothrombin in blood plasma being more than 48 hours [38], this finding cannot be explained by uncarboxylated prothrombin formation. The detected difference between PI and EI values on the 1st day after irradiation indicates prethrombin generation and gives evidence of thrombin formation with subsequent low-grade inflammation and induction of coagulation. It was found that thrombin generation was greatly suppressed by enterosorption that acted by complete inhibition of prethrombin generation – no difference between PI and EI was observed in the group of irradiated rats that received ES (Fig. 5).

On the other hand, the detection of FIFPs on the 9th day after irradiation is probably due also to the accumulation of decarboxy-prothrombin (Fig. 5). This effect can be observed during liver injury caused by inflammation or the action of toxic agents. As it was demonstrated, administration of ES diminished this harmful effect (Fig. 5).

Thus we detected the accumulation of FIFPs that indicated thrombin generation on the 1st day and liver disease on the 9th day after irradiation. Study
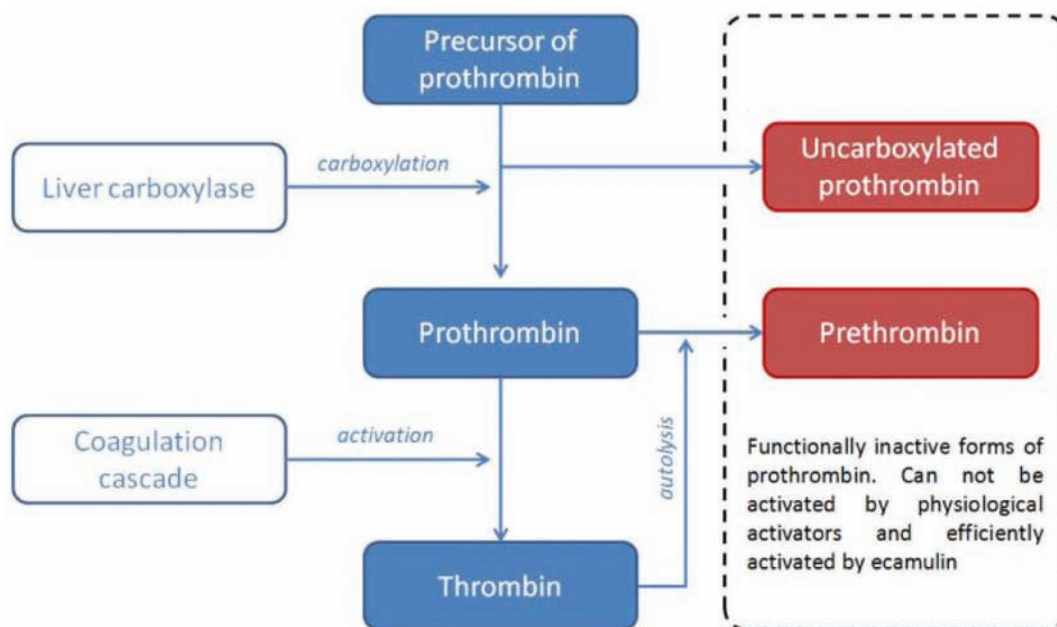

Fig. 4. Scheme of formation of functionally inactive forms of prothrombin (FIFPs): uncarboxylated prothrombin and prethrombin that cannot be activated by physiological activators in the coagulation cascade or under the action of thromboplastin, but can be efficiently activated by non-physiological activators (e.g. ecamulin)
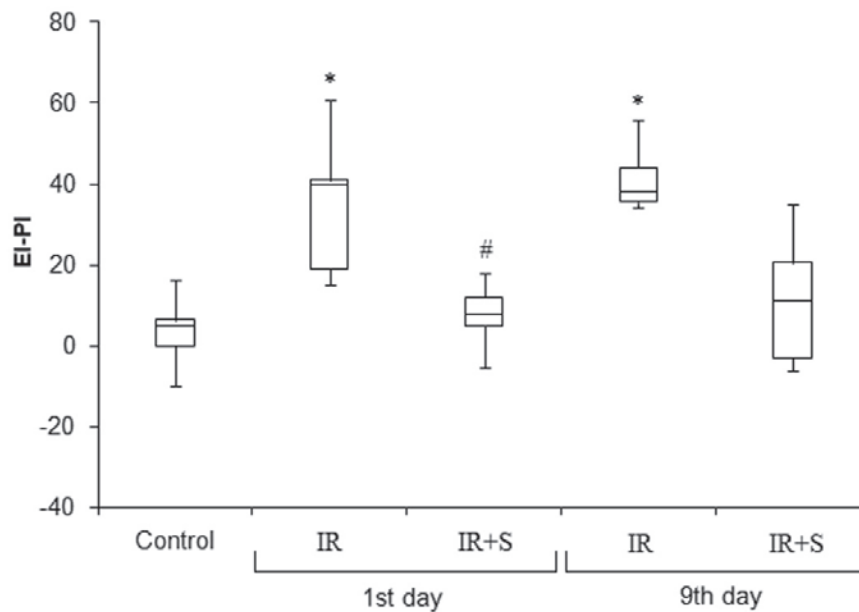
Fig. 5. Differences between the prothrombin index (PI) and ecamulin index (EI) for blood plasma of irradiated rats and irradiated rats that had received activated carbon (AC) (n = 40). PI and EI were calculated by the formulas: EI = Ap/An; PI = Ap/An, where An is the blood plasma thrombin activity of a healthy control animal; Ap is the blood plasma thrombin activity of an exposed animal. Control – the PI-EI of healthy animals including this parameter of blood plasma in healthy animals that received activated carbon (AC) admixed with food 3 days before irradiation and until the end of the experiment. IR – The PI-EI in the blood plasma of irradiated rats on the 1st and 9th day after the irradiation. IR+S – The PI-EI in the blood plasma of irradiated rats that received AC (denoted by "S" for sorbent) admixed with food 3 days before irradiation, on the day of irradiation and after irradiation until the end of the experiment. *According to Wilcoxon-Mann-Whitney (WMW) test the result is significant at P < 0.05 (in comparison to control); #According to WMW test the result between columns is significant at P < 0.05 (in comparison to data from 1st and 9th day IR group)

of the FIFPs allowed us to detect anti-inflammatory and hepatoprotective properties of the AC. Simultaneous application of the PI and EI was shown to be extremely sensitive for the detection of radiation-induced changes in the blood coagulation system.

