EVALUATION OF THE USE OF OZONE THERAPY IN TREATMENT OF INFLAMMATORY PROCESSES IN DIABETES MELLITUS IN AN EXPERIMENT

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Despite some advances in the treatment of Diabetic patients with severe localized wound infection, surgical treatment results can not be considered satisfactory [2,7,10]. Resulting experience data in the medical practice on physical factors application for purpose of influence reparative processes stimulation associated with targeted pharmacotherapy, confirm the correctness of the search attempts. Ozonotherapy have been widely used for this purpose in recent years [1,8,11].

According to the data of the authors [2,3,6,12], local influence of ozone therapeutic concentrations during 3-7 days [7], and ozone-containing medications possess an extremely strong bacterial and fungicidal, immunomodulating, anti-inflammatory, virolytic, cytostatic, analgesic properties and open up new prospects for the use of ozone for the treatment of different inflammatory processes of various types. But the problem consists in that fact that purulent inflammation of soft tissues in patients with diabetes is diagnosed 20 times more frequently than those without a history of diabetes. Purulent infection rate is 10-25% [2,3,6]. It is known that the indicia of the blood coagulation system play an essential role in the wounds healing in the surgical treatment [1,5-7]. Special studies devoted to the influence of ozone on the blood coagulation system, as a factor affecting the wound healing in diabetes mellitus with purulent-inflammatory complications, have not been found in the available scientific literature. Hence, it is important to study the ozone influence on blood clotting in diabetes with pyoinflammatory processes [2-4,6].

Objective of the research is to study the effect of ozone therapy on the course of purulent-inflammatory processes in modeled diabetes.

Material and methods. Experimental studies were carried out on 30 white old rats, weight 300-450 gr, aged 24-30 month. The first group contained the rats, which received fecal suspension introduction (10 rats), rats of the second group received fecal suspension and modeled diabetes was simulated (10 rats), in the third group diabetes was modeled after them, the rats received fecal suspension and ozone was also used (10 rats). Diabetes was simulated by means of subcutaneous injection of alloxan (100 mg per kg of body weight). Animals of the control group (10 rats) in the same weight and age were subcutaneously administered 0,9% sterile sodium chloride solution (1,0 ml per 100g of body weight). On the 14th day after alloxan administration the animals were subcutaneously injected 10% fecal suspension (stool pool from 20 animals in 0,9% sodium chloride solution) at a dose of 0,5 ml per 100g of body weight. Rats of the first studied group received a subcutaneous injection of fecal suspension. The animals of the second group (10) were subcutaneously injected a fecal mixture and thereafter non-ozonized 0,9% sodium chloride solution (1,0ml per 100g of body weight) was administered intraperitoneally for three days once daily.

Ozonation of sterile 0,9% sodium chloride solution was performed using "Boson" apparatus with ozone concentration in isotonic sodium chloride solution 20 mg/ml. 10 minutes after ozonation isotonic sodium chloride solution was administered intraperitoneally to rats of the third test group (10) once a day during three days. Three days later, under ether anesthesia a laparotomy was performed. Blood sampling was carried out with a silicone syringe from the abdominal aorta (stabilizer – 3,8% sodium citrate solution). Condition of platelet-vascular hemostasis was assessed by the percentage of adhesive platelets (norm in human 180-360 *109, in rats to 368) and by the index of spontaneous platelet aggregation. Total coagulation potential of the blood (plasma recalcification, prothrombin and thrombin time, activated partial thromboplastin time), Hageman-dependent fibrinolysis, potential plasminogen activity, fibrinogen level in the blood plasma, antithrombin-III activity in blood was determined by reagent kits of "Simko Ltd" firm (Ukraine). During the study of the antithrombin activity diluted citrate plasma was incubated with a standard amount of thrombin with activity of 10 NIH/mL (part of thrombin combines with antithrombin). Norm condition of the regulation system of blood aggregation state in old rats: plasma recalcification time(101,5±2,38), activated partial thromboplastin time(42,5±2,32), prothrombin time(16,4±1,38), thrombin time(14,5±0,97), fibrinogen concentration in blood plasma(4,48±0,179), antithrombin-III activity(98,2±3,53), factor XIII of blood coagulation activity(94,9±3,74), percentage of adhesive thrombocytes(2,69±0,362), index of spontaneous platelet aggregation(28,3±1,91), hageman dependent fibrinolysis(16,9±0,68), potential plasminogen activity(15,8±1,26).

