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Hepatic Microcirculation in Inflow and Inflow–Outflow Occlusion of the Liver

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ABSTRACT

Background. Total vascular exclusion (TVE) causes warm liver ischemia. The aim of this study was to investigate the patterns of injury caused by inflow-outflow obstruction in the rat liver.

Materials and Methods. Twenty-four Wistar-Albino rats were divided into three groups: liver inflow occlusion (Group A), inflow-outflow occlusion (Group B) and intermittent inflow-outflow occlusion applied for 15 minutes. Microcirculation was measured with laser Doppler flowmetry during the procedure. Samples for biochemical and histopathological analyses were collected at the end of the ischemia period.

Results. Significant alterations in microcirculation were determined by application of vascular control maneuvers. Microcirculation in the central and dome segments were affected adversely compared with the dome segments in all experimental groups. TVE induced severe disturbances in hepatic microcirculation with more prominent hepatocellular damage. Damage to central segments of the rat liver was more prominent with inflow occlusion; whereas inflow-outflow occlusion produced more prominent damage to dome segments. Intermittent application of TVE clamping was associated with more hepatocellular damage compared with continuous TVE.

Conclusion. Our mapping methodology within the liver parenchyma suggested that hepatovenous back-perfusion is a principle source of continuity of microcirculation in the rat liver during inflow occlusion. Inflow-outflow occlusion caused more tissue damage compared with inflow occlusion. Ischemic preconditioning during TVE did not increase the tolerance of the liver against ischemia.

ISCHEMIA-reperfusion injury of the liver is a challenging clinical problem during transplantation and hepatic surgery. Vascular control maneuvers are performed during liver surgery to reduce the blood loss. Two alternative methods for vascular control are hepatic inflow occlusion (Pringle maneuver) and total vascular exclusion (TVE).[1–4] The mechanisms responsible for the cellular damage associated with ischemic and hypoxic liver injuries due to vascular control maneuvers are under investigation. Some workers have focused on the topographic distribution of liver injury during ischemic and hypoxic liver injury.[5–21] Controversies about the distribution of cellular damage during ischemia-reperfusion of the liver continue. Our prior experimental studies on selective application of TVE to the rat liver[21–23] have now investigated the segmental distribution of liver microcirculation and injury in rat liver inflow and inflow-outflow obstruction models. Ischemic preconditioning, a well-studied approach to control adverse effects of inflow occlusion has not been well studied in animal models of TVE. The effects of intermittent application of inflow-outflow obstruction of the liver were also examined in the current study.

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0041-1345/13/$–see front matter
http://dx.doi.org/10.1016/j.transproceed.2012.07.155

METHODS

Twenty-four male *Wistar-Albino* rats weighing between 180 and 200 g were used for the study. They were kept under routine laboratory conditions receiving standard laboratory chow with free access to food and water. The study protocol was approved by our Ethics Committee for Experimental Studies (document no: 15/2007).

Experimental Design

The animals were divided into three groups, each composed of 8 animals: Group A hosts underwent 15-minutes of hepatic inflow occlusion with Laser Doppler Flowmetry (LDF) microcirculation measurement during clamping and blood sampling at the end of the ischemia period for biochemical analyses as well as tissue sampling for microscopic analyses. Group B experienced 15-minutes of hepatic inflow-outflow occlusion with LDF microcirculation measurements during clamping and blood sampling at the end of the ischemic period for biochemical analyses of liver injury as well as tissue sampling for microscopic analyses. After 15-minute intermittent hepatic inflow-outflow occlusion cycles, Group C had microcirculation measurements with LDF during clamping, blood sampling at the end of the second ischemic period for biochemical analyses of liver injury and tissue sampling for microscopic analyses. All animals were sacrificed at the end of the ischemic period.

