Tsymbaliuk G. Y., Krylyuk V. O. Features of transport of potassium ions in ischemia-reperfusion syndrome of lower extremities, abdominal trauma and hypovolemic shock. Journal of Education, Health and Sport. 2018;8(9):1785-1795. eISNN 2391-8306. DOI http://dx.doi.org/10.5281/zenodo.1473054
http://ojs.ukw.edu.pl/index.php/johs/article/view/6235

The journal has had 7 points in Ministry of Science and Higher Education parametric evaluation. Part b item 1223 (26/01/2017).
1223 Journal of Education, Health and Sport eISSN 2391-8306 7

The authors declare that there is no conflict of interests regarding the publication of this paper.
Received: 17.09.2018. Revised: 24.09.2018. Accepted: 27.09.2018.

UDC 616.711-001.3-616-092-616.61-008.64] – 092.9

FEATURES OF TRANSPORT OF POTASSIUM IONS IN ISCHEMIA-REPERFUSION SYNDROME OF LOWER EXTREMITIES, ABDOMINAL TRAUMA AND HYPOVOLEMIC SHOCK

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Abstract

80 pubescent white male Wistar rats weighing 190-220 g were used in the experiment. Rats have been on a standard vivarium diet.

Animals were divided into 1 control (CG) and 3 experimental groups (EG) (8 animals per group). Haemostatic tourniquets were applied for 120 minutes proximal to the lower paws of animals of the first experimental group under the thiopental-sodium anesthesia (40 mg kg-1). Applying of tourniquets caused the development of ischemic-reperfusion syndrome. Blunt abdominal trauma was simulated in the second experimental group by applying two strikes to the abdomen; Hypovolemic shock was modeled in a group by cutting the femoral vessels and bloodletting from 20 to 22% of the circulating blood volume. Injuries of the third experimental group were combination of injuries of the first two groups. Animals of the control group were inducted into the anesthesia without any injury.

The functional status of the kidneys of experimental animals was determined by the method of water loading at 1st, 3rd and 7th day. The concentration of potassium ions was determined in urine and blood serum and the level of its proximal and distal transport, as well as excretion, were calculated.

Animals of experimental groups were withdrawn from the experiment by the method of total blood-flow from the heart under thiopental sodium anesthesia.

Thus, simulated injuries are accompanied by a significant increasing potassium level in serum in all experimental groups. Moreover, in EG-1 there was a tendency of gradual decrease of indicator on 7 day of observation, in contrast to the indicators in EG-2 and EG-3 where increased on 7 day. In all EG the maximum increase of the indicator was observed on the 1 day of the post-traumatic period.

The simulation of various causes of mechanical trauma leads to a significant increase of the excretion of potassium ions in the urine, with the exception of injuries of the abdominal trauma with massive blood loss and applied haemostatic tourniquets on both lower limbs, when on the 7 day the indicator is approaching the level of control group. This can be explained by a significant decrease of diuresis in the experimental group.

Keywords: **potassium ions, trauma, reperfusion syndrome, experiment.**

Introduction

A combined trauma causes an adaptation changes in metabolic processes of body, which result in changes in both intracellular metabolism and composition of internal environments [1, 2]. It is also known that systemic disorders are important in the pathogenesis of severe trauma. In particular, they include disorders of the water-electrolyte, osmotic and colloid-osmotic balance. In this case pathological changes also appear in systems and organs that are not directly exposed to the traumatic factors. It is known that one of these target organs is kidneys [3, 4].

In recent times the number of severe combined trauma, accompanied by massive bleeding and ischemic-reperfusion syndrome of the extremities, has increased significantly due to the widespread use of haemostatic tourniquets [3]. It is known that reperfusion injury of extremities is one of the most common acute pathological condition that develops with impaired peripheral blood flow [5 - 9]. The influence of hypovolemic shock and ischemic-reperfusion limb syndrome was thoroughly studied [10, 11]. However, the functional state of the internal organs, including kidneys, in case of combined trauma and ischemic-reperfusion

syndrome has been studied insufficiently. In particular, the analysis of scientific sources indicates that there is no data of impaired renal transport of potassium ions.

