*Огляди літератури, **оригінальні дослідження**, погляд на проблему, ювілеї*
УДК 612.015.11-02:616.137.8/.9-001.4-005.1-089.814]-092.9
DOI 10.11603/1811-2471.2019.v0.i1.10043

CHANGES IN THE GLUTATHIONE SYSTEM'S ACTIVITY OF INTERNAL ORGANS IN THE FIRST HOURS OF EXPERIMENTAL LIMB ISCHEMIA-REPERFUSION SYNDROME, COMBINED WITH BLOOD LOSS AND MECHANICAL INJURY

©N. V. Volotovska, T. Cliff Nhokwara, I. V. Zhulkevych

I. Horbachevsky Ternopil State Medical University

SUMMARY. Blood loss of various genesis irreversibly leads to hypoxia, which in turn triggers the activation of lipid peroxidation and, as a result, cell membranes are damaged. The use of a hemostatic tourniquet, which can complicate the course of traumatic illness under these pathological conditions, has not been fully studied.

The aim of the study – to investigate the features of the response of glutathione system's enzymes of internal organs on the pathogenetic stimulation from modifications of ischemic-reperfusion syndrome in the first hours after its application.

Materials and methods. The determination of the content of glutathione peroxidase (GP), glutathione reductase (GR) and reduced glutathione (RG) in homogenates of internal organs of rats on the base of experimental acute ischemia-reperfusion (IR); IR, associated with mechanical trauma (MT); IR, combined with blood loss (IR+B), and IR combined with blood loss and mechanical trauma (IR+MT) and comparison of results with only blood loss. The acquired exponents are processed statistically.

Results and Discussion. It has been experimentally established that the greatest reduction in the activity of the glutathione system's enzymes – its exhaustion – occurs on the background of ischemia-reperfusion syndrome, combined with blood loss and mechanical trauma of the limb. Already in the first hours in the liver, kidney, heart and lung, a decrease of recovered glutathione was found at 28.8, 30.6, 31.0 and 19.8 times reteably.

Conclusions. An active response for all of examined parameters was found. Towards the activity of enzymes, the following pattern was established: in the first hours, the isolated overlay of the tourniquet led to compensatory changes. The tourniquet, combined with blood loss, has reduced the activity slightly lower than the blood loss itself, which in our opinion, can be explained by the fact that a large circle of blood flow has received a significant concentration of pathogenic factors – due to ischemia, but at the first hour they still managed to evoke, obviously only local damage, whereas hypoxic processes activated by proper blood loss occurred on its background at the system level.

KEY WORDS: ischemia-reperfusion; tourniquet; bleeding; experiment; ischemia; mechanical trauma; glutathione system.

Introduction. The use of a hemostatic tourniquet can be the cause of complications, united under the name "ischemia-reperfusion syndrome" [1]. IRS has been studied a long time ago as a pathological manifestation of the post-operative period associated with the necessity to stop blood supply of operated organ [2-4]. By itself, the application of a tourniquet for therapeutic purposes has been used since the seventeenth century [5], but there are many questions without answers concerning the duration and conditions of the tourniquet in the first medical care, in anticipation of hospitalization before the complications associated with ischemia-reperfusion (I-P), will cause complications [6]. In particular, the cause of even more severe bleeding can be incorrect use of the tourniquet, which leads to bleeding of the destroyed distal veins or increase in hematoma, if the veins are pressed under the tourniquet, but the flowing of arterial blood is not blocked [7]. Acute bleeding leads to tissue hypoxia [8]. However, use of tourniquet can potentiate the effects of circular hypoxia. In addition, the active release of metabolic and lipid-peroxidation products into the general blood flow from the damaged area after release from the tourniquet can actively affect the bloodless exhausted organism of the wounded. Nowadays, the increased attention to this topic shows that the processes of post-traumatic reperfusion on the background of already existing injuries can trigger an additional chain of damage [9-11] much earlier than the "safe" 2 hours of application the tourniquet. Nevertheless, the use of wrists to stop the bleeding of extremities may be the first measure taken on the battlefield under fire, in conditions of darkness, or in the case of massive human casualties [12, 13]. This examination (experiment) is intended to establish a connection between pathological mechanisms that are launched not only against the background of bleeding as such, but also by the supplying of a hemostatic tourniquet, which can also cause damage or exacerbate violations in the body.