Surgical Procedure

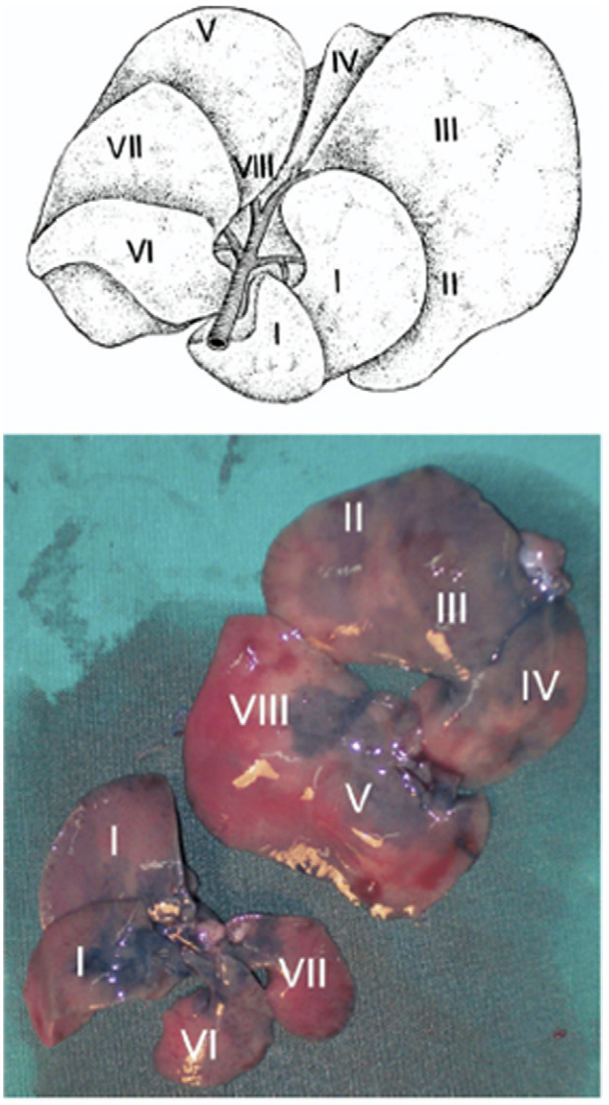
Animals were fasted for 12 hours before the experiments; however, they were allowed to drink water. All surgical procedures were performed using anesthesia with 40 mg/kg ketamine (Ketalar, Parke-Davis, Ann Arbor, Mich) and 4 mg/kg xylazine (Rompun, Bayer AG, Frankfurt, Germany). A midline abdominal incision was preferred for laparotomy. Portal pedicle clamping was applied to group A using a non-crushing vascular clamp for 15 minutes. At the end of the ischemic period, a blood sample was obtained by IVC (inferior vena cava) puncture and total hepatectomy performed.

In contrast to previous method used to evaluate experimental ischemia-reperfusion models in rat livers, we examined the whole organ instead of one or two lobes. The hepatic segments of rats were grouped according to the previous macroscopic description by *Köckerling.*[24] The rat liver was visually divided into three parts: hilar segments I, VI, and VII; central segments V and VIII, and dome segments II, III, and IV, (Fig 1).[21] Laser Doppler measurements and histopathological examinations were performed according to the abovementioned division of the rat liver.

The TVE method was applied to the rat liver as described previously.[21–23] Hepatic inflow-outflow occlusion was performed for 15 minutes in group B. Intermittent application of TVE (5-minute clamping, 5-minute reperfusion and 5-minute clamping) was applied to animals in group C. At the end of the TVE period, blood samples were obtained by IVC puncture and total hepatectomy performed.

Laser Doppler Flowmetry

Hepatic microcirculation was assessed with a commercially available LDF device (OxyLab LDF; Oxford Optronix Ltd, Oxford, UK), using a needle LDF probe (MNP 100XP, SN PR 90990) with a table stabilization device. Data inputs were processed with a special program (Biopac Student Lab Pro Software and MP35/30 Hardware, Biopac Systems Inc., Goleta, Calif) and measurements recorded as blood perfusion units (BPU). After an adaptation period of 20 minutes, the LDF device was calibrated to the company's specifications and recommendations. A distinct mapping of the liver surface and baseline liver perfusion were performed at the beginning of each experiment to achieve sufficient reliability and validity of the repeated measurements. After application of the vascular control maneuvers, the needle