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#### INDICATORS OF COAGULATION HEMOSTASIS AND DEGREE OF BLOOD OXYGENATION IN RATS UNDER THE OPIOID INFLUENCE IN THE LATE TERMS OF CORRECTION

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A long-term use of opioid analgesics is the cause of hematological changes that lead to trophicity and oxygenation disorders of tissues and organs of various body systems. In the study 50 white, sexually mature rats (160–270 g) were used. With the help of generally accepted methods, we investigated the dynamics of changes in hematological indicators of coagulation hemostasis and the degree of oxygenation of the blood of rats in the chronic period of opioid exposure and during correction. Correction was carried out with the Pentoxifylline. The results of our study significantly expand the data on the negative influence of opioids on the body during their long-term use and testify that it leads to the development of significant changes in coagulation hemostasis indicators (number of platelets, prothrombin time, prothrombin index, whole blood recalcification time, total fibrinogen, hemoglobin, hematocrit), which leads to impaired vascularization and progression of the phenomenon of dyscirculatory hypoxia.

Key words: rheology change, oxygenation disorder, opioid analgesic, rat, chronic period, correction.

# €.В. Пальтов, З.З. Масна, Ю.Я. Кривко, К.І. Войценко, А.Р. Вергун ПОКАЗНИКИ КОАГУЛЯЦІЙНОГО ГЕМОСТАЗУ ТА СТУПІНЬ ОКСИНГЕНАЦІЇ КРОВІ У ЩУРІВ ПІД ВПЛИВОМ ОПІОЇДІВ НА ПІЗНІХ ТЕРМІНАХ КОРЕКЦІЇ

Тривале застосування опіоїдних анальгетиків є причиною гематологічних змін, що призводять до порушення трофіки та оксигенації тканин і органів різних систем організму. Матеріалом для дослідження була мікропопуляція 50 статевозрілих безпородних щурів-самців віком 4,5–7,5 місяців масою 160–270 г. За допомогою загальноприйнятих методів досліджено динаміку змін гематологічних показників коагуляційного гемостазу та ступеня оксигенації крові щурів у хронічному періоді опіоїдної дії та під час корекції. Корекцію проводили пентоксифіліном. Результати наших досліджень значно розширюють дані щодо негативного впливу опіоїдів на організм при їх довготривалому застосуванні та свідчать про те, що це призводить до розвитку значних змін показників коагуляційного гемостазу (кількість тромбоцитів, протромбіновий час, протромбіновий індекс, час рекальцифікації цільної крові, загальний фібриноген, гемоглобін, гематокрит), що призводить до порушення васкуляризації та прогресування явища дисциркуляторної гіпоксії. Ключові слова: зміна реології, порушення оксигенації, опіоїдний анальгетик, щур, хронічний період, корекція.

ключові слова. зміна реології, порушення оксигенації, опогдний аналыстик, щур, хронічний період, корекція.

The work is a fragment of the research project "Morphofunctional features of organs in pre- and postnatal periods of ontogenesis, under the influence of opioids, food supplements, reconstructive surgery and obesity", state registration No. 0120U002129.

According to the statistics of the European Monitoring Center for Drugs and Drug Addiction, there are about 275 million people aged 15 to 64 in the European region who use strong drugs at least once a year. [1, 13]. According to narcological studies, the prevalence of mental and behavioral disorders, primarily mental and behavioral disorders due to PAS use, is 41.7 % per 100,000 population [1]. According to the latest biobehavioral and psychiatric studies in the government-controlled territory of Ukraine, the estimated number of people who use injection drugs was 317,000, of which 200,661 (63.3 % of the total) were opioid users alone [3]. Opioid addiction and secondary pathological changes in addicts are not only a psychiatric and narcological problem, but also a secondary therapeutic, general surgical, angiological and even ophthalmological problem etc. In recent years, publications of experimental studies of opioid drugs are increasingly appearing in domestic and foreign sources. In particular, scientific publications highlight data on the pathology of the structures of the organ of vision, in particular, visual organ relating regarding to the iris-corneal angle [2, 4, 10, 11].

Taking into account the above, we believe that this study is relevant both from the point of view of experimental morphology and from the point of view of practical ophthalmology. A number of questions remain open regarding the comparison of retinal angiopathy indicators with changes in blood rheological indicators at the late stages of correction of experimental opioid retinal angiopathy.

**The purpose** of the study was to establish the specificity of a changes in the dynamic indicators of coagulation hemostasis and the dynamics of biochemical deviations in the experiment during opioid exposure and in the its correction of some later stages.