The aim of the study – to impose a tourniquet on a time interval admitted as not causing damage to the organism (2 hours) to detect pathogenic or sanogenic reactions triggered on this background in the body; determining the state of the glutathione system in the nearest (after 1 hour after the removal of the tourniquet) post-tourniquet period.

Materials and Methods. Experiments were performed on non-linear white male rats with weight 250–270 g. Animals were divided into the following groups: control, which were only entered into thiopental-sodium anesthesia (40 mg/kg), 1 experimental group (tourniquet was applied to the thigh for 2 hours, reperfusion lasted for 1 hour, after that animals were eliminated from experiment), 2 experimental group (blood loos in volume of 40 % from volume of circulating blood was simulated; after 1 hour animals were eliminated), 3 experimental group (tourniquet on thigh was combined with 40 % blood loss from vena femoralis on another lower limb), 4 experimental group (tourniquet on thigh was combined with fracture of femoral bone of another lower limb), 5 experimental group (tourniquet on thigh was combined with 40 % blood loss and fracture of femoral bone of another lower limb). Animals were exluded from the experiment by total bleeding from the heart. Condition of the gluthatione system of internal organs was determined by the level of glutathione peroxidase (GP), glutathione reductase (GR) and reduced glutathione (RG).

Results and Discussion. As it can be seen from Table 1, GP in the liver mostly has no changed on the background G1-G4 – activity of the enzyme has decreased not significantly in the limits of 2.25 % – 22.5 %. Only on the background of G5 – it has been decreased up to 50 %. The activity of the GR has already undergone variations in the first hours: on the background of the G1 - it did not change significantly (decrease by 4.5 %), but on the background of G2-G5 - decreased by 2.8 times, by 2.1 times, by 5, 2 times and by 3.8 times. Concentration of RH on the background of G1 increased by 25.7 %, on the background of G2, G3 amd G5 basically unchanged (decrease by 5.8 % and 8.7 %), but on the background of G4 – decreased by 28.8 times.

Table 1. Dynamics of activity of glutathione system of internal organs in the first hours on the background of modifications of ischemic-reperfusion limb syndrome in comparison with isolated blood loss

Index	Control (n=10)	G1(T) (n=10)	G2(B) (n=10)	G3(T+B) (n=10)	G4(T+F) (n=10)	G5(T+B+F) (n=10)
10 % liver homogenate						
GP	0.089±0.002	0.087±0.002	0.078±0.001	0.076±0.001	0.044±0.002	0.069±0.001
GR	0.580±0.002	0.554±0.001	0.204±0.003	0.275±0.001	0.153±0.004	0.111±0.003
RG	4.350±0.009	5.470±0.02	4.098±0.04	3.973±0.02	3.363±0.008	0.151±0.001
10 % kidney homogenate						
GP	0.168±0.01	0.300±0.007	0.120±0.002	0.055±0.002	0.089±0.001	0.094±0.001
GR	0.151±0.002	0.176±0.002	0.105±0.002	0.162±0.002	0.161±0.002	0.098±0.003
RG	5.083±0.002	6.740±0.05	4.065±0.03	4.210±0.05	3.120±0.05	0.166±0.004
10 % heart homogenate						
GP	0.170±0.004	0.112±0.006	0.150±0.002	0.457±0.002	0.147±0.001	0.111±0.002
GR	0.267±0.005	0.202±0.002	0.207±0.005	0.216±0.005	0.164±0.001	0.098±0.003
RG	3.767±0.09	5.270±0.05	2.00±0.05	2.373±0.05	2.133±0.04	0.118±0.02
10 % lung homogenate						
GP	0.187±0.005	0.162±0.002	0.130±0.002	0.069±0.004	-0.070±0.003	0.071±0.001
GR	0.292±0.001	0.302±0.002	0.138±0.004	0.179±0.004	0.166±0.002	0.111±0.007
RG	2.927±0.04	4.670±0.04	2.058±0.04	2.517±0.03	2.000±0.01	0.148±0.005

