The influence of non-selective arginase inhibitors on some reparative regeneration indexes of experimentally resected liver

La influencia de los inhibidores no selectivos de arginasa en algunos índices de regeneración reparadora del hígado resecado experimentalmente

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Abstract

The impact of L-norvaline, a non-selective arginase inhibitor, on the state of the resected liver of *Wistar* strain white male rat, was evaluated. The resection of the liver was made on the second day of the experiment to the extent of 70%. L-norvaline ("WIRUD JmgH", Hamburg) was intragastrically administered, at a daily dose of 18mg/kg for the first seven days of the experiment, every 48 hours. The effect of the drug on the state of the resected liver was assessed according to the mortality rate, time of the volume recovery, the structure and functions of the organ. The mortality rate was being assessed in the experimental animal groups for the first ten days after the surgery. The animals were withdrawal from the experiment on the second, seventh, fourteenth, twenty-first, and twenty-eight days after the surgery. The cytolysis degree was assessed according to the AlAT, AsAT, and LDH indexes in the blood of the experimental animals, by an ultraviolet kinetic method. The synthesis function of the liver was evaluated according to the coagulogram indexes (APPT, INR, fibrinogen). The level of microcirculation in the liver was determined by laser doppler fluorometry. The morphological study was performed on the material of the standard liver sites taken after the animal had been withdrawal from the experiment. It was shown, that the non-selective arginase inhibitor, L-norvaline, stimulates liver regeneration after resection, which is manifested by decreased lethality, a significant decrease in the post-resection liver failure in comparison with the control group; significant earlier restoration of the volume, structure, and functions of the resected organ compared with the control group.

Keywords: non-selective arginase inhibitor, L-norvaline, liver resection, reparative regeneration, laser doppler fluorometry, nitric oxide.

Resumen

Se investigó el impacto la L-norvalina, un inhibidor no selectivo de arginasa, sobre el estado del hígado resecado de la rata macho blanca de la cepra Wistar. La resección del hígado se realizó el segundo día del experimento hasta un 70%. La L-norvalina ("WIRUD JmgH", Hamburgo) se administró intragástricamente a una dosis diaria de 18 mg/kg, durante los primeros siete días del experimento, cada 48 horas. El efecto del fármaco en el estado del hígado resecado se evaluó de acuerdo con la tasa de mortalidad, el tiempo de recuperación del volumen, la estructura y las funciones del órgano. La tasa de mortalidad se evaluó en los grupos de animales experimentales durante los primeros diez días después de la cirugía. Los animales fueron sacados del experimento los días segundo, séptimo, catorce, veintiuno y veintiocho después de la cirugía. La magnitud de la citólisis se evaluó de acuerdo con los índices AlAT, AsAT y LDH en sangre de los animales experimentales, mediante el método cinético ultravioleta. La función sintética del hígado se evaluó según los índices de coagulograma (APPT, INR, fibrinógeno). El nivel de microcirculación en el hígado se determinó por fluorometría láser doppler. El estudio morfológico se realizó sobre el material de los sitios estándar de hígado tomados después de que el animal había sido retirado del experimento. Se estableció que el inhibidor no selectivo de arginasa, la L-norvalina, estimula la regeneración del hígado resecado, que se demuestra por la disminución de la letalidad, disminución significativa de la insuficiencia hepática posterior a la resección en comparación con el grupo de control; la restauración significativamente temprana del volumen, estructura y funciones del órgano resecado comparado con el grupo de control.

Palabras clave: inhibidor de arginasa no selectivo, L-norvalina, resección hepática, regeneración reparadora, fluorometría láser doppler, óxido nítrico.