**Materials and methods.** The study material was sexually mature, outbred male rats in the amount of 50 animals weighing 160–270 g, aged 4.5–7.5 months. Animals were injected with Nalbufine intramuscularly, daily 1 time a day in one time interval (10–11 a.m.) for 70 days. The initial dose of Nalbufine for the first 2 weeks was 0.212 mg/kg, with the following dynamics: II–IV weeks – 0.225 mg/kg, IV–VI weeks – 0.252 mg/kg, VI–VIII weeks – 0.260 mg/kg, VIII–X weeks – 0.283 mg/kg. Thus, conditions of chronic opioid exposure were created [5].

The animals were divided into 5 groups. The 1st group of animals received Nalbufine for 70 days with subsequent collection of study material (end of the 10th week of experimental opioid exposure); the 2nd control group, which for 70 days received injections of saline solution intramuscularly in one time interval (10-11 am); the 3rd group – 6 weeks of Nalbufine administration with subsequent withdrawal at the 4th week; the 4th group – 6 weeks of Nalbufine administration with the addition of Pentoxifylline at the 4th week; the 5th group – 6 weeks of administration of opioid with withdrawal followed by 4 – week administration of Pentoxifylline. Correction was performed at a late stage, the dose of Pentoxifylline was 2.857 mg/kg.

All animals were kept in a vivarium and work related to maintenance, care, labeling and other manipulations was carried out in compliance with the provisions of the "European Convention for the protection of vertebrates used for experimental and other scientific purposes" [Strasbourg, 1985], "General ethical principles of animal experiments", adopted by the First National Congress on Bioethics [Kyiv, 2001]. The Commission on Bioethics of the Danylo Halytsky Lviv National Medical University found that the conducted scientific research meets ethical requirements in accordance with the order of the Ministry of Health of Ukraine No. 231 of 01.11.2000 (Protocol No. 10 of 26.12.2011). Blood collection and hematological examination indicators (platelet count, prothrombin time, prothrombin index, whole blood recalcification time, total fibrinogen, hemoglobin determination, hematocrit value) were performed according to generally accepted methods Baluda V.P. (1980).

R V 4.0.3 and R Studio v 1.2.5042 software were used to perform statistical counting and create plots. [14, 15]. At the first stage of statistical analysis, all the results obtained from studies of animal blood counts have been screened to meet normal blood values using the Shapiro-Wilk test. Based on the results of this test, as well as taking into account the small volume of samples and the discrete nature of partial data due to the peculiarities of laboratory methods used to obtain them, the median and quartiles in the format Me [25 %; 75 %] were used for representation, where Me is the median (50th percentile), 25 % is the first quartile (25th percentile), 75 % is the third quartile (75th percentile) Serdyuk A.M. (2010) For graphical representation, we used boxes (box-and-whiskers), the central line of which indicated the median, the box borders - quartiles, the whiskers – 1.5 range from the corresponding quartile and the points – outliers (values lying outside the 1.5 range) [12]. In addition, the non-parametric Kruskal-Wallis H test was used to determine the validity of differences between groups for three or more independent groups, followed by post-hoc analysis using Dunn's test. In order to better interpret the data obtained and compare different blood parameters of animals in the dynamics of the experiment, line plots of decimal logarithms of the median values of indicators were used.

Results of the study and their discussion. As a result of the correction performed in the period of the 7th, 8th, 9th and 10th week of opioid exposure, the indicators of the prothrombin blood index of rats of all subgroups at the 10th week were significantly higher than the values of the control group and slightly ranged from 97.38 [91.99; 107.93] in the "Pentoxifylline+Nalbufine" subgroup to 99.50 [99.00; 100.00] in the "withdrawal" subgroup. Such indicators were as close as possible to the indicator of the blood prothrombin index in animals of the general subgroup of the 10th week, which was 99.00 [94.00; 100.00], without having a statistically significant difference in comparison with it. (Fig. 1A). Indicators of recalcification time in the animal subgroups, "withdrawal" and "Pentoxifylline+Nalbufine" at the 10th week of the experiment were as close as possible to the value of this indicator in animals of the general subgroup and amounted to 26.50 [24.25; 27.00] and 15.00 [14.25; 15.75], respectively. While the value of the recalcification time in the "withdrawal+Pentoxifylline" subgroup was 45.94 [44.05; 46.70], which was significantly higher than the value of this index both in other experimental subgroups and in the general group of animals at the 10th week. At the same time, the studied indicator was close to the value of the recalcification time of animals of the control group (49.50 [48.25; 50.00], p=0.46). (Fig. 1B). Dynamics in index'es subgroups of total fibrinogen at the 10th week, it was similar to that in most biochemical parameters. In the subgroup "withdrawal+Pentoxifylline", the level of total fibrinogen in the blood of animals was 2.00 [1.93; 2.00], which was as close as possible to the control group (1.75 [1.62; 1.84]) and had no statistically significant difference (p=0.31). In the "Pentoxifylline+Nalbufine" subgroup, the level of total blood fibrinogen was 2.23 [2.08; 2.68], and in the "withdrawal" subgroup, it was the highest among all subgroups of this period and was 2.80 [2.73; 2.90]. This increase compared to the control group was confirmed statistically (p<0.01). (Fig.1C).

