IJABR

International Journal of Advanced Biotechnology and Research (IJABR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol-10, Issue-2, 2019, pp429-434
http://www.bipublication.com

Research Article

Functional features of platelets in vivo in rats long-term fructose

Kharitonov E.L.[1], Zavalishina S.Yu.[1], Medvedev I.N.[1]*,

Nontina E.A.[2], Bykova I.A. [2], Avdotyin V.P. [2], Zharov A.N.[2],

Sakhno N.V.[3], Kochneva M.V.[2], Lukina D.M.[2] and Zuev E.A.[2]

[1] All-Russian Research Institute of Animal Physiology, Biochemistry and Nutrition, Branch, Federal Science Center for Animal Husbandry named after Academy Member L.K. Ernst, Borovsk, Institute village, 249013,Russian Federation

[2] Peoples' Friendship University of Russia (RUDN University), 6 Miklukho-Maklaya St, Moscow, 117198, Russian Federation

3 Orel State Agrarian University named after N.V. Parakhin. 32019, Orel region, Orel, General Rodin St. 69, Russian Federation

***Corresponding author:** Medvedev IlyaNikolaevich, Tel +79102732263, E-mail: ilmedv1@yandex.ru

[Received: 26/03/2019; Accepted: 04/05/2019; Published: 09/05/2019]

ABSTRACT

Objective:to follow the development of increased intravascular platelet activity in rats during the experimental formation of their metabolic syndrome.

Material and methods:The study included 61 male rats aged 2.5-3 months, which were obtained from completely healthy females by the first-second litter. When included in the study, the body mass of the rats reached 261.1 ± 1.18 g, the circumference of their abdomen was 14.7 ± 0.26 cm. Before our study, the animals did not participate in other studies and had no pathology. By chance, all rats were divided into 2 groups: 32 rats received in unlimited quantities as a drink 10% fructose solution. This solution is derived from crystalline fructose (Novaprodukt, Russia). The remaining 29 rats formed a control group. The study was conducted for 8 weeks.

Results:In the animals taken in the experiment that received the fructose solution, after 2 weeks the plasma lipid composition and peroxidation activity in it underwent a tendency to deterioration, and after 4 weeks their negative changes reached the level of confidence, then gradually deteriorated. In rats treated with a solution of fructose, after 4 weeks of the experiment their apparent imbalance of arachidonic acid metabolites was detected, reaching a maximum of 8 weeks of observation. These changes were accompanied by an increase in the plasma level of endothelin-1 and a decrease in the synthesis of nitric oxide. In experimental rats, already after 2 weeks, there was a tendency to an increase in the intravascular activity of platelets, which gradually increased during the whole observation. Within 8 weeks of experimental observation, the level of aggregates of any size freely moving through their vessels increased significantly, creating the danger of blockade of the microvasculature.

Conclusion:Under conditions of experimental loading with fructose in rats, a rapid simultaneous violation of biochemical parameters and a rapid increase in the intravascular activity of platelets characteristic of the metabolic syndrome were established. These changes should be attributed to the growth of prostacyclin and nitric oxide levels in the blood of experimental animals against the background of the fructose load and an increase in the concentrations of thromboxane, endothelin-1, thiobarbituric acid-active products and acylhydroperoxides.

Keywords:Fructose, Rats, Model, metabolic syndrome, Platelets, Intravascular platelet activity.

INTRODUCTION

The effective operation of the hemostasis system is largely ensured by sufficient platelet activity, which is highly dependent on a large number of environmental influences[1,2]. In vivo, it

manifests itself as an intravascular platelet activity[3,4,5].