Introduction

The phenomenon of regeneration of the liver after being damaged was firstly described by Eshil in the tragedy "Prometheus Bound"[23]. A lot of works was devoted to learning this process. The ability of damaged liver regeneration was studied on different models. Regenerative processes in them have specific features. Respective models of the liver-damaging were widespread in studying the ability of regeneration of the organ after surgical treatments. Radical surgical treatments for the liver contribute to the reduction of blood flow and organ volume, which leads to a dangerous complication - post-resection liver failure. Lethality from this complication varies quite widely[1-5]. Liver regeneration as an organ in general, with the restoration of function, is one of the most important indicators in surgical hepatology, that defines the results of surgical treatments in many aspects. Nitric oxide (NO) plays an important role in the recovery of the liver. NO is formed from the amino acid L-arginine catalyzed by the enzyme NO-synthase (NOS)[6]. However, there is another way of metabolizing L-arginine, that is the enzyme arginase metabolizes L-arginine to urea and L-ornithine. The enzymes arginase and NO-synthase compete for the common substrate of L-arginine[7,8]. The activity of arginase is thousands of times greater than that of NO-synthase[9-13]. Arginase in the body is represented as two isoforms: arginase I - hepatic form, and arginase II - extra-hepatic form[14,15], localized more often in the kidneys, prostate, and small intestine. They catalyze the same biochemical reactions, but they differ in localization. There is evidence that liver ischemia-reperfusion (I/R) injury is associated with profound arginine depletion due to arginase release from injured hepatocytes[9,13,16]. Furthermore, arginase inhibits NOS, thus preventing the production of nitric oxide. Thus, it is suggested the inhibition of arginase would induce a reparative regeneration of the resected liver[7-12,14,17-19]. The purpose of this study was to determine whether arginase inhibition with the non-selective arginase inhibitor L-norvaline, would revert hepatic damage during liver resection.

Material and Methods

Experiment based on the Research Institute of Pharmacology of Living Systems of the Federal Autonomous Educational Institution "Belgorod National Research University" on the 170 white male Wistar rats, with an initial weight of 210-220 g. The supplier is a nursery «Stolbovaia» of the federal state budget institution of science "Scientific Centre of Biomedical Technologies of the Federal Medical-Biological Agency".

Experimental animals were divided into groups using the randomization method: 1. Intact (n=20); 2. Sham operated (n=50); 3. The liver resection (n=50); 4. The liver resection + L-norvaline (n=50).

Liver resection was performed on the second day of the experiment in a volume of 70%. The choice of this volume is based on literature. A model of liver resection in rats was first described in 1931 by Higgins and Anderson[4]. Later, adult rats were shown to have a directly proportional relationship between the volume of resection in 40 to 70% of their resection volume and DNA synthesis: in case of resection in the volume less than 30% regeneration lasts slower, and in the case of resections of more than 85% volume, regeneration is inefficient and highly lethal[20]. However, models with a small amount of liver resection (<45%) cannot be considered adequate, because the degree of liver damage in such cases is negligible. In contrast, high-resection models (≥70%) are the most successful, because they allow us to model the problem situation closest to it in clinical practice.

The rats were anesthetized with the intraperitoneal injection of zoletyl, and placed in position on its back. The fur on the abdomen was carefully lined; the skin was treated with a 70% solution of ethyl alcohol. The median laparotomy was performed at the swordfish process length of 4 cm. The liver was mobilized by crossing the ligaments. The left and medial lobes were removed after the pre-ligation of the receptacles. The laparotomy was sutured after abdominal sanitization and control of the hemostasis layered with a knotted suture.

Liver regeneration was stimulated in animal group 4 by intragastric injection of L-norvaline ("WIRUD Jmgh", Hamburg) at a daily dose of 10 mg/kg the first seven days of the experiment, every 48 hours. The first drug was administered 24 hours before the hepatectomy[17,21,22].

The level of microcirculation in the liver was determined by Biopac systems equipment: MP100 polygraph with the LDF100C laser doppler fluorometry module and the surface sensor TSD 140 on the 2nd, 7th, 14th, 21st and 28 days after the operation. Laser Doppler Fluorometry (LDF) results were recorded and processed using Acknowledge 4.2., Microcirculation values were expressed in perfusion units (PU). The microcirculation curve was recorded over the entire liver surface for 30 seconds at each point. From the values obtained, the average was derived, which was entered into the report and assumed as the level of microcirculation in the liver of the animal. Further, the average microcirculation level of a given group of animals at this time of the study was calculated.

Cytolysis expressed by the level of performance AlAT, AsAT, and LDH indexes, in experimental animals in the 2nd, 7th, 14th 21st, 28th days after the surgery. The indicators were determined by the UV kinetic method. The synthesizing function of the liver was assessed by coagulogram (APPT, INR, and fibrinogen) on the 7th, 14th, 21st, and 28th days after the operation.

The morphological study was performed on the material of the standard liver sites taken after the animal had been withdrawal from the experiment. The material was treated using standard formalin fixation, paraffin casting, hematoxylin, and eosin staining. General morphological and morphometric studies were carried out using a system for scanning and archiving images MiraxDesk.

Statistical analysis of the data was carried out in Microsoft Excel version 10.0 with the help of analytical package tools. «Descriptive statistics» were used to find the mean (M) of the

indicators and the standard error of the mean (m). «Double-sampling t-test with different variances» was used to compare the corresponding indicators in different animal groups and to determinethevalidityofthe differences betweenthem. A value of p<0.05 was considered statistically significant.