Purpose: to study the effect of ischemic-reperfusion syndrome on renal transport of potassium ions in the early post-traumatic period.

Materials and methods. The working hypothesis of the experimental study is the assumption that the use of a haemostatic tourniquet and subsequent reperfusion of ischemic tissues carries systemic effects on the body with a disruption of functions of the internal organs in case of abdominal trauma with hypovolemic shock and may be the cause of the development of multiple organ failure.

To achieve the goal 80 nonlinear male rats weighing 190-220 g were used in performed experimental study. All animals were divided into groups: 1 control and 3 experimental (for 8 animals in each group).

In the animals of the first experimental group (EG-1) haemostatic tourniquets were applied for 120 minutes proximal to the lower paws of animals of the experimental groups under the thiopental-sodium anesthesia (40 mg kg-1). Elastic strips of tourniquet SWAT-T (USA) width of 10 mm were used as tourniquets, which corresponds to the width of the tourniquet for human thigh. Tourniquets were wrapped in accordance with the effective pressure index on it.

In the second experimental group (EG-2) combined injury to the abdominal organs was simulated: after anesthesia with sodium thiopental (40 mg kg-1) the animals were hit twice in the abdominal region using a special device - the impact force was calculated as follows so that there was no internal bleeding; Blood loss was achieved by performing operative access and crossing the femoral vessels in a volume from 20 to 22% of the circulating blood volume of the animal for one minute (acute blood loss), after which the bleeding was stopped by vascular ligation.

In the third experimental group (EG-3), a combined injury to the abdominal cavity and ischemic reperfusion syndrome was modeled according to the methods described above.

Animals of experimental groups were withdrawn from the experiment under conditions of thiopental sodium anesthesia (60 mg • kg-1) by total bleeding from the heart after 1, 3 and 7 day after reperfusion. Kidneys were taken from animals bodies for study. In the control group (CG) animals were only injected into anesthesia using an equivalent dose of thiopental sodium and subsequently withdrawn from the experiment in 2 hours. The obtained data were compared with the CG.

The functional status of the animal's kidneys was determined by the method of water loading at 1, 3 and 7 day of experimental study. The method was used 2 hours prior to euthanasia by performing the following actions: heated to 30°C water was injected in the stomach in a volume of 5% of the body weight of the test animal. Urine of experimental animal was collected during 2 subsequent hours. After urine collection under thiopental-sodium anesthesia, in accordance with the provisions of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (Strasbourg, 1985), euthanasia was performed by the method of total blood-flow from the heart. The concentration of potassium ions was determined in urine and blood serum and the level of its proximal and distal transport, as well as excretion, were calculated.

The research of these indicators was determined in the Central Scientific Laboratory of I.Horbachevsky Ternopil State Medical University.

Reliability of indicators differences between experimental and control groups was estimated using Mann-Whitney's non-parametric criterion. The differences were considered true when the reliability of a null hypothesis was less than 5% ($p<0.05$).

Results and discussion. As can be seen from Table. 1, the level of potassium ions in blood serum in EG-1 in 1 day significantly exceeded the control group (by 32.7%, $p<0.01$), after 3 days by 6.1% ($p<0.05$) and through seven days by 4.1% ($p> 0.05$). In EG-2, this indicator in all terms of observation was significantly higher (correspondingly to 33.7, 36.7 and 39.8%, $p<0.001$). Similarly, it turned out to be higher in EG-3 (42.9, 69.4 and 72.4% respectively, $p<0.001$).

Comparison of the experimental groups on observation points showed that after 1 day of the post-traumatic period in the EG-1 the indicator was lower than in other experimental groups, although the difference was unreliable statistically. ($p_{1-2}>0,05$, $p_{1-3}>0,05$). After 3 day of post-traumatic period level of potassium ions was the highest in EG-3, which exceeded EG-1 by 59,6% ($p_{1-3}<0,001$), EG-2 - by 23,8% ($p_{2-3}<0,001$). After 7 day this pattern was persisting, when the indicator in EG-3 was higher than in EG-1 and EG-2 by 65.7% ($p_{1-3}<0,001$) and 23.4% ($p_{2-3}<0,01$) respectively.