Earlier studies on many aspects of platelet physiology have provided a clear picture of the course of its regulation in the conditions of any pathology[6,7]. Its dynamics was clarified in the presence of arterial hypertension and when combined with many metabolic disorders[8,9]. It has been established that, under conditions of combination of arterial hypertension with metabolic syndrome, ultimately, an excessive level of functional activity of platelets develops, which forms a high risk of onset of thrombosis[10,11]. To reduce thrombocytopathy and the risk of thrombosis in patients with arterial hypertension with metabolic syndrome, various experimental and clinical studies were performed[12,13,14,15]. At the same time, the phenomena of thrombocytopathy at the very beginning of the development of the metabolic syndrome remain poorly elucidated. It is still difficult to trace these changes in people due to the loss of patients with the first manifestations of the metabolic syndrome from the medical field. This requires systematic experimental observations using laboratory animals and simulating the phenomena of the metabolic syndrome[16]. This information is intended to create a basis for further clinical studies, allowing to supplement aspects of pathogenesis and find out the timing of the earliest onset of therapeutic interventions in individuals with the first manifestations of the metabolic syndrome[17].

The aim of the paper is to trace the development of increased intravascular platelet activity in rats during the experimental formation of their metabolic syndrome.

MATERIALS AND METHODS

The study was conducted in strict accordance with the ethical principles established by the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (adopted in Strasbourg on March 18, 1986 and confirmed in Strasbourg on June 15, 2006).

The study included 61 male rats aged 2.5-3 months, which were obtained from completely healthy females by the first-second litter. The value of the body weight of rats when included in the study reached 261.1 ± 1.18 g, the circumference of their abdomen was 14.7 ± 0.26 cm. Prior to our study, animals did not participate in other studies and had no pathology. By chance, all rats were divided into 2 groups: 32 rats received in unlimited quantities as a drink 10% fructose solution. This solution is derived from crystalline fructose (Novaprodukt, Russia)[18]. The remaining 29 rats formed a control group. The study was conducted for 8 weeks. Blood from animals was taken from the tail vein at the beginning of the observation, as well as after 2,4,6 and 8 weeks of the fructose load. The rats of the control group were examined twice: at the beginning of the observation and at the age of 4.5-5 months, that is, at the end of the observation process for the animals taken in the experiment. Due to the lack of statistically significant differences between the results obtained in these two surveys of rats of the control group, the data are presented in the form of a single figure, which is their arithmetic average.

All rats were evaluated body weight during their weighing on standard laboratory scales, which are expressed in grams. The value of the abdominal circumference was evaluated during the registration of its coverage in the middle and expressed in centimeters. The content of total cholesterol and triglycerides in the blood of animals was recorded by an enzymatic colorimetric method, using a kit produced by the Russian company Vital Diagnosticum. The level of high-density lipoprotein cholesterol in their blood was recorded using an enzymatic colorimetric set made by the Russian company Olvex Diagnosticum. The low-density lipoprotein cholesterol level was ascertained by standard calculation. Very low density lipoprotein cholesterol was calculated using the formula: triglyceride level/2.2.

The expression of plasma lipid peroxidation was determined by the level of thiobarbituric acid-active products in a set manufactured by Agat-Med (Russia) and by the level of plasma acyl hydroperoxides when recording the state of plasma antioxidant activity[19]. In the plasma of all rats, the amount of endothelin-1 was

ascertained using a radioimmunoassay method using DRG reagents (USA). The amounts of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ were evaluated during the enzyme immunoassay using an Enzo Life science kit (USA). The total level of nitric oxide metabolites was ascertained from the examined animals[20]. The severity of intravascular platelet activity was ascertained using a phase contrast microscope[21]. Statistical processing of the results of the observation was carried out by the criterion (t) of Student.

RESULTS AND DISCUSSION

Initially, the normal body weight of rats taken under observation already after 2 weeks of participation in the experiment underwent a tendency to increase, and after 4 weeks its growth reached a level of confidence. After 6 weeks of ingestion of a solution of fructose in rats, their body weight increased to 283.4±1.27 g, and the value of the circumference of their abdomen was 16.4±0.19 cm. These figures increased further by the end of the experiment (table).

In the animals taken in the experiment that received the fructose solution, after 2 weeks the plasma lipid composition underwent a tendency to its deterioration, and after 4 weeks its negative changes reached the level of confidence, then steadily deteriorating. At the same time, already after 2 weeks of the experiment, animals showed a significant decrease in plasma antioxidant activity against the background of an increase in the level of acylhydroperoxides and thiobarbituric acid-active products in it. These changes continued throughout the consumption of the fructose solution by rats (table).