Results and discussion

Lethality was estimated in experimental animal groups for the first 10 days after the surgery. In the group of animals with liver resection, the lethality was 40%, while in the group of animals with liver resection treated with L-norvaline there was no lethality.

Among the group of intact animals, the microcirculation in the liver was 877±17 PU. The micro-circulation levels of the animals in the experimental groups are presented in Table 1.

Table 1. Microcirculation in the liver of experimental animals (M±m). Perfusion units (PU) at the 2nd, 7th, 14th, 21st and 28th days after the surgery

A group	The level of microcirculation in the liver (PE)				
	2nd day	7th day	14th day	21st day	28th day
Sham-operated	881±14	860±19	872±21	875±9	880±12
The liver resection	421±14*	429±9*	501±11*	591±17*	637±19*
The liver resection + L-norvaline	740±19**	738±18**	783±14**	933±21**	1110±31**

*p<0.05 compared to the group of intact animals (877±17 PU); **p<0.05 compared with animals with the resection of the liver at the appropriate time; sham-operated - laparotomy followed by contusion of the front abdominal wall on the second day of the experiment; liver resection at 70% on the second day of the experiment; L-norvaline - L-norvaline intragastrically at a daily dose of 10 mg/kg the first seven days of the experiment every 48 hours.

The average level of microcirculation in the liver of dipped animals at all times does not differ reliably from that of intact rats (p>0.1).

After liver resection, the rate drops by 50% and remains unchanged for the first 14 days. By 21 days, the rate starts to rise, however, on the 28th day, the average microcirculation level in the resected liver of rats is lower than that of intact animals (p<0.05). L-norvaline administration contributed to the preservation of blood flow in the cut liver at a fairly high level (the rate drops by 15%) and recovered it for up to 21 days after resection.

AlAT in the control group on the 2nd day was 108±4 ME/L and was reduced to normal values by 21 days - 38±2 ME/L. In the group, L-norvaline on the 2nd day was 60±2 ME/L and was normalized on the 7th day and remains within normal values on all other dates of the experiment.

AsAT in the control group on the 2nd day was 184±7 ME/L, and it was reduced to normal values by 21 days. In the group with L-norvaline on the 2nd day was 82±3 ME/L, and was normalized on the 14th day and stays within normal values on all other dates of the experiment.

The LDH in the control group on the 2nd day was 564±3 ME/L and dropped to normal by 28 days with a value of 250±6 ED/L. In the group treated with L-norvaline on the 2nd day was 300±8 ME/L and it was normalized on the 7th day and remains within normal values on all other dates of the experiment.

On the 7th day after the liver resection, there was a reduction in the synthetic function of the liver, which is shown by the extension of the APPT to 60±1s, increase of the INR to 4±0.2, a decrease of the fibrinogen level to 1.7±0.002 g/l. The largest changes in the indicators were detected on the 21st day (APPT - 75±1 sec, INR - 6±0.1, fibrinogen 0.9±0.003 g/L). Some recovery is recorded on the 28th day (APPT - 55±1 sec, INR 4±0.1, fibrinogen 1,2±0,002 g/l).

L-norvalinetreatmentcontributed to alesspronouncedreduction inthe synthetic function ofthe liver after its resection. The most marked changes were recorded in the 7th day (APPT-39±1 seconds, INR - 2.5±0.02, fibrinogen 1.9±0.01 g/l). By the 14th day, the numbers had recovered and remained within the normal range at all other times of the experiment. The mass of the liver in intact animals was 9.2±0.002 gr. In the group of false-dipped animals, the indicator does not differ reliably from that of intact animals.

A mass of the liver immediately after resection was 2.8±0.003g. The mean mass of the liver of animal experimental groups at different test dates showed in Table 2.

Table 2. An average mass of the liver of the experimental groups' animals (M ± m) in grams at the 2nd, 7th, 14th, 21st and 28th days after the surgery

A group	An average mass of the liver in grams				
	2nd day	7th day	14th day	21st day	28th day
The liver resection	5.1±0.01*	4.2±0.01*	5±0.01*	5.9±0.01*	6.3±0.02*
The liver resection + L-norvaline	5.4±0.01**	7.3±0.01**	8.3±0.01**	9.3±0.021**	10.2±0.02**

*p<0.05 compared to the group of intact animals; **p<0.05 compared with animals with the resection of the liver at the relevant time; liver resection at 70% for the second day of the experiment; L-norvaline - L-norvaline intragastrically at a daily dose of 10 mg/kg the first seven days of the experiment every 48 hours.

In a group of animals with liver resection, 2 days after the liver mass increased when compared with the mass immediately after resection almost doubled. By the 7th day, it decreases slightly, then it started to increase, but by the 28th day after resection, the liver mass was slightly lower than that of intact rats. In the group of animals with liver resection L-norvaline administration induced an increase of liver mass, indeed in two days increases exactly two times, continuing to grow and reaching mass in intact animals by 21 days (p<0.001).

In the histological study on the 2nd day in the control group, revealed edema of stroma, necrosis of groups of hepato-

cytes, an erratic hemorrhaging, and in some cases, there was a sign of a shock liver. In the later years, irregular blood filling, discomplexing of the finning and girder structure, dystrophic changes, monocellular necrosis. In microscopy - at a distant time in the control group there was uneven blood filling of all types of vessels, Pockets of granular dystrophy of hepatocytes, of sclerosis, and inflammatory infiltration of portal pathways was observed. Recovery of hepatic tissue was achieved by hypertrophy of single-nucleus hepatocytes, polyploidia, and amytic division of binuclear hepatocytes. In the experimental group with L-norvaline administration, the stalk-balcony structure is preserved at an early date; there is no sign of shock liver. There's no evidence of microcirculatory disorders or hepatocyte damage in the long term, evenly expressed cytoplasmic hypertrophy and hepatocyte nuclei are determined. The recovery of hepatic tissue was due to polyploidia, amytic division of two-nuclear hepatocytes, and mitotic division of single-nuclear hepatocytes, which means that all kinds of liver regeneration were involved. The main load was on single-core hepatocytes with a core size of 9 µm on the 2nd and 7th day, indicating the stimulation of the cell's genetic apparatus.

Conclusion

1. L-norvaline administration to animals with 70% liver resection contributed to reduced mortality from post-resection hepatic insufficiency, a significant reduction in symptoms of post-resection hepatic insufficiency compared to the control group, recovery of the volume, structure, and functions of the resected organ up to 21 days after resection, which was not observed in the control group

2. The Nonselective arginase inhibitor, L-norvaline, at a dose of 10 mg/kg stimulates reparative regeneration of the resected rat liver.

References

1. Bobyntsev, I.I., Kryukov, A.A., Belykh, A.E., Dudka, V.T., 2016. Effect of delta sleep-inducing peptide on functional state of hepatocytes in rats during restraint stress. Bull. Exp. Biol. Med., 160(4): 421-424.
2. Chan, J., Perini, M., Fink, M., Nikfarjam, M., 2018. The outcomes of central hepatectomy versus extended hepatectomy: a systematic review and meta-analysis.HPB (Oxford), 20(6): 487-496.
3. Lee, E.S., Chen, H., Hardman, C., Simm, A., Charlton, C., 2008. Excessive S-adenosyl-L-methionine-dependent methylation increases levels of methanol, formaldehyde and formic acid in rat brain striatal homogenates: possible role in S-adenosyl-L-methionine-induced Parkinson's disease-like disorders. Life Sciences, 83(25-26):821-827.
4. Higgins, G.M., Anderson, R.M., 1931. Experimental pathology of the liver. I. Restoration of liver of white rat following partial surgical removal. A.M.A. Arch. Path., 12: 186-202.
5. Kountouras, J., Boura, P., Lygidakis, N.J., 2001. Liver regeneration after hepatectomy. Hepatogastroenterology, 48: 556-562.
6. Korokin, M.V., Pokrovsky, M.V., Novikov, O.O., Gureev, V.V., Denisyuk, T.A., Korokina, L.V., Polyanskaya, O.S., Ragulina, V.A., Pokrovskaya, T.G., Danilenko, L.M., Belous, A.S., 2011. Effect of L-arginine, vitamin B6 and folic acid on parameters of endotheli- al dysfunction and microcirculation in the placenta in modeling of L-NAME-induced NO deficiency. Bull. Exp. Biol. Med., 152(1):70-72.
7. Bachetti, T., Comini, L., Francolini, G., Bastianon, D., Valetti, B., Cadei, M., Grigolato, P.G., Suzuki, H., Finazzi, D., Albertini, A., Curello, S., Ferrari, R., 2004. Arginase pathway in human endothelial cells in pathophysiological conditions.J. Mol. Cell. Cardiol., 37(2):515-523.
8. Huynh, N.N., Harris, E.E., Chin-Dusting, J.F.P., Andrews, K.L., 2009.The vascular effects of different arginase inhibitors in rat isolated aorta and mesenteric arteries. Br. J.Pharmacol., 156(1):84-93.
9. Kurganov, N.A., Blinova, E.V., Semeleva, E.V., Gromova, I.A., Blinov, D.S., Novikov, A.V., Mashkova, J.N., Vasilkina, O.V., 2018. 2-aminoaethanesulfonic acid compounds possess protective property in reperfusion-induced heart injury. Research Results in Pharmacology, 4 (2): 19-26.
10. Ming, X., Pajapakse, A.G., Carvas, J.M., Ruffieux, J., Yang, Z., 2009.Inhibition of S6K1 accounts partially for the anti-inflammatory effects of the arginase inhibitor L-norvaline. BMC Cardiovascular Disorders, 12: https://doi.org/10.1186/1471-2261-9-12.
11. Pokrovskiĭ, M.V., Pokrovskaia, T.G., Gureev, V.V., Barsuk, A.A., Proskuriakova, E.V., Korokin, M.V., Gudyrev, O.S., Belous, A.S., Kochkarov, V.I., Danilenko, L.M., Levashova, O.V., Mal'tseva, N.V., Polianskaia, O.S., 2012. Correction of endothelial dysfunction by L-arginine under experimental pre-eclampsia conditions. Eksp. Klin. Farmakol., 75(2):14-16.
12. Voronkov, A.V., Pozdnyakov, D.I., 2018. Endothelotropic activity of 4-hydroxy-3,5-di-tret-butylcinnamic acid in the conditions of experimental cerebral ischemia. Research Results in Pharmacology, 4 (2): 1-10.
13. Zweier, J.L., Samouilov, A., Kuppusamy, P., 1999. Non-enzymatic nitric oxide synthesis in biological systems. Biochim. Biophys. Acta., 1411 (2-3): 250-262.
14. Pokrovskii, M.V., Korokin, M.V., Kudryavtsev, K.V., Pokrovskaya, T.G., Gudyrev, O.S., Gureev, V.V., Korokina, L.V., Povetkin, S.V., 2017. Study of endothelial protective activity of phenol-derived thrombin and arginase-2 inhibitors KUD-259 and KUD-974.Bull. Exp. Biol. Med., 163(4):436-438.
15. Scibior, D., Czeczot, H., 2004. Arginine--metabolism and functions in the human organism. PosteryHig. Med. Dosw., 58:321-332.
16. Sommer, C., 2008. Ischemic preconditioning: postischemic structural changes in the brain.J. Neuropathol. Exp. Neurol.,67 (2):85-92.
17. Kolesnik, I.M., Pokrovskiy, M.V., Lucenko, V.D., Sernov, L.N., Povetkin, S.V., Dolzhikov, A.A., Reznikov, K.M.,2016. New possibility of application of L-arginine and L-Norvaline in surgery. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(6):3272-3276.
18. Korokin, M.V., Pokrovskiy, M.V., Kochkarov, V.I., Gudyrev, O.S., Korokina, L.V., Pokrovskaia, T.G., Gureev, V.V., 2014.Endothelial and cardio protective effects of tetrahydrobiopterin, L-norvaline, L-arginine and their combinations by simulation of hyperhomocysteine induced endothelial dysfunction. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 5(6):1375-1379.
19. Solin, A.V., Lyashev, Y.D., Tsygan, N.V., 2019. Hepatoprotective effect of opioid peptides in stress. Research Results in Pharmacology, 5 (1): 77-96.

20. Alikhanov, R.B., Vishnevskiy, V.A., Kubyshkin, V.A., Ikramov, R.Z., Eshcanov, R.G., Kozyrin, I.A., 2013. Risk factors predisposing and producing hepatic failure after major hepatic resection. Kubanskii Nauchnyi Meditsinskii Vestnik, 7:51-53.

21. Sharquie, I. K. (2020). BCG is a Good Immunotherapeutic Agent for Viral and Autoimmune Diseases: Is it a New Weapon against Coronavirus (COVID-19)?. Electron J Gen Med. 2020; 17 (6): em229.

22. Gupta, P. K., Bhaskar, P., & Maheshwari, S. (2020). Coronavirus 2019 (COVID-19) Outbreak in India: APerspective so far. J Clin Exp Invest, 11(4).

23. Ziman, J. M. (1994). Prometheus bound. Cambridge University Press.

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