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SHORT COMMUNICATION

Role of Hepatovasculature in Warm Ischaemia-Reperfusion Injury of Rat Liver

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Summary

Liver haemodynamics were studied after warm (37 °C) ischaemia of isolated rat livers for periods of 30 s (Group 1), 30 min (Group 2), and 60 min (Group 3) using a constant pressure system with a recirculating blood-free perfusate. Portal flow recovered to basal values within 6 min in livers from Group 1, whereas it was significantly reduced in Group 2 during the initial 15 min and in Group 3 during the first 33 min of reperfusion. Thus, the recovery of liver flow was proportional to the duration of ischaemia. By using the same mode of liver perfusion, the effect of norepinephrine on portal resistance was also studied in normal livers. At the beginning of reperfusion, the values of portal resistance in ischaemic livers were comparable to the values of portal resistance mediated by norepinephrine at concentrations between 10^{-7} and 10^{-6} mol/l in normal livers. The results suggest that vasoconstriction of the hepatovasculature may be a contributing factor to the reperfusion injury of the liver following warm ischaemia.

Key words
Ischaemia – Reperfusion – Liver – Portal flow and resistance

Ischaemia and reperfusion involve a cascade of pathophysiological events leading to irreversible loss of cell viability. In an *in vivo* model of liver ischaemia, two phases of injury have been identified, namely a moderate or first phase (1 hr of reperfusion) and a severe inflammatory phase (24 hr of reperfusion) (Jaeschke *et al.* 1990). Kupffer cells are thought to play a major role in the first phase of reperfusion-related liver injury, whereas neutrophils appear to be mainly involved in the inflammatory phase of injury (Jaeschke *et al.* 1990). During the first phase of injury, the velocity of leukocytes in liver sinusoids was decreased as early as 5–15 min after starting reperfusion (Koo *et al.* 1989) and liver blood flow failed to recover completely (Chávez-Cartaya *et al.* 1994a). Neutropoenia significantly improved liver blood flow suggesting that neutrophils may also be involved in the first phase of reperfusion injury (Chávez-Cartaya *et al.* 1994b). Since the contributing effect of the hepatovasculature to the decreased flow through the liver after ischaemia is not possible to determine in an *in vivo* study, the aim of the present investigation was to determine this effect following different periods of ischaemia of the isolated rat liver.

Pentobarbital sodium and heparin sulfate were purchased from Spofa (Prague, Czech Republic). Norepinephrine bitartarate and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO). All other chemicals were of the highest purity commercially available. Male Wistar non-fasted rats, 200–300 g, (SPF, standard) were used. The rats were given pentobarbital sodium intraperitoneally (50 mg/kg) to induce anaesthesia prior to surgery. The surgical procedure for the preparation of the rat liver for perfusion has already been described (Bezek *et al.* 1990, Kukan *et al.* 1995). The perfusion system was modified according to Ballet *et al.* (1988). During surgery, the livers were perfused at 37 °C with about

400 ml of blood-free Krebs-Henseleit bicarbonate buffer pH 7.4, fortified with glucose (10 mM) and saturated with 95 % oxygen and 5 % carbon dioxide.

In the first set of experiments, three groups of rat livers were used in which ischaemia was induced by stopping the perfusate flow for 30 s (Group 1; n=5), for 30 min (Group 2; n=6) and for 60 min (Group 3; n=6). The livers were kept at 37 °C in Krebs-Henseleit bicarbonate buffer during periods of ischaemia. All three groups of livers were then reperfused in a recirculating system with the same perfusion medium as given above at a constant pressure of 12 cm H_2O, which was maintained by an overflow mechanism (for details see Fig. 1 of Ballet *et al.* 1988). The volume of the perfusion medium was 200 ml. To determine the perfusate steady-state dynamics, portal perfusate flow was measured every 3 min. This was accomplished by measuring the volume of perfusate collected over 1 min after diversion of the outflow. Portal resistance (R) was calculated by dividing perfusion pressure/perfusion rate:

$$R = (P_1 - P_2)/Q \quad (\text{mm Hg.ml}^{-1}\text{.min.g liver})$$

where Q is portal perfusate flow, P_1 is the perfusion pressure (12 cm H_2O and P_2 is the venous pressure, which was considered to be negligible as the hepatic vein was sectioned and the venous perfusate flowed freely into the reservoir. Statistical analysis was carried out by the unpaired Student's t-test.