Dynamics in Indicator's subgroups of prothrombin time at the 10th week, it was also similar in most biochemical parameters. Thus, in the subgroup "withdrawal+Pentoxifylline", this indicator was 14.50 [14.00; 15.00], which was the highest among all subgroups at this period and statistically did not differ from the control group (16.76 [16.12; 17.00]). At the same time, this indicator in the subgroups "Pentoxifylline+Nalbufine" and "withdrawal" was 12.00 [12.00; 13.00] and 12.85 [11.31; 14.39], respectively, which was significantly less than the control group. (Fig. 1D).



Fig.1. A – Changes in prothrombin index when corrected during the 7th, 8th, 9th, and 10th weeks of opioid exposure. B – Changes in blood recalcification time index when corrected during the 7th, 8th, 9th, and 10th weeks of opioid exposure. C – Changes in the total fibrinogen index when corrected during the 7th, 8th, 9th, and 10th weeks of opioid exposure. D – Changes in prothrombin time index when corrected during the 7th, 8th, 9th, and 10th weeks of opioid exposure.

Like other metrics, the blood haemoglobin contents in subgroups of the 10th week was closest to the value of the control group in the subgroup "withdrawal+Pentoxifylline" – 130.50 [130.00; 132.00] (control group 151.89 [150.00; 153.53], the difference is not reliable, p=0.35). In other subgroups, the hemoglobin index was significantly lower and amounted to 119.00 [119.00; 120.00] in the "Pentoxifylline+Nalbufine" subgroup and 124.00 [124.00; 125.00] in the "withdrawal" subgroup. Note that the hemoglobin index in these subgroups was close to the value of the general group at Week 10, which was 118.87 [114.94; 124.43]. (Fig. 2A). In subgroups of the 10th week, the lowest number of platelets was in the subgroup "withdrawal+Pentoxifylline" – 177.00 [175.25; 178.00]. Like other blood parameters, this value was close to the control group and had no significant difference compared to it (control group 153.05 [142.91; 154.64], p=0.24). In other subgroups, the platelet count was significantly higher than in the control group and was 196.50 [195.25; 197.75] in the "Pentoxifylline+Nalbufine" subgroup and 209.50 [182.50; 210.00] in the "withdrawal" subgroup. These indicators were also close to the number of platelets in the general group of Week 10 – 213.00 [210.00; 214.00]. (fig. 2B). Indicator of blood hematocrit in rats of the "withdrawal+Pentoxifylline" subgroup was close to the control group and amounted to 44.00 [43.00; 44.00] (control group 42.07 [40.13; 43.75], p=0.54). While the hematocrit level in other subgroups was significantly lower than the value of this indicator in the control group -25.00 [23.50; 26.00] in the "Pentoxifylline+Nalbufine" subgroup and 33.50 [33.00; 34.00] in the "withdrawal" subgroup, respectively. In these subgroups, the hematocrit level was close to the value of this indicator in animals of the general group at Week 10 – 27.00 [27.00; 28.00] (Fig. 2C).

The tendency to approach the value of the control group of most indicators at this period in the subgroup "withdrawal+Pentoxifylline" is exponential, which is indicated by a red arrow on separate graphs. In the remaining subgroups, the values were close to those in the animals of the general group of the 10th weeks.







Fig.2. A – Changes in hemoglobin index when corrected during the 7th, 8th, 9th, and 10th weeks of opioid exposure. B – Changes of platelet count when corrected during the 7th, 8th, 9th, and 10th weeks of opioid exposure. C – Changes of hematocrit value when corrected during the 7th, 8th, 9th, and 10th weeks of opioid exposure.