Results and their discussion. Assessment of the blood aggregation system in terms of purulent processes of the skin and subcutaneous tissue in aged rats detected a growth of percentage of adhesive platelets, Hageman dependent fibrinolysis and antiplasmin concentration (Table 1).
It has been found that diabetes in human causes mosaic disorders of the hemostatic system in the presence of purulent processes. An increase in plasma recalcification time, activated partial thromboplastin time, adhesive platelets, index of spontaneous platelet aggregation, potential plasminogen activity, reducing of prothrombin time, fibrinogen concentration in the blood plasma, activity of clotting factor XIII, Hageman dependent fibrinolysis have been noted.

Table 1. Blood aggregation state under pyoinflammatory processes of soft tissues in alloxan-modeled diabetes against the background of ozone therapy in 24-30 month rats, weight 300-450 gr

Factors	FS introduction I group (n=10)	FS introduction + simulated diabetes II group (n=10)	FS introduction + simulated diabetes + ozone III group (n=10)
Plasma recalcification time, sec	77,5±4,12	103,8±4,26 d 1-2 <0,05	70,9±4,31 d 2-3 <0,05
Activated partial thromboplastin time, sec	37,1±2,62	51,3±4,41 d 1-2 <0,05	25,8±1,37 d 2-3 <0,05 d 1-3 <0,05
Prothrombin time, sec	23,5±1,74	16,5±0,91 d 1-2 <0,05	10,5±0,63 d 2-3 <0,05 d 1-3 <0,05
Thrombin time, sec	13,4±0,89	15,8±1,02	9,74±0,64 d 2-3 <0,05 d 1-3 <0,05
Fibrinogen concentration in blood plasma, g/L	3,79±0,19	2,04±0,07 d 1-2 <0,05	3,40±0,17 d 2-3 <0,05
Antithrombin-III activity, %	91,8±3,51	68,6±3,51 d 1-2 <0,05	102,6±5,60 d 2-3 <0,05 d 1-3 <0,05
Factor XIII of blood coagulation activity, %	88,1±3,03	71,6±5,29 d 1-2 <0,05	99,5±6,37 d 2-3 <0,05 d 1-3 <0,05
Percentage of adhesive thrombocytes	23,6±1,68	51,7±2,78 d 1-2 <0,05	14,8±0,78 d 2-3 <0,05 d 1-3 <0,05
Index of spontaneous platelet aggregation	31,7±3,38	59,7±1,69 d 1-2 <0,05	51,9±3,25 d 1-3 <0,05
Hageman dependent fibrinolysis, m	15,6±0,72	10,0±0,89 d 1-2 <0,05	7,11±0,79 d 2-3 <0,05 d 1-3 <0,05
Potential plasminogen activity, m	15,7±1,19	22,0±1,52 d 1-2 <0,05	7,94±0,55 d 2-3 <0,05 d 1-3 <0,05

note: FS – fecal suspension; d 1-2 – difference between the first and the second experimental groups; d 2-3 – difference between the second and the third experimental groups; d 1-3 – difference between the first and the third experimental groups

Ozone administration in the presence of ulcers of soft tissues against the background of diabetes in old rats was not accompanied by a significant improvement of regulation indices of blood aggregation state. An increase in manifestations of hypercoagulation have been noted: decrease in plasma recalcification time, activated partial thromboplastin time, prothrombin time, thrombin time, Hageman dependent fibrinolysis, potential plasminogen activity. In addition, fibrinogen concentration in plasma and the activity of coagulation factor XIII were increasing. At the same time, ozone therapy improved the condition of the vascular-platelet hemostasis, growth of antithrombin (Table 1).