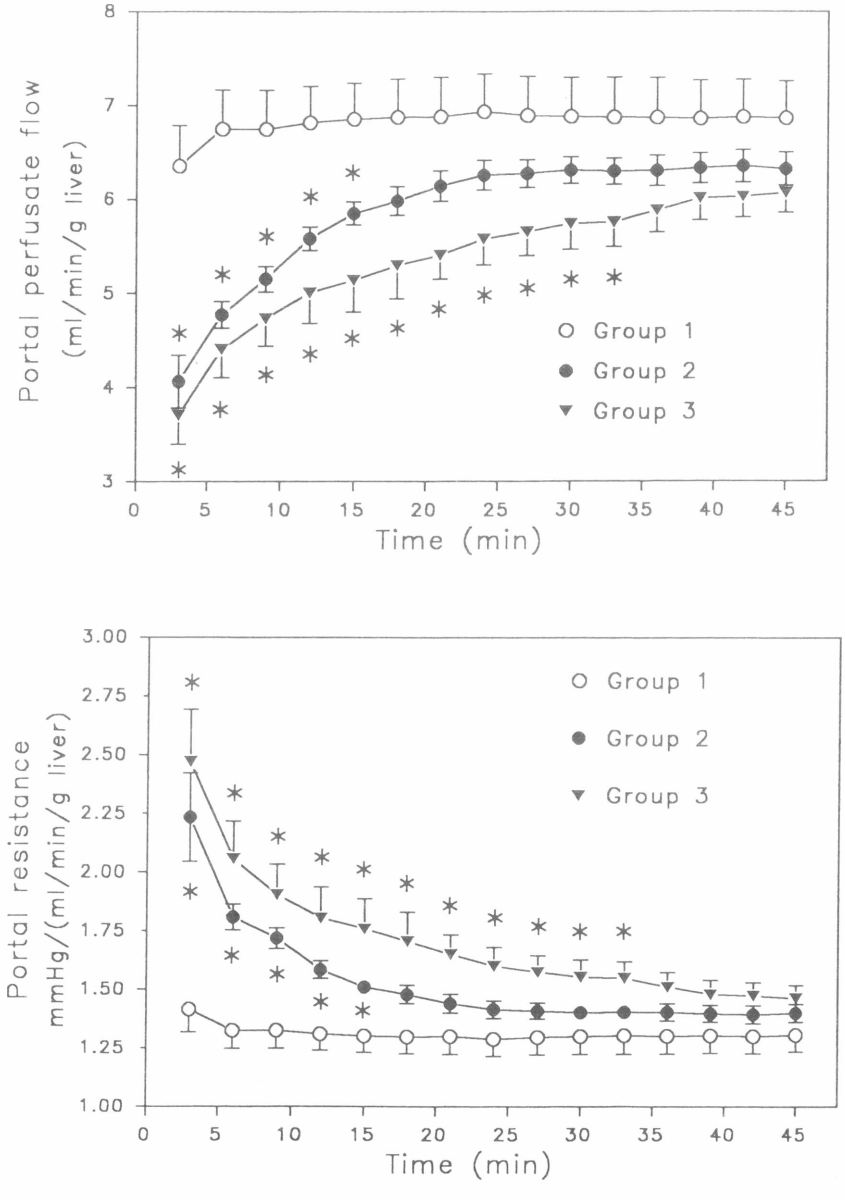

Fig. 1
*The effect of different periods of ischaemia on portal perfusate flow (upper panel) and portal resistance (lower panel) in isolated rat liver. Ischaemia was performed by stopping the perfusate flow for 30 s (Group 1; n=5), for 30 min (Group 2; n=6) and for 60 min (Group 3; n=6). All three groups of livers were then reperfused in a recirculating system with blood-free Krebs-Henseleit bicarbonate buffer. Each point represents mean ± S.E.M. * Significantly different (p<0.05) from Group 1.*

The finding of a substantial reduction of portal perfusate flow after ischaemia in both Group 2 and 3 (see Fig. 1) led us to examine the effect of one of the major vasoactive agents – norepinephrine – on the hepatovasculature, using our model of liver perfusion. Thus, in this second set of experiments, norepinephrine

(dissolved in Krebs-Henseleit buffer containing 20 μg/ml of ascorbic acid to prevent oxidation) was added stepwise from 10^{-9} to 10^{-3} mol/l into the reservoir at 5 min intervals and the response was measured. These experiments were performed using normal livers that had not been subjected to ischaemia.