The works of numerous authors are devoted to the study of structural changes that occur in various organs and tissues of the body with prolonged opioid use [2, 4, 10, 11]. However, we have not found any data on changes in rheological parameters of blood, in particular, the dynamics of components of coagulation hemostasis and the degree of blood oxygenation against the background of prolonged opioid exposure in available literature references. However, the results of experimental and clinical studies convincingly show that disorders in the hemostatic system that develops against the background of various pathological conditions are one of the main pathogenetic mechanisms that contribute to the progression of numerous diseases, complicate their course and treatment [6–9]. That is why the results of our research significantly complement the data on the exposure of opioid drugs to the body, convincingly certifying that their long-term use also leads to the development of significant changes in the indicators of coagulation hemostasis – platelet count, prothrombin time, prothrombin index, time of recalcification of whole blood, total fibrinogen, hemoglobin and hematocrit value. In our opinion, this approach provides for study of the risks of bleeding and thromboembolic complications in people who use opioid drugs for a long time, and the data obtained can become a theoretical basis for correcting their treatment and developing preventive activities.

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Changes in indices in the "withdrawal+Pentoxifylline" subgroup were more systemic at the10th week – in terms of value, the indicators of this subgroup in most cases were as close as possible to the values of the corresponding indicators of the control group. The only exceptions were the indicators of the prothrombin index – in all subgroups, its value fluctuated at the level of the general group of Week 10 and was higher than the value of the control group. The remaining indicators at this time in the "withdrawal" and "Pentoxifylline+Nalbufine" subgroups did not have a significant difference compared to the general group at the 10th week, both in absolute values and in the results of verification using statistical criteria.

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## PECULIARITIES OF THE PROJECTILE PENETRATION DEPTH WHEN FIRED WITH "FORT 9R" AND "FORT 17R" PISTOLS WHILE USING DIFFERENT CLOTHING FABRIC

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The study of the protective qualities of various types of clothing when fired from non-lethal firearms, which are mainly used by law enforcement agencies, is one of the promising directions of the so-called humanitarian direction of forensic medicine. The conducted experimental research with the use of human body simulators covered with different types of fabric and "Fort 9R" and "Fort 17R" pistols made it possible to establish that at close-range shot distances of 50 cm and any type of fabric, a wound channel is formed to a depth of at least 1 cm; at the same time, "Fort 9R" in any case does not cause the formation of a wound channel longer than 6 cm, and "Fort 17R".

Key words: gunshot wound, gunshot injury, gunshot weapon, elastic balls, different fabrics.

## А.М. Перебетюк, В.І. Гунас, Л.В. Фоміна, В.Ф. Зверховська, С.В. Прокопенко ОСОБЛИВОСТІ ГЛИБИНИ ПРОНИКНЕННЯ СНАРЯДУ ПРИ ПОСТРІЛАХ З ПІСТОЛЕТІВ «ФОРТ 9Р» ТА «ФОРТ 17Р» ПРИ ЗАСТОСУВАННІ РІЗНИХ МАТЕРІАЛІВ ТКАНИНИ ОДЯГУ

Вивчення захисних властивостей різних видів одягу при пострілах з нелетальної зброї, що перебуває на озброєнні переважно органів правопорядку є одним з перспективних напрямків так званого гуманітарного напрямку судової медицини. Проведене експериментальне дослідження з застосуванням імітаторів тіла людини вкритих різним видом текстильного матеріалу та пістолетів «Форт 9Р» та «Форт 17Р» дозволило встановити, що при дистанціях пострілу впритул – 50 см та будь яких видів текстильного матеріалу утворюється рановий канал на глибину щонайменше 1 см; в той же час «Форт 9Р» в будь якому випадку не викликає утворення ранового каналу довжиною більше 6 см, а «Форт 17Р» глибиною більше 5 см; бавовняна тканина має кращі захисні властивості при пострілах з «Форт 9Р», а шкірозамінник при пострілах з «Форт 17Р». Ключові слова: вогнепальна рана, вогнепальні ушкодження, вогнепальна зброя, еластичні кулі, різні тканини.

The study is a fragment of the research project "Characteristics of damage to human body tissue simulators caused by non-lethal weapons", state registration No. 0121U107924.

Among all types of firearms, non-lethal firearms are still the least researched. Its appearance was primarily due to the need of arm law enforcement agencies for an effective means that can stop offenders but at the same time not cause serious physical harm. In addition, in the future, such types of weapons became available to a wider range of people, in particular, representatives of journalistic professions, judicial bodies, etc.

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