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Research Article

INFLUENCE RECOMBINANT ERYTHROPOIETIN SPEED VOLUME PERFUSION AND MORPHOLOGICAL CHANGES DURING REPERFUSION LIVER INJURY

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Abstract

Recombinant erythropoietin (EPO) ("Epokrin") for 30 min before ischemia in liver at 5-500 IU/kg dose-dependent protective effect exerted during ischemia reperfusion liver. Thus, in controlling the volume of perfusion rate measured by lazerdopplerovskoyflowmetry 15 min reperfusion increased up to 1983 PE, and then decreased to 500 PE 30 min reperfusion. EPO at a dose of 500 IU/kg prevented the severity of reactive hyperemia and volumetric perfusion rate at 15 minutes was 1308 PE. A similar prevention of reactive hyperemia was found in modeling and liver ischemic preconditioning (1338 PE). Introduction EPO dose-dependently reduced the severity of hepatocellular damage during ischemia and reperfusion, resulting in virtually no necrobiotic changes to 30 minutes, and their low severity by 30 minute reperfusion.

Key words: Ischemia, reperfusion, liver, blood flow, morphological changes.

Ischemic and reperfusion complications take leading role in developmentinternal organs different diseasesas liver injury during surgical interventions with decreasing blood supply. The possibility of direct and remote ischemic preconditioning simulation is an experimental pharmacology keystone. The greatest interest among a number ofsubstances for pharm modeling ischemic preconditioning [1-4], it is a recombinant human erythropoietin showed to be effective inprotecting to myocardial and brain tissues ischemia/reperfusion injuries protection [5-13].

However data of human recombinant erythropoietin usage in liver ischemia/reperfusion correction are rather contradictory [14,15].

The goal of our investigation was to assess the influence of human recombinant erythropoietin different dosage on blood flow velocity as microcirculation changes evaluation screening method.

Materials and methods: 70 white (male and female) 280-300g rates were used. Animals were grouped in 7 sets by 10 rates each. I/R group: reperfusion 30 minutes followed by 30 minutes of ischemia. I/R+EPO in dose 5 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 5 IU/kg human recombinant erythropoietin. I/R+EPO in dose 25 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 25 IU/kg human recombinant erythropoietin. I/R+EPO in dose 50 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 50 IU/kg human recombinant erythropoietin. I/R+EPO in dose 100 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 100 IU/kg human recombinant erythropoietin. I/R+EPO in dose 200 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 200 IU/kg human recombinant erythropoietin. I/R+EPO in dose 500 IU/kg group: reperfusion 30 minutes followed by 30 minutes of ischemia pretreated with 500 IU/kg human recombinant erythropoietin. All interventions were made under general anesthesia («Zolitel 100» 60 mg/kg with chloral hydrate 125 mg/kg intraperitonealy).

Transientdeep liver ischemia reproduced by temporary hepatoduodenal ligament compression for 30 min [16, 17].

Human recombinant erythropoietin («Epocrin» obtained from StateSRU ultrapure biological drags FMBA FGUP, Russia)injected intraperitonealy 50 IU/kg 30 min before ischemia.

Blood flow velocity was measured by Biopaq systems MP150 with TSD144 probe in perfusion units (PU).

Direct ischemic preconditioning was reproduced 30 min ahead of deep ischemia episode by 10 min hepatoduodenal ligament compression.

For control method we used standard histological investigation with hematoxylin/eosin dye.

During research we found blood flow velocity were on 850.48± 19.75 PU level. Deep ischemia episode leads to perfusion dropping to zero level with restoration on 1 minute till 120.17±4.7 PU, changed with transient hyperemia 1983.22±63.35 PU on 15 minute and decreasing till 611.63±27.43 PU on reperfusion 30 minute. According obtained data the best time for assessing is reperfusion 15 minute as maximum volatile point. Direct ischemic preconditioning largely decrease transient hyperemia till 1338.46± 14.06 PU on 15 minute, changed by 500.16± 16.41 PU on 30 minute blood flow velocity investigation. Human recombinant erythropoietin injection in doses 5, 25, 50, 100, 200, 500 IU leads to transient hyperemia decreasing with maximum effect in 200 and 500 IU/kg doses (Table 1). Statistic analysis revealed no differences between groups with 200 and 500 IU/kg, moreover small distinctions between groups with 50 and 100 IU/kg were foundand dose of 50 IU/kg we decided to use later as safer one. Histological

examination showed complex of nonspecific changes caused by ischemic damage and characterized by portal vessels and sinusoids desolation combined with pronounced dystrophic, necrobiotichepatocytes changes and microcirculation impairment (Fig.1.). Reperfusion injury appear as severe sinusoidal dilation with diapedetic bleeding increasing dystrophic and necrobiotic changes (Fig.2.). Human recombinant erythropoietin injection (Epocrin) 50 IU/kg decreased hepatocelular damage and manifested in necrobiotic changes absence in late stages of 30 minute of ischemia and their small presence at 30 minute of reperfusion. It's characteristic in group with EPO to haven't microthrombosis and stromal leakage (Fig.3, Fig.4.)

Table 1: Human recombinant erythropoietin different dosage effect on blood flow velocity in liver microvascular vessels during ischemia and reperfusion (PU) (M±m, n=10).

Groups	15reperfusonminute
I/R	1983.22±63.35*
I/R+EPO in dose 5 IU/kg	1856.38±72.12**
I/R+EPO in dose 25 IU/kg	1789.28±22.58**
I/R+EPO in dose 50 IU/kg	1447.93±23.72**
I/R+EPO in dose 100 IU/kg	1367.81±34.28**
I/R+EPO in dose 200 IU/kg	1295.26±54.82**
I/R+EPO in dose 500 IU/kg	1308.14±31.87**
I/R+IPC	1338.46±14.06**

* - $p \leq 0.05$ versus against intact group data, ** - $p \geq 0.05$ – versus against ischemia/reperfusion group data.

Conclusion. Thereby the study found that the recombinant erythropoietin dose-dependently prevented the development of reactive hyperemia 15 minute reperfusion. Prekonditsionuyuschey optimal dose is 50 IU/kg. The positive effect of recombinant erythropoietin also confirmed by morphological study and is manifested in the absence of thrombosis and hemorrhage, minimum changes in the severity of necrobiotic a tsentrolobulyarnyh necrosis and venous plethora

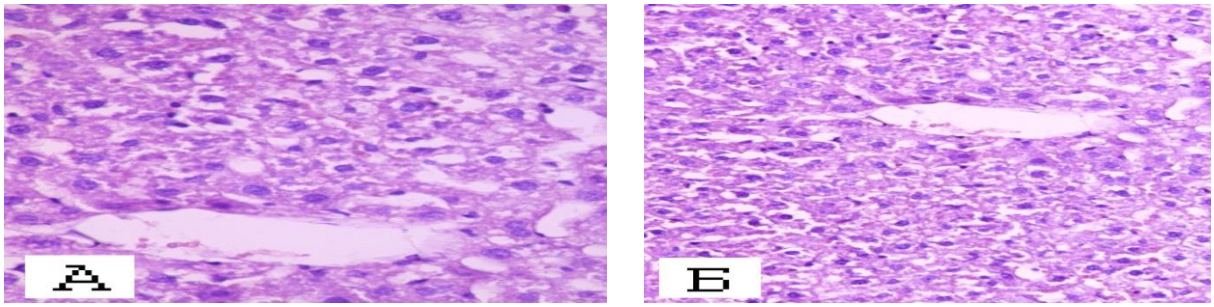

Figure 1. Ischemic liver injury: centrolobularanaemia, compact grain liver dystrophy. Hematoxylin and eosin dye. Microphoto. А) Х 200. Б) Х400.