The introduction of ozonated sodium chloride solution in purulent processes of soft tissues against the background of diabetes in old rats did not contribute to the protective properties; that was indicated by the growth of azoalbumin lysis, azocasein, total fibrinolytic activity and proteinase activity by Kunitz in blood plasma. Ozone therapy led to an increase in the degree of intoxication provided there were ulcers in diabetic old rats, it was indicated by the growth of the concentration of molecules of average weight (Table 2).

Thus, the application of ozone therapy under conditions of presence of soft tissue ulcers in diabetic old rats did not show any significant protective properties with reduced azoalbumin lysis, total non-enzymatic fibrinolytic activity and proteinase activity by Kunitz in blood plasma.

Such changes in the aged rats can be estimated as an exacerbation of purulent- inflammatory process, an increase in toxicity, reduced protective properties, an increase in hypercoagulation manifestations against the background of chronic diabetes caused by age-related changes.

Table 2. Unlimited proteolysis system, fibrinolytic pancreatic activity under conditions of purulent-inflammatory processes of soft tissues in alloxan-modeled diabetes against the background of ozone therapy in 24-30 month rats, weight 300-450 gr rats (x±Sx)

Indices	Fecal suspension introduction (n=10)	Fecal suspension introduction against the background of ozone therapy (n=10)
Azoalbumin lysis, mg/g ·time	28,5±4,31	28,9±4,21
Azocasein lysis, mg/g ·time	37,3±4,76	47,6±4,22
Azocollagen lysis, mg/g ·time	23,6±2,87	24,2±2,66
Total fibrinolytic activity, mg/g ·time	20,0±3,02	21,4±2,67
Non-enzymatic fibrinolytic activity, mg/g time	7,82±1,01	6,51±0,87
Proteinase activity by Kunitz	1,40±0,18	1,23±0,12

Conclusions 1. The use of ozone therapy in conditions of chronic inflammatory processes diabetic old rats with alloxan-modeled diabetes did not manifest protective properties on the hemostasis system and proteolysis of blood plasma.
2. Ozone therapy enhances intoxication manifestations against the background of diabetes mellitus with purulent-inflammatory processes in old rats, causing the need for cautious use of this method of treatment.

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SUMMARY

EVALUATION OF THE USE OF OZONE THERAPY IN TREATMENT OF INFLAMMATORY PROCESSES IN DIABETES MELLITUS IN AN EXPERIMENT

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Studying the effect of ozone therapy on the course of pyoinflammatory processes with diabetes mellitus in the experiment, conducted on 30 white 24-30 month rats, weight 300-450 gr with purulent-inflammatory processes, it was found out that diabetes, which was simulated by subcutaneous injection of alloxan, causes mosaic disturbances of hemostasis system in the presence of pyoinflammatory processes. Complicated changes in blood condition were also detected against the background of diabetes mellitus: chronometric hypocoagulation on the intrinsic pathway of blood coagulation in association with chronometric hypercoagulation by the external thrombinogenesis mechanism and fibrinogenesis depression against the background of hypofibrinogenaemia. Thus, the use of ozone therapy in the presence of soft tissues abscesses in old rats with diabetes does not demonstrate significant protective properties with reduced azoalbumin lysis, total non-enzymatic fibrinolytic activity and proteinase activity by Kunitz in blood plasma.

Such changes in old rats can be considered as an exacerbation of purulent inflammation, increase in toxicity, reduced protective properties, increase in manifestations of hypercoagulation against the background of chronic course of diabetes, caused by age-related changes.

Keywords: diabetes mellitus, pyo-inflammatory complications, fibrinolysis, ozone therapy.