The normal balance of plasma arachidonate metabolites in rats that received the fructose solution deteriorated rapidly: after 4 weeks of the experiment, there was a clear imbalance that reached its peak at 8 weeks of observation - the level of thromboxane B_2 increased by 37.3% 6-keto-prostaglandin $F_{1\alpha}$ decreased by 21.8%. These changes were accompanied in rats of the experimental group by an increase in the plasma level of endothelin-1 to a value of 12.5±0.36 pg/ml and a decrease in the total volume of the level of nitric oxide derivatives by 20.2% (table).

In the course of creating in the experiment in rats in the fructose model, a picture of the metabolic syndrome already after 2 weeks, there was a tendency for an increase in the intravascular activity of platelets. Subsequently, it additionally grew and after 4 weeks of the experiment underwent an additional increase in the course of the observation. As a result of all 8 weeks of the experiment, a decrease in the number of platelet-discocytes to the level of 61.3±0.24% and an increase in the level of active platelet species to a total level of 38.7±0.23% were observed. Within 8 weeks of experimental observation, the level of small aggregates freely moving in rat vessels increased to 14.6±0.08 per 100 free platelets, while the number of medium and large aggregates increased to 2.3±0.06 per 100 free platelets. At the time of completion of the experiment in the blood of experimental rats, the number of platelets in the composition of the aggregates was higher than the control values by 80.6% (11.2±0.05%).

CONCLUSION

Under conditions of experimental loading with fructose in rats, a rapid simultaneous increase in body weight and impaired biochemical parameters characteristic of the metabolic syndrome were established. This was combined with a rapid increase in the level of intravascular platelet activity in their blood. These changes should be attributed to the growth of prostacyclin and nitric oxide levels in the blood of experimental animals against the background of the fructose load and an increase in the concentrations of thromboxane, endothelin-1, thiobarbituric acid-active products and acylhydroperoxides.

REFERENCES

1. Bikbulatova AA, Andreeva EG. Impact of daily wearing of medioprophylactic clothes on the state of locomotor system of 5-6-year old children with scoliosis of I-II degree. Bali Medical Journal. 2018; 7(3): 621-625. DOI:10.15562/bmj.v7i3.948