Analysis of the dynamics of the researched indicator in the experimental groups showed (Fig. 1) that in EG-1 indicator sharply decreased on 3 day by 26,6% ($p<0,05$) in comparison with the 1 day. The difference between the indicators on 3 and 7 day was unreliable. In EG-2 the indicator after 3 and 7 days was greater than in 1 day, however, the

difference was unreliable (p> 0.05). In EG-3 the indicator sharply increased to the 3 day, with more than 26.5% (p> 0.05) compared with the 1 day, then slightly increased to the 7 day.

Table 1. - The content of potassium ions in serum (mmol / l-1) in the dynamics in the study groups, Me (LQ; UQ) - median (lower and upper quartile)

Experimental groups	Control group	Observation points		
		1 day	3 day	7 day
EG – 1	4,90 (4,55;5,18) (n=8)	6,50** (6,15;6,75) (n=8)	5,20* (4,88;5,53) (n=8)	5,10 (4,88;5,45) (n=8)
EG – 2		6,55*** (6,13;6,63) (n=8)	6,70*** (5,85; 7,28) (n=8)	6,85*** (6,43;7,30) *(n=8)
EG – 3		7,00*** (6,75;7,38) (n=8)	8,30*** (7,88;8,43) (n=8)	8,45*** (7,40;8,80) (n=8)
p_{1-2}		>0,05	<0,001	<0,001
p_{1-3}		>0,05	<0,001	<0,001
p_{2-3}		>0,05	<0,001	<0,01

Notes: here and in other tables:

1. * Reliability of index differences compare to control group (* – p<0,05; ** – p<0,01; *** – p<0,001).

2. p_{1-2} – reliability of index differences between EG –1 and EG – 2; p_{1-3} – between EG – 1 and EG – 3; p_{2-3} – between EG – 2 and EG – 3.

Thus, simulated injuries are accompanied by a significant increasing potassium level in serum in all experimental groups. Moreover, in EG-1 there was a tendency of gradual decrease of indicator on 7 day of observation, in contrast to the indicators in EG-2 and EG-3 where increased on 7 day. In all EG the maximum increase of the indicator was observed on the 1 day of the post-traumatic period.

In urine (Table 2, Figure 2) was noted highly increased potassium level, regardless of the type of injury and the duration of the observation (p<0.001). In EG-1 on 1 day the indicator exceeded CG by 151.6%, on 3 day-by 121.1% and on 7 day - by 160.9%. In ED-2 it was higher by 166.8%, 175.1% and 184.2% respectively. In EG-3 it was higher by 226.6%, 241.8% and 248.4% respectively.

The analysis of differences of indicator levels in the experimental groups during observation periods has showed: on 1 day the highest level was observed in EG-3, which was

significantly higher compare to the indicators in EG-1 and EG-2 (p_{1-3}<0,01, p_{2-3}<0,01). A similar situation was observed after the 3 and 7 day of observation.