РЕЗЮМЕ

ОЦЕНКА ПРИМЕНЕНИЯ ОЗОНОТЕРАПИИ В ЛЕЧЕНИИ ГНОЙНО-ВОСПАЛИТЕЛЬНЫХ ПРОЦЕССОВ ПРИ ЭКСПЕРИМЕНТАЛЬНОМ ДИАБЕТЕ

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Изучено влияние озонотерапии на течение гнойно-воспалительных процессов при экспериментальном диабете (аллоксановая модель) на 30 белых 24-30-месячных крысах весом 300-450 гр. Обнаружены мозаичные нарушения в системе гемостаза. На фоне аллоксанового диабета обнаружены сложные изменения в крови: 1) хронометрическая гипокоагуляция по внутреннему пути свертывания крови сочетается с хронометрической гиперкоагуляцией за внешним механизмом тромбиногенеза, 2) угнетение фибриногенеза на фоне гипофибриногенемии. Применение озонотерапии при наличии гнойников мягких тканей при экспериментальном диабете у крыс старческого возраста не оказывает существенного защитного свойства; отмечается снижение лизиса азоальбумина, уменьшение суммарной, неферментативной фибринолитической активности и активности протеиназ в плазме крови (по Кунитцу).

Изменения, происходящие в организме крыс, очевидно, следует объяснить обострением гнойно-воспалительного процесса, нарастанием интоксикации, снижением протекторных свойств, увеличением проявлений гиперкоагуляции на фоне хронического течения сахарного диабета.

რეზიუმე

ოზონოთერაპიის გამოყენების ეფექტურობის შეფასება ჩირქოვანი ანთებითი პროცესების მკურნალობაში ექსპერიმენტული დიაბეტის პირობებში

ს. კარატეევა, ო. იურკივი, ს. სემენენკო, ი. კოზლოვსკაია, ს. იაკობჩუკი

უკრაინის უმაღლესი სასწავლო დაწესებულება ბუკოვინის სახელმწიფო სამედიცინო უნივერსიტეტი, ჩერნოვცი, უკრაინა

კვლევის მიზანს წარმოადგენდა ოზონოთერაპიის გავლენის შესწავლა ჩირქოვან ანთებით პროცესებზე ექსპერიმენტული დიაბეტის პირობებში.

კვლევა ჩატარდა 300-450 გრ წონის 30 თეთრ 24-30 თვის ვირთაგვაზე ჩირქოვანი ანთებითი პროცესით. შაქრიანი დიაბეტის მოდელირება განხორციელდა ალოქსანის კანქვეშ შეყვანით. გამოვლინდა, რომ შაქრიანი დიაბეტი ჩირქოვანი ანთებითი პროცესების არსებობის პირობებში იწვევს ჰემოსტაზის სისტემაში მოზაიკური ტიპის ცვლილებებს. ექსპერიმენტული დიაბეტის ფონზე ასევე გამოვლინდა რთული ცვლილებები სისხლში: სისხლის შიდა გზით შედედების ქრონომეტრული ჰიპერკოაგულაცია მიმდინარეობდა პარალელურად ჰიპოფიბრინოგენეზის ფონზე განვითარებულ ქრონომეტრულ ჰიპერკოაგულაციასთან. ამგვარად, ოზონოთერაპიის გამოყენება რბილი ქსოვილების ჩირქოვანი პროცესების არსებობის პირობებში ექსპერიმენტული დაიბეტის დროს ხანდაზმულ ვირთაგვებში არ ავლენს არსებით პროტექტორულ თვისებებს სისხლის პლაზმაში აზოალბუმინების ლიზისის, ჯამური არაფერმენტული ფიბრინოლითური აქტივობის და კუნიცის პროტეინაზის აქტივობის დაქვეითების თვალსაზრისით. ხანდაზმულ ვირთაგვებში განვითარებული ზემოაღნიშნული ცვლილებები შეიძლება აიხსნას ჩირქოვან-ანთებით პროცესების გამწვავებით, ინტოქსიკაციის მოვლენების ზრდით, პროტექტორული თვისებების დაქვეითებით და ჰიპერკოაგულაციის გამოვლინებათა მატებით ქრონიკული შაქრიანი დიაბეტის ფონზე.