Conclusions. Characterization of the hemostasis system of rats that were exposed to a semilethal dose of X-rays allowed us to select parameters that can be used for monitoring of ARS development. Apart from basic coagulation tests (APTT) and the measurement of platelet aggregation, fibrinogen and protein C level we can recommend the determination of FIFPs as a useful tool for estimation of the hemostasis response after irradiation with X-rays. This test indicates the intravascular thrombin generation and can help predict thrombotic complication or disseminated intravascular coagulation. Determination of FIFPs in blood plasma of irradiated rats allowed us to study the enterosorption effect on the develop-

ment of irradiation-induced changes. It was shown that enterosorption with AC prevented accumulation of FIFPs which appears to be a newly discovered anti-thrombotic effect of therapy with AC.

ПАРАМЕТРИ ЗСІДАННЯ ПЛАЗМИ КРОВІ ЩУРІВ ІЗ ГОСТРОЮ ПРОМЕНЕВОЮ ХВОРОБОЮ ЗА УМОВ ЕНТЕРОСОРБЦІЇ

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Радіаційно-індукована коагулопатія (РІК) є однією з основних причин смерті під час гострої променевої хвороби (ГПХ). Метою цієї статті було охарактеризувати реакцію системи гемостазу на ГПХ помірного рівня на 1-шу і 9-ту добу після опромінення, виявити молекулярні маркери системи зсідання крові, які найбільше постраждализаГПХ,таоцінитиефектентеросорбції на розвиток індукованих опроміненням змін. Рівень агрегації тромбоцитів, активований частково тромбопластиновий час (АЧТЧ) і концентрацію фібриногену визначали стандартними методами. Рівень протеїну С (ПС) вимірювали за допомогою хромогенного субстрату S2366 (p-Glu-Pro-Arg-pNa) та ензиму-активатора з отрути *Agkistrodon halys halys.* Функціонально неактивні форми протромбіну (ФНФП) визначали, використовуючи паралельно два активатори – тромбопластин та активатор протромбіну з отрути *Echis multisqumatis.* Щури обох опромінених груп мали підвищений ризик внутрішньосудинного зсідання крові порівняно з обома контрольними групами. Виявлено статистично вірогідне скорочення часу зсідання крові в тесті АЧТЧ (24 ± 4 с порівняно з 33 ± 5 с) і підвищення концентрації фібриногену (4,2 ± 0,6 мг/мл порівняно з 3,2 ± 0,3 мг/мл). Обидва параметри нормалізувалися на 9-ту добу після радіаційного опромінення. Однак кількість тромбоцитів зменшувалася (0,3·10⁶ ± 0,05·10⁶ 1/мкл порівняно з 0,145·10⁶ ± 0,04·10⁶ 1/мкл) внаслідок порушення функції мегакаріоцитів. Рівень ПС знижувався після опромінення (70 ± 10%) і частково відновлювався на 9-ту добу (87 ± 10%). Введення щурам активованого вугілля (АВ) запобігало зниженню концентрації ПС після опромінення (86 ± 15%) і прискорювало її відновлення на 9-ту добу (103 ± 14%). Статистично вірогідне накопичення ФНФП було виявлено в плазмі крові опромінених щурів (група 1) на обох досліджуваних етапах. ФНФП не були виявлені у жодного з опромінених щурів, які отримували АВ (група 2). Характеристика системи гемостазу щурів, які піддавалися напівлетальній дозі опромінення, дозволила вибрати параметри, які можуть бути використані для моніторингу розвитку ГПХ. Крім основних коагуляційних тестів (АЧТЧ), вивчення агрегації тромбоцитів, рівня фібриногену та протеїну С, виявлення ФНФП може бути рекомендованим як дієвий інструмент для оцінки відповіді системи гемостазу на рентгенівське опромінення. Поява ФНФП свідчить про внутрішньосудинне утворення тромбіну і може вказувати на тромботичні ускладнення або дисеміноване внутрішньосудинне зсідання. Визначення ФНФП у плазмі крові опромінених щурів дозволило вивчити ефект ентеросорбції на розвиток змін, спричинених опроміненням. Показано, що ентеросорбція АВ перешкоджає накопиченню ФНФП. Антитромботичний ефект терапії АВ виявлено вперше. Також встановлено, що ГПХ спричинює активацію системи гемостазу, утворення тромбіну (виявлене за рахунок утворення ФНФП), незначне запалення (на що вказує зниження концентрації ПС) і тромбоцитопенію. Ентеросорбція АВ мінімізує запальні та прокоагулянтні процеси, спричинені помірною дозою опромінення. Накопичення ФНФП можна вважати одним із найчутливіших маркерів відповіді системи зсідання крові на опромінення.

К л ю ч о в і   с л о в а: ентеросорбція, активоване вугілля, тромбоцити, зсідання крові, гострий променевий синдром помірного рівня.

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