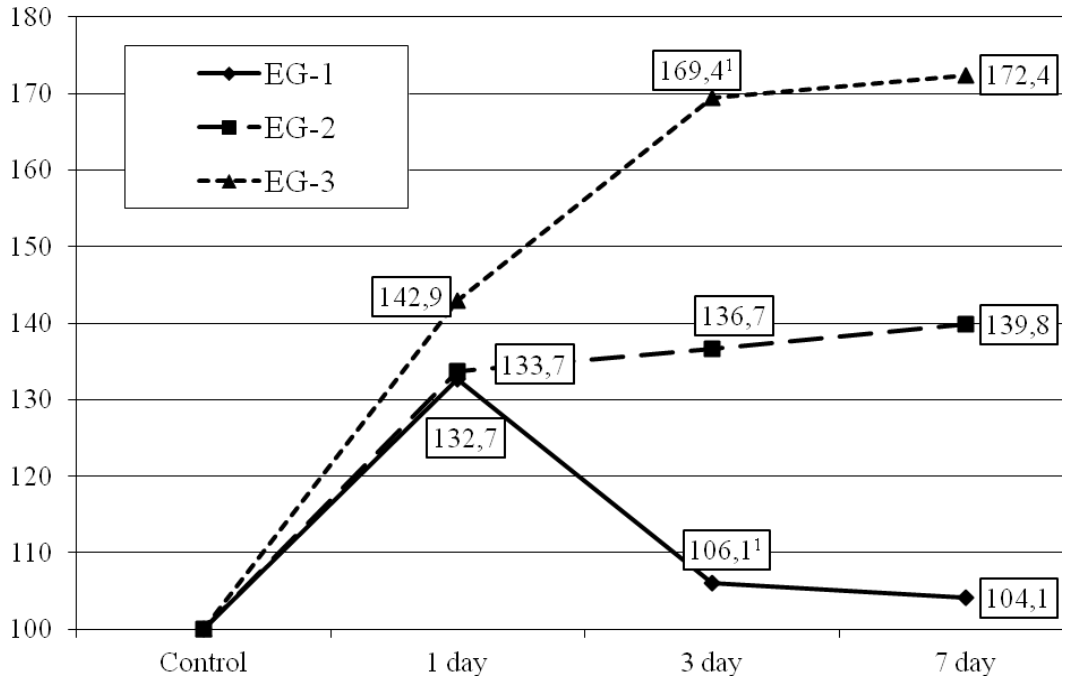

Figure 1. - Dynamics of potassium ion level in serum (as a percentage of the control group level) in the experimental groups. (Here and in other chapters: 1,3 - the differences regarding 1 and 3 day of the post-traumatic period are statistically reliable, p <0.05).

Table 2. - The dynamics of potassium ions level in urine (mmol·L^{-1}) in the experimental groups, Me (LQ; UQ) - median (lower and upper quartile)

Experimental groups	Control group	Observation points		
		1 day	3 day	7 day
EG – 1	9,20 (8,88;9,45) (n=8)	23,15*** (21,13;25,53) (n=8)	20,35*** (19,78;21,53) (n=8)	24,00*** (22,53;24,98) (n=8)
EG – 2		24,55*** (23,13;27,10) (n=8)	25,30*** (23,93; 27,38) (n=8)	26,15*** (25,28;26,90) *(n=8)
EG – 3		30,05*** (28,50;31,78) (n=8)	31,45*** (30,15;32,73) (n=8)	32,05*** (30,63;33,28) (n=8)
p_{1-2}		>0,05	<0,01	<0,05
p_{1-3}		<0,01	<0,001	<0,001
p_{2-3}		<0,01	<0,001	<0,001

The dynamics of changes in the EG-1 were as follows: on the 1 day there was a significant increasing of indicator, for the 3 day the indicator was reduced by 13.8% (p> 0.05), however, by the 7 day it actually returned to the 1 day level. In EG-2 and EG-3 there was a unidirectional tendency of the growth of the indicator up to the 7 day of observation.

Thus, simulated injuries are accompanied by a significant increase of potassium level in the urine, which does not depend on the type of injury and the period of observation.

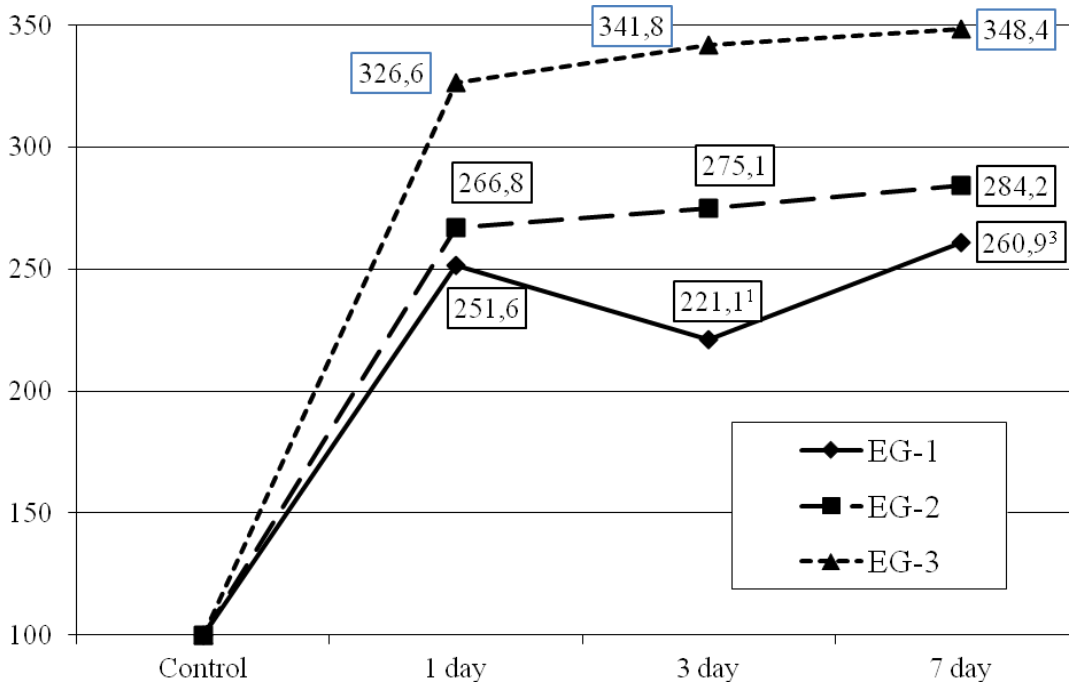

Figure 2. - Dynamics of potassium ion level in urine (as a percentage of the control group level) in the experimental groups.

The revealed deviations also led to disorders of potassium excretion with urine (Table 3.). In almost all observation points, regardless of the type of injury, the excretion of potassium ions with urine was statistically significantly higher than in the CG ($p<0.001$). Thus, in EG-1 at the 1 day the indicator exceeded CG by 29.7%, in the 3 day- by 27.1% and after the 7 day - by 72.9%. In EG-2 in observation points excretion exceeded the CG by 72.9%, 56.6% and 54.1% respectively ($p<0.001$). The exception was in EG with the trauma of the abdominal cavity with massive blood loss and ischemic-reperfusion syndrome. After the 7 day the investigated parameter level in EG-3 did not actually differ from the control group ($p>0.05$). The foregoing may be explained by a sharp decrease of diuresis in EG-3.

Comparison of experimental groups at the observation points showed that after the 1 day of post-traumatic period the excretion of potassium ions was higher in EG-2 than in EG-1

and EG-3 (33.3%, $p_{1-2}<0,01$ and 8.5%, $p_{2-3}>0,05$ respectively). After the 3 day similar trend was observed: the indicator in EG-2 exceeded those in EG-1 and EG-3, however, it was negligible. It should also be noted that levels of the indicator in EG-1 and EG-3 were practically the same ($p> 0,05$). After the 7 day the indicator was clearly lower in EG-3: by 82.7% compared to EG-1 ($p_{1-3}<0,001$), by 58.3% - compared to EG-2 ($p_{2-3}<0,001$).

Table 3. The dynamics of excretion of potassium ions with urine (mkmol · min-1) per 100g of animal body weight in the experimental groups, Me (LQ; UQ) - median (lower and upper quartile)

Experimental groups	Control group	Observation points		
		1 day	3 day	7 day
EG – 1	0,37 (0,34;0,39) (n=8)	0,48*** (0,43;0,52) (n=8)	0,47*** (0,46;0,50) (n=8)	0,64*** (0,61;0,65) (n=8)
EG – 2		0,64*** (0,57;0,70) (n=8)	0,58*** (0,54; 0,59) (n=8)	0,57*** (0,53;0,63) *(n=8)
EG – 3		0,59*** (0,50;0,64) (n=8)	0,48*** (0,43;0,56) (n=8)	0,36 (0,33;0,41) (n=8)
p_{1-2}		<0,01	<0,01	>0,05
p_{1-3}		<0,05	>0,05	<0,001
p_{2-3}		>0,05	>0,05	<0,001

Analysis of the dynamics of this indicator in experimental groups showed (Fig. 3) that in EG-1 it increased and reached the maximum level on the 7 day, exceeding the indicator at the 1 day by 33.3% ($p_{2-3}<0,001$). In EG-2 after the maximum growth at the 1 day there was a slight decrease later. Thus, after the 7 day its level was minimal, however, the difference with the indicator after the 1 day was 12.3% ($p<0.05$). The most dynamic changes of the indicator were in EG-3: after the 3 day it was lower by 20,8% of the indicator on the 1 day ($p<0,05$) and after the 7 day it was lower than on the 3 days by 33,3% ($p<0.01$) and actually equaled the indicator in the control group.

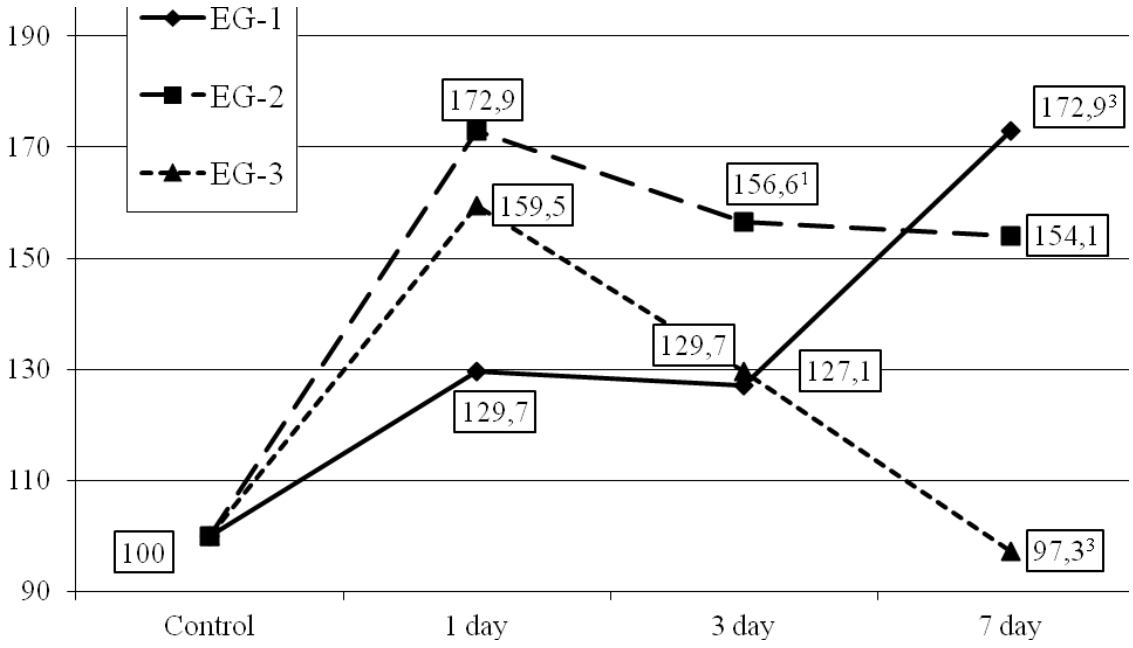

Figure 3. - Dynamics of excretion of potassium ions with urine (as a percentage of the control group level) in the experimental groups.

Thus, the simulation of various causes of mechanical trauma leads to a significant increase of the excretion of potassium ions in the urine, with the exception of injuries of the abdominal trauma with massive blood loss and applied haemostatic tourniquets on both lower limbs, when on the 7 day the indicator is approaching the level of control group. This can be explained by a significant decrease of diuresis in the experimental group.

Conclusions.

1. Ischemic-reperfusion syndrome in experimental animals with trauma of the abdominal cavity and hypovolemic shock significantly affects the level of potassium ions in urine and blood serum, which manifests by significantly growth of these levels to the 7 day after the traumatic period.

2. Excretion of potassium ions with urine increased in experimental animals, but in the experimental group of animals with abdominal cavity injuries, hypovolemic shock and ischemic-reperfusion syndrome levels of excretion decreased to the 7 day, which had an unreliable difference with the control group, which can be explained by a sharp decrease of diuresis in this group.

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