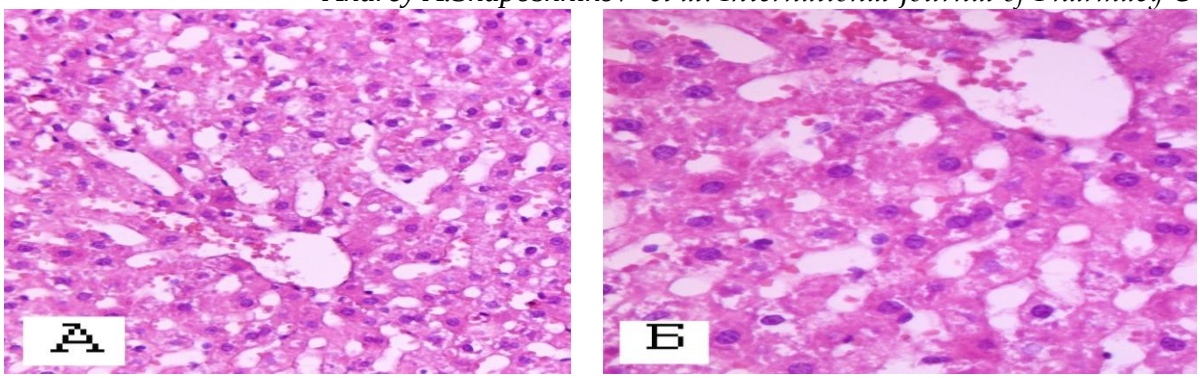

Figure 2. Reperfusion liver injury: severe necrobiotic and dystrophic changes, diapedetichaemorrhage focus. Hematoxylin and eosin dye. Microphoto. А) Х 200. Б) Х400.

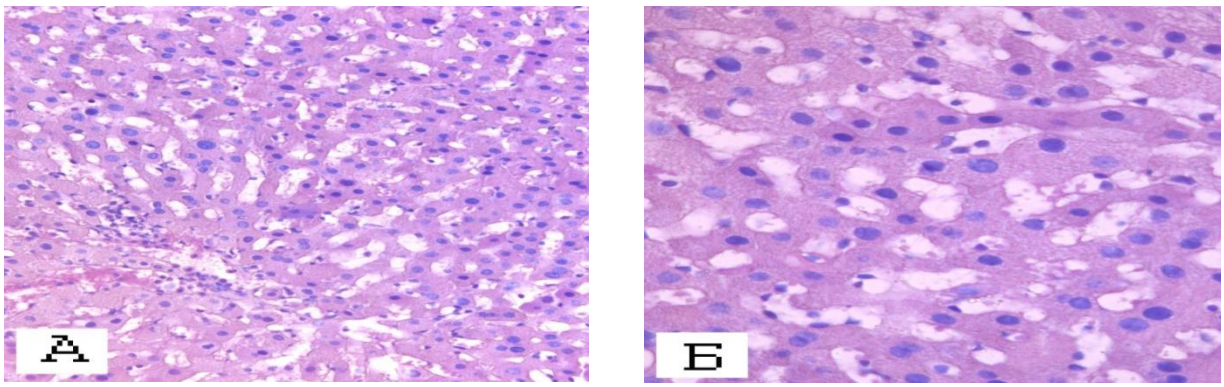

Figure 3. 50 IU/kg erythropoietin influence on ischemic liver injury: mild centrolobularenimia and absence of dystrophic changes. Hematoxylin and eosin dye. Microphoto. А) Х 200. Б) Х400.

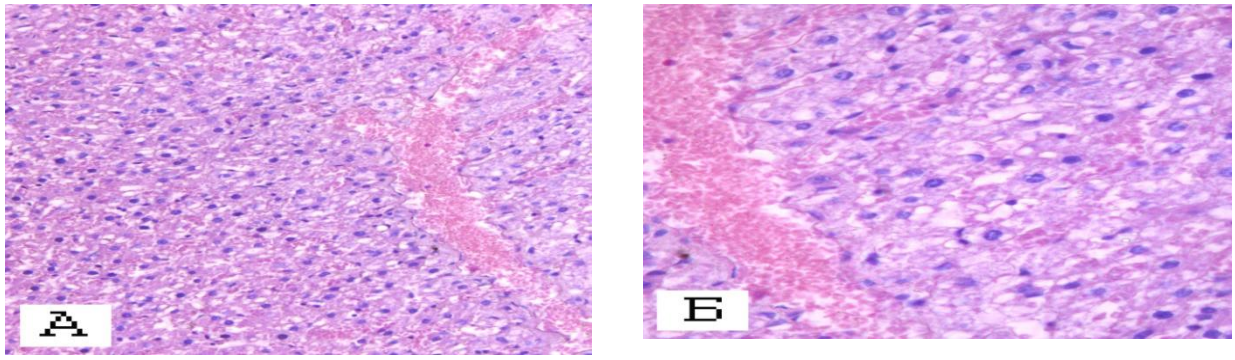

Figure 4. IU/kg erythropoietin influence on reperfusion liver injury: venous hyperaemia, absence of hemorrhage. Hematoxylin and eosin dye. Microphoto. А) Х 200. Б) Х400.

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