2. Bikbulatova AA. Peculiarities of abnormalities of locomotor apparatus of children at preschool age with scoliosis of I-II degree living in Central Russia. Bali Medical Journal. 2018; 7(3): 693-697. DOI:10.15562/bmj.v7i3.738
3. Vorobyeva NV, Mal GS, Skripleva EV, Skriplev AV, Skoblikova TV. The Combined Impact OfAmlodipin And Regular Physical Exercises On Platelet And Inflammatory Markers In Patients With Arterial Hypertension. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018 ; 9(4) : 1186-1192.
4. Bikbulatova AA.Creating Psychological Comfort In Women Who Wear Corrective Clothing For A Long Time. Research Journal of Pharmaceutical, Biological and Chemical Sciences.2018; 9(6) : 1112-1121.
5. Yousefi M, Hoseini SM, VatnikovYuA, Kulikov EV, Drukovsky SG.Rosemary leaf powder improved growth performance, immune and antioxidant parameters, and crowding stress responses in common carp (Cyprinuscarpio) fingerlings. Aquaculture. 2019; 505 : 473-480. https://doi.org/10.1016/j.aquaculture.2019.02.070
6. Vorobyeva NV, Skripleva EV, Makurina ON, Mal GS. Physiological Reaction of The Ability of Erythrocytes to Aggregate to Cessation of Prolonged Hypodynamia. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018; 9(2) : 389-395.
7. Skripleva EV, Vorobyeva NV, KipermanYaV, Kotova OV, Zatsepin VI, Ukolova GB.The Effect Of Metered Exercise On Platelet Activity In Adolescents. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018 ; 9(3) : 1150-1154.
8. Makhov AS. Specificity Of Requirements Of Russian And Foreign Hockey Players With Hearing Impairment To The Process Of Training And Competition. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018;9(5):157-163.
9. Makhov AS. The Basic Needs Of Hearing Impaired People In Organizing Football Training. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018;9(5):121-126.
10. YousefiM, HoseiniSM, VatnikovYuA, NikishovAA, KulikovEV.Thymol as a new anesthetic in common carp (Cyprinuscarpio): Efficacy and physiological effects in comparison with eugenol, Aquaculture. 2018; 495 (2018) 376-383.
11. Bikbulatova AA. Functional Features Of Microcirculatory Processes In Obese Women Against A Background Of Long Daily Wearing Of Corrective Clothing. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018; 9(6) : 785-793.
12. Bikbulatova AA, Andreeva EG. Achievement of psychological comfort in 5-6-Year-Old children with scoliosis against the background of daily medicinal-prophylactic clothes' wearing for half a year. Bali Medical Journal. 2018; 7(3): 706-711. DOI:10.15562/bmj.v7i3.947
13. Lenchenko E, Lozovoy D, Strizhakov A, Vatnikov Y, Byakhova V, Kulikov E, Sturov N, Kuznetsov V, Avdotin V, Grishin V. Features of formation of Yersinia enterocolitica biofilms, Veterinary World. 2019; 12(1): 136-140.
14. Suleymanov SM, Usha BV, Vatnikov YA, Sotnikova ED, Kulikov EV, Parshina VI, Bolshakova MV, Lyshko MU, Romanova EV. Structural uterine changes in postpartum endometritis in cows. Veterinary World. 2018; 11(10): 1473-1478.
15. YousefiM, VatnikovYA, KulikovEV, GhelichpourM. Change in blood stressand antioxidant markers and hydromineral balance of common carp (Cyprinuscarpio)anaesthetized with citronellal and linalool: comparison with eugenol. Aquac. Res.2019. https://doi.org/10.1111/are.14007.
16. Makhov AS. The Importance Of The Needs Arising In People When Organizing

Classes Rink Bandy (Mini Hockey). Research Journal of Pharmaceutical, Biological and Chemical Sciences. 2018;9(5):96-101.
17. GilmutdinovRYa, Kurbanov RZ. Blood physiology. Kazan, 1999:199.
18. Reshetnyak MV, Khirmakov VN, Zybina NN, Frolova MV, Sakuta GA, Kudryavtsev BN. Model of metabolic syndrome caused by fructose feeding: pathological relationships of metabolic disorders. Medical Academic Journal. 2011; 11(3) :23-27.
19. Volchegorsky IA, Dolgushin II, Kolesnikov OL, Tseylikman VE. Experimental modeling and laboratory evaluation of adaptive reactions of the body. Chelyabinsk, 2000. 167.
20. Metelskaya VA, Gumanova NG. Nitric oxide: role in the regulation of biological functions, methods for determining in human blood. Laboratory medicine. 2005;7 :19-24.
21. Barkagan ZS, Momot AP. Diagnosis and controlled therapy of hemostatic disorders. Moscow, 2008 : 292.

Table. Changes in the recorded parameters in experimental rats treated with fructose solution as a drink