Portal flow and portal resistance during 45 min reperfusion after various periods of ischaemia are shown in Figure 1. In Group 1, a plateau of the flow and thus of resistance was attained shortly after the onset of reperfusion. Portal flow was significantly reduced in both Group 2 and 3 in comparison to Group 1. As can be seen from Figure 1, the duration of ischaemia prolonged the time for reaching recovery of portal perfusate flow. The concentration-effect relationship of norepinephrine on portal resistance on using normal (non-ischaemic) livers is shown in Table 1. An increase in portal resistance at the beginning of reperfusion in ischaemic livers corresponded to norepinephrine-induced vasoconstriction in the concentration range between 10^{-7} and 10^{-6} mol/l of norepinephrine in normal livers (cf. Fig. 1, lower panel and Table 1).

Table 1
The concentration-effect relationship of norepinephrine on portal resistance using normal non-ischaemic livers.

Norepinephrine concentration (mol/l)	Portal resistance mm Hg.ml^{-1}.min.g liver
Without	1.31 ± 0.19
10^{-9}	1.33 ± 0.21
10^{-8}	1.36 ± 0.21
10^{-7}	1.86 ± 0.32
10^{-6}	3.90 ± 0.89*
10^{-5}	11.89 ± 5.26*
10^{-4}	10.99 ± 2.85*
10^{-3}	8.23 ± 2.10*

Values are the means ± S.E.M. from three experiments.
** Significantly different ($p < 0.05$) from the basal value.*

In the present study, the livers from fed rats were used for the characterization of the effect of ischaemia on portal perfusate flow. Since a constant pressure system was adopted for the experiments, the portal perfusate flow could be monitored, portal resistance calculated, and the role of the hepatovasculature in ischaemia-reperfusion injury was determined.

In Group 1, the steady-state portal resistance was lower by about 50 % than that reported by Ballet *et al.* (1988) who used a perfusion buffer diluted with whole rat blood. It was, however, nearly identical to values obtained by Minor and Isselhard (1993) who used Krebs-Henseleit for perfusion buffer. The low viscosity of the Krebs-Henseleit buffer may account for the difference of our data and those published by Ballet *et al.* (1988). Considering the results of portal resistance in our ischaemia groups of livers during reperfusion, two discrepancies with the study of Minor and Isselhard (1993) were noted. First, in their study the steady-state of portal resistance was reached 10 min after reperfusion and it remained stable during the whole reperfusion period (cf. Fig. 1 of Minor and Isselhard (1993) and Fig. 1 of this paper). Second, the portal resistance was doubled after 60 min of ischaemia, whereas our results showed only a moderate increase in resistance under steady-state conditions. Minor and Isselhard (1993) subjected the livers to warm ischaemia for a period of 60 min followed by cold ischaemia for 60 min. This combination of both warm and cold ischaemia or warm ischaemia with cooling of the liver may damage the hepatovasculature substantially. Very recently, Bilzer and Lauterburg (1994) found a transient increase in portal pressure after 45 min of warm hepatic ischaemia in isolated rat livers (using a constant flow system) both in the presence or absence of human polymorphonuclear leukocytes. In an *in vivo* study, Chávez-Cartaya *et al.* (1994a) found that rat liver blood flow was inversely proportional to the duration of ischaemia and improved by neutropoenia (Chávez-Cartaya *et al.* 1994b). Although in this study portal perfusate flow recovered (Fig. 1), the longer was the period of ischaemia the longer time was needed for reaching the recovery of flow. Confrontation of our data on portal perfusate flow (see Fig. 1) and the data on liver blood flow measured *in vivo* (see Fig. 1 of Chávez-Cartaya *et al.* 1994a) indicate that the hepatovasculature contributes substantially to the reduction of flow through the liver during the first 15 min of reperfusion. The comparison of norepinephrine-induced vasoconstriction in our model of liver perfusion with that found at the beginning of reperfusion following ischaemia indicates a profoundly impaired hepatovasculature. The platelet activating factor thromboxane(s) and other vasoconstrictors released by activated Kupffer cells after ischaemia might account for this effect (Ballet 1990).

In conclusion, the results of this investigation showed that reduction of portal perfusate flow correlated with the duration of hepatic ischaemia even in the absence of blood constituents. Thus vasoconstriction of the hepatovasculature may contribute to liver injury following ischaemia by reducing portal flow.

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Reprint Requests

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