Note: Difference in reference values $p \leq 0.05$

In the kidneys, the dynamics was different – the activity of the GP on the background of G1 – increased by 78.6 %; and on the background of other models was reduced: against the background of G2-G5 – by 26.8 %, by 67.3 %, by 44 % and 47 %. On the background of G1, G3 and G5, the GR has increased slightly by 16.6 %, 7.3 % and 6.3 %, while on the background of G2 and G4 it has declined to 30.5 % and 35.1 %. Concentration of RH on the background of G1 increased by 32.6 %, while on the background of G1, G3 and G5 – decreased by 20 %, 17 % and 38.7 % appropriately. Expressive decrease in the concentration of RH has fixed on the background of the most severe trauma, combined with the IR – on the background of G4 – a decrease by 30.6 times.

In the heart, the dynamics was variable: the activity of the GP decreased by 34.1 %, 11.8 % and 13.5 % on the background of G1, G2 and G 5 appropriately. However, on the background of G3 – expressed increase in 2.7 times and a slight decrease on the background of G4 –by 34.7 % were fixed. As for GR, its activity has decreased by 24.3 %, 22.5 %, 19.1 % and 38.6 % on the background of G1-G3 and G 5 appropriately, while on the background of G4, the changes were the most expressed – by 2.7 times. RH on the background of G1 – has increased by 40 %, but on the

background of the G2-G5 – it has decreased by 47 %, by 37 %, by 31.9 times and by 43.4 % appropriately.

In the lungs, GP activity decreased in all examined groups – by 13.4 %, by 30.5 %, by 2.7 times, by 2.6 times and by 2.6 times on the background of G1-G5. The activity of the GR grew insignificantly – by 13.1 % on the background of G1, while on the background of G2-G5 – it decreased by 48.3, 33 %, 58.4 % and 31.7 % appropriately. The concentration of RG on the background of isolated IR (G1) increased by 1.6 times, on the background of G2, G3 and G5 – decreased by 30 %, by 14 % and and by 31.7 % appropriately. Finally, the combination of the most severe injury has led to a decrease in concentration of 19.8 times.

Conclusions. Thus, studies have shown that already in the first hours after the very overlay of the tourniquet, the antioxidant protection chain reacts vigorously, manifested by the variable reaction of the glutathione system. At the same time, on the background of the IP, most of the decline in the activity of the enzymes GR and GP were observed, while the concentration of VH increased, with the most active in the lungs. Probably an explanation for this is the full compensation of the condition of ischemia. The condition of modeling hypovolemia led to the expected decrease in both the activity of anzymes and the concentration of RG – the most reacted liver, where the level of GR decreased by 2.8 times. On the background of blood loss associated with the IR, the depressive dynamics were similar, however, all investigated organs reacted to varying degrees – the most significant result was expressed in the liver (decrease in GR decreased by 2.1times), in the kidneys and heart (GP), and in the lungs (GP decreased by 2.5 times). The most significant changes were recorded on the background of blood loss – a drop in the concentration of RH in the range of 19.8-31.9 times, as well as a significant decrease in HR and GP indicates the destabilization of the compensatory mechanisms of the glutathione system in the first hours after the injury and the IR syndrome, which is very important taking into account the conditions of real traumatic situations and carry out preventive and therapeutic measures.

Prospects for further research. Thus, the continuous two-hour use of a hemostatic tourniquet is, though effective, but probably the factor that complicates the course of an injury. It is advisable to continue the study to study the state of the antioxidant system in the remote reperfusion period, as well as the state of the pro-oxidant link.

LITERATURE

1. New effective tournicets for potential use in the military environment: A servimg soldier study / Alastair Beaven, Rob Ballard, Paul Parker // Military Medicine. – 2017. – Vol. 182 (7). – P. 1929–1932.

2. Remote ischaemic preconditioning and sevoflurane postconditioning synergistically protect rats from myocardial injury induced by ischemia and reperfusion partly via inhibition TLR4/MyD88/NF-κB Signaling Pathway / J. A. Zhang, J. B. Zhang, P. C. Yu [et al.] // Cellular Physiology and Biochemistry. – 2017. – Vol. 41. – P. 22–32.

3. Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy / T. Aslan, M. D. Turer, A. Joseph. M. D. Hill // The American Journal of Cardiology. – 2010. – Vol. 106, Issue 3. – P. 360–368.

4. Eltzschig H. K. Vascular ischaemia and reperfusion injury / H. K. Eltzschig, C. D. Collard // British Medical Bulletin. – 2004. – Vol. 70 (1). – P. 71–86.

5. Mabry R. L. Tourniquet use on the battlefield // Mil. Med. – 2006. – Vol. 171. – P. 352–356.

6. Джон Ф. Крех, Девід Дж. Бае, Томас Джей Уолтерс Тривале (упродовж 16 годин) застосування джгута в умовах бойового поранення: Клінічний випадок та огляд актуальної літератури Інтернет-ресурс «Проект Віктора та Олени Пінчук» 8.11.2014 http://www.medsanbat.info/16-godinne-vikoristannya-dzhguta/

7. Bunker T. D. Uncontrollable bleeding under tourniquet / T. D. Bunker, A. H. Ratliff // Br. Med. J. (Clin. Res. Ed). – 1984. – Vol. 288. – P. 1905

8. Генинг Т.П., Ксейко Д.А. Показатели перекисного окисления липидов и антиоксидантной защиты в системе «сыворотка крови – эритроцит» при острой циркуляторной гипоксии // Успехи современного естествознания. – 2004. – № 4. – С. 17-20

9. Acute limb ischemia: contemporary approach / I. Fukuda, M. Chiyoya, S. Taniguchi, W. Fukuda // The Journal of Thoracic and Cardiovascular Surgery. – 2015. – Vol. 63 (10). – P. 540–548.

10. Effects of adrenomedullin and vascular endothelial growth factor on ischemia/reperfusion injury in skeletal muscle in rats / M. Kirisci, G. L. Oktar, C. Ozogul [et al.] // Journal of Surgical Research. – 2013. – Vol. 185 (1). – P. 56–63.

11. Protective effects of Sonic Hedgehog against ischemia/reperfusion injury in mouse skeletal muscle via AKT/mTOR/p70S6K Signaling / Q. Zeng, Q. Fu, X. Wang [et al.] // Cellular Physiology and Biochemistry. – 2017. – Vol. 43 (5). – P. 1813–1828.

12. Погляди на тимчасові методи зупинки кровотечі в умовах бойових дій / В. С. Тарасюк, М. В. Матвійчук, І. В. Паламар [та ін.] // Вісник вінницького національного медичного університету. – 2017. – № 1, Ч. 2 (Т. 21). – С. 220–227.

13. Березан С. Тактична медицина для підрозділів спеціального призначення / С. Березан, С. Ротчук // К. : ПП "МВЦ «Медінформ", 2016. – 33 с.

REFERENCES

1. Beaven, A., Ballard, R., & Parker, P. (2017). New effective tournicets for potential use in the military environment: A servimg soldier study. *Military Medicine,* 182 (7), 1929-1932.

2. Zhang, J., Zhang, J., Yu, P., Chen, M., Peng, Q., Wang, Z., & Dong, N. (2017). Remote ischaemic preconditioning and sevoflurane postconditioning synergistically protect rats from myocardial injury induced by ischemia and reperfusion partly via inhibition TLR4/MyD88/NF-κB Signaling Pathway. *Cellular Physiology and Biochemistry,* 41, 22-32.

3. Aslan T., Turer, A., & Joseph, H. (2010). Pathogenesis of myocardial ischemia-reperfusion injury and rationale for therapy. *The American Journal of Cardiology,* 106 (3), 360-368.

4. Eltzschig, H.K., & Collard, C.D. (2004). Vascular ischaemia and reperfusion injury. *British Medical Bulletin,* 70 (1), 71-86.

5. Mabry, R.L. (2006). Tourniquet use on the battlefield. *Mil. Med.*, 171, 352-356.

6. Dzhon, F., Krekh, Bae, D.Dzh., Uolters, T. D., Tryvale (uprodovzh 16 hodyn) zastosuvannia dzhhuta v umovakh boiovoho poranennia: Klinichnyi vypadok ta ohliad aktualnoi literatury [Prolonged (within 16 hours) use of a tourniquet in the conditions of a wounded wound: Clinical incident and review of actual literature Web-site "Project Victor and Olena Pinchuk"]. *Retrieved from:* http://www.medsanbat.info/16-godinne-vikoristannya-dzhguta/ [in Ukrainian].

7. Bunker, T.D., & Ratliff, A.H. (1984). Uncontrollable bleeding under tourniquet. *Br.Med. J. (Clin. Res. Ed.),* 288, 1905.

8. Gening, T.P., & Kseyko, D.A. (2004). Pokazateli perekisnogo okisleniya lipidov i antioksidantnoy zashchity v sisteme "sivorotka krovi – eritrotsit" pri ostroy tsirkulyatornoy gipoksii [Indicators of lipid peroxidation and antioxidant protection in the "blood serum – erythrocyte" system in acute circulatory hypoxia]. *Uspekhi sovremennogo estestvoznaniya – Advances in Modern Natural Science,* 4, 17-20 [in Russian].

9. Fukuda, I., Chiyoya, M., Taniguchi, S., & Fukuda, W. (2015). Acute limb ischemia: contemporary approach. *The Journal of Thoracic and Cardiovascular Surgery,* 63 (10), 540-548.

10. Kirisci, M., Oktar, G.L., Ozogul, C., Oyar, E.O., Akyol, S.N., Demirtas, C.Y., & Arslan, M. (2013). Effects of adrenomedullin and vascular endothelial growth factor on ischemia/reperfusion injury in skeletal muscle in rats. *Journal of Surgical Research*, 185 (1), 56-63.

11. Zeng, Q., Fu, Q., Wang, X., Zhao, Y., Liu, H., Li, Z., & Li, F. (2017). Protective effects of Sonic Hedgehog against ischemia/reperfusion injury in mouse skeletal muscle via AKT/mTOR/p70S6K Signaling. *Cellular Physiology and Biochemistry,* 43 (5), 1813-1828.

12. Tarasiuk, V.S., Matviichuk, M.V., Palamar, I.V., Korolova, N.D., Poliarush, V.V., Podolian, V.M., … Chorna, V.V. (2017). Pohliady na tymchasovi metody zupynky krovotechi v umovakh boiovykh dii [Views on temporary methods of stopping bleeding in combat situations]. *Visnyk vinnytskoho natsionalnoho medychnoho universytetu – Bulletin of Vinnytsia National Medical University,* 1, 2 (21), 220-227 [in Ukrainian].

13. Berezan, S., & Rotchuk, S. (2016). *Taktychna medytsyna dlia pidrozdiliv spetsialnoho pryznachennia [Tactical medicine for special purpose units].* Kyiv: PP "MVTS "Medinform" [in Ukrainian].

ЗМІНИ АКТИВНОСТІ ГЛУТАТІОНОВОЇ СИСТЕМИ ВНУТРІШНІХ ОРГАНІВ У ПЕРШІ ГОДИНИ ЕКСПЕРИМЕНТАЛЬНОГО ІШЕМІЧНО-РЕПЕРФУЗІЙНОГО СИНДРОМУ КІНЦІВКИ, ПОЄДНАНОГО З КРОВОВТРАТОЮ ТА МЕХАНІЧНОЮ ТРАВМОЮ

©Н. В. Волотовська, Т. Кліфф Нхоквара, І. В. Жулкевич

ДВНЗ «Тернопільський державний медичний університет імені І. Я. Горбачевського МОЗ України»

РЕЗЮМЕ. Крововтрата різного ґенезу незворотно призводить до гіпоксії, яка, в свою чергу, запускає активацію перекисного окиснення ліпідів і, як результат, ушкоджує мембрани клітин.

Мета – вивчити особливості відповіді ферментів глутатіонової системи внутрішніх органів на патогенетичний поштовх модифікацій ішемічно-реперфузійного синдрому в перші години після його застосування.

Матеріали і методи. Здійснено визначення вмісту глутатіонпероксидази (ГП), глутатіонредуктази (ГР) та відновленого глутатіону (ВГ) в гомогенатах внутрішніх органів щурів за умов експериментальної гострої ішемії-реперфузії (ІР), ІР, що була поєднана з механічною травмою (МТ), ІР, поєднаної з крововтратою (К), та ІР, поєднаної з К та МТ, та порівняння результатів із ізольованою крововтратою (40 % від ОЦК). Отримані показники оброблено статистично.

Результати. Експериментально встановлено, що найбільше зниження активності ферментів глутатіонової системи – її виснаження – відбувається на тлі синдрому ішемії-реперфузії, поєднаного із крововтратою та механічною травмою кінцівки. Уже в перші години в печінці, серці, нирках та легенях виявлено зниження рівня відновленого глутатіону в 28,8, в 30,6, в 31,0 та в 19,8 разів відповідно.

Висновки. Встановлено активну відповідь усіх досліджуваних показників. Що стосується активності ферментів, то встановлено наступну закономірність: у перші години ізольоване накладання джгута призводило до компенсаторних змін. Джгут, поєднаний з крововтратою, дав зниження активності дещо нижче, ніж сама крововтрата, що, на нашу думку, можна пояснити тим, що у велике коло кровообігу поступила значна концентрація патогенних

чинників – внаслідок ішемії, однак у першу годину вони встигли викликати, очевидно, тільки локальне ушкодження, тоді як гіпоксичні процеси, активовані власне крововтратою, відбувалися на її тлі на системному рівні.

КЛЮЧОВІ СЛОВА: ішемія-реперфузія; турнікет; кровотеча; експеримент; ішемія; механічна травма; глутатіонова система.

ИЗМЕНЕНИЯ АКТИВНОСТИ ГЛУТАТИОНОВОЙ СИСТЕМЫ ВНУТРЕННИХ ОРГАНОВ В ПЕРВЫЕ ЧАСЫ ЭКСПЕРИМЕНТАЛЬНОГО ИШЕМИЧЕСКОЙ-РЕПЕРФУЗИОННОГО СИНДРОМА КОНЕЧНОСТИ, СОЕДИНЕННЫХ С КРОВОПОТЕРЕЙ И МЕХАНИЧЕСКОЙ ТРАВМОЙ

©Н. В. Волотовская, Т. Клифф Нхоквара, І. В. Жулкевич

ГВУЗ «Тернопольский государственный медицинский университет имени И. Я. Горбачевского МОЗ Украины»

РЕЗЮМЕ. Кровопотеря различного генеза необратимо приводит к гипоксии, которая, в свою очередь, запускает активацию перекисного окисления липидов и, как результат, повреждает мембраны клеток.

Цель – изучить особенности реакции ферментов глутатионовой системы внутренних органов на патогенетический толчок модификаций ишемической-реперфузионного синдрома в первые часы после его применения.

Материалы и методы. Осуществлено определение содержания глутатионпероксидазы (ГП), глутатионредуктазы (ГР) и восстановленного глутатиона (ВГ) в гомогенатах внутренних органов крыс на фоне острой экспериментальной ишемии-реперфузии (ИР), ИР, осложненной механической травмой (МТ), ИР, осложненной кровопотерей (К), и ИР, осложненной К и МТ, и сравнение результатов с изолированной кровопотерей (40 % от ОЦК). Полученные показатели обработаны статистически.

Результаты. Экспериментально установлено, что наибольшее снижение активности ферментов глутатионовой системы – ее истощение – происходит на фоне синдрома ишемии-реперфузии, осложненного кровопотерей и механической травмой конечности. Уже в первые часы в печени, сердце, почках и легких обнаружено снижение уровня восстановленного глутатиона в 28,8, в 30,6, в 31,0 и в 19,8 раз соответственно.

Выводы. Установлен активный ответ всех исследуемых показателей. Что касается активности ферментов, то установлена следующая закономерность: в первые часы изолированное наложения жгута приводило к компенсаторным изменениям. Жгут, сочетанный с кровопотерей, дал снижение активности несколько ниже, чем сама кровопотеря, что, по нашему мнению, можно объяснить тем, что в большой круг кровообращения поступила значительная концентрация патогенных факторов – вследствие ишемии, однако в первый час они еще успели вызвать, очевидно, только локальное повреждение, тогда как гипоксические процессы, активированные собственно кровопотерей происходили на ее фоне на системном уровне.

КЛЮЧЕВЫЕ СЛОВА: ишемия-реперфузия; турникет; кровотечение; эксперимент; ишемия; механическая травма; глутатионовой система.

Отримано 11.02.2019