Registered indicators	**Dynamics of parameters during the experiment, n=32, M±m**					**Control, n=29, M±m**
	The initial state	2 week load of fructose	4 week load of fructose	6 week load of fructose	8 week Load of fructose	
Bodyweight, g	262.1±1.24	268.5±1.10	276.3±1.23 p<0.05	283.4±1.27 p<0.01	296.6±1.34 p<0.01	260.1±1.12
Abdominal circumference, cm	14.7±0.22	15.1±0.28	15.8±0.12 p<0.05	16.4±0.19 p<0.01	17.2±0.20 p<0.01	14.8±0.31
Total cholesterol, mmol / l	2.19±0.06	2.30±0.09	2.54±0.07 p<0.01	2.79±0.05 p<0.01	2.92±0.03 p<0.01	2.22±0.06
HDL cholesterol, mmol / l	1.12±0.05	1.06±0.04	1.01±0.003 p<0.05	0.96±0.004 p<0.01	0.94±0.005 p<0.01	1.10±0.004
LDL cholesterol, mmol / l	0.59±0.04	0.67±0.05 p<0.05	0.82±0.07 p<0.01	1.09±0.08 p<0.01	1.15±0.04 p<0.01	0.63±0.02
Cholesterol VLDL, mmol / l	0.48±0.003	0.57±0.06 p<0.05	0.71±0.05 p<0.01	0.78±0.006 p<0.01	0.83±0.002 p<0.01	0.49±0.004
Triglycerides, mmol / l	1.05±0.05	1.26±0.06 p<0.05	1.56±0.04 p<0.01	1.72±0.03 p<0.01	1.83±0.02 p<0.01	1.08±0.04
Acyl hydroperoxide, D_{233}/1 ml	1.37±0.12	1.64±0.06 p<0.05	1.97±0.07 p<0.01	2.50±0.05 p<0.01	2.85±0.04 p<0.01	1.41±0.03
Thiobarbituric acid-active products, µmol / l	2.27±0.06	2.83±0.06 p<0.05	3.39±0.09 p<0.01	3.98±0.07 p<0.01	4.48±0.08 p<0.01	2.30±0.04
Antioxidant activity,%	29.2±0.05	27.6±0.08	26.0±0.08 p<0.05	24.6±0.06 p<0.01	22.4±0.05 p<0.01	29.7±0.04
Thromboxane B_2, pg /ml	145.9±0.21	168.7±0.50	184.7±0.59 p<0.01	208.1±0.42 p<0.01	232.6±0.69 p<0.01	148.1±0.28
6-keto-prostaglandin $F_{1\alpha}$, pg / ml	75.9±0.20	72.4±0.26	69.6±0.32 p<0.05	65.4±0.38 p<0.01	62.3±0.44 p<0.01	76.5±0.22
Nitric oxide metabolites, µmol / l	27.9±0.28	27.1±0.16	26.4±0.09 p<0.05	24.7±0.19 p<0.01	23.2±0.06 p<0.01	28.5±0.29
Endothelin -1, pg / ml	6.9±0.18	8.2±0.23 p<0.05	10.1±0.27 p<0.01	11.4±0.29 p<0.01	12.5±0.36 p<0.01	6.8±0.16
The number of discocytes, %	81.0±0.14	78.8±0.12	72.5±0.16 p<0.01	65.0±0.21 p<0.01	61.3±0.24 p<0.01	80.4±0.16
Basal number of disco-echinocytes, %	14.5±0.14	15.5±0.06 p<0.05	17.3±0.07 p<0.01	21.7±0.06 p<0.01	21.0±0.08 p<0.01	15.2±0.13
The number of spherocytes, %	2.3±0.12	2.9±0.09	5.6±0.07 p<0.01	7.9±0.09 p<0.01	10.9±0.10 p<0.01	2.2±0.10

The number of sphero-echinocytes, %	1.7±0.05	2.2±0.04 p<0.05	3.8±0.07 p<0.01	4.4±0.08 p<0.01	5.5±0.09 p<0.01	1.6±0.06
The total number of activated platelets, %	19.0±0.11	21.2±0.24	27.5±0.18 p<0.01	35.0±0.16 p<0.01	38.7±0.23 p<0.01	19.6±0.10
Platelet count in aggregates, %	6.3±0.07	6.9±0.05 p<0.05	8.5±0.06 p<0.01	9.9±0.07 p<0.01	11.2±0.05 p<0.01	6.2±0.06
The number of aggregates of small size, 2-3 platelets per 100 free platelets	2.4±0.03	3.8±0.05 p<0.01	7.4±0.06 p<0.01	11.2±0.05 p<0.01	14.6±0.08 p<0.01	2.3±0.04
The number of medium and large platelet aggregates of 4 or more cells per 100 free platelets	0.1±0.03	0.8±0.04 p<0.05	1.3±0.02 p<0.01	1.8±0.03 p<0.01	2.3±0.06 p<0.01	0.1±0.02

Legend: p - the reliability of differences in performance in experimental rats from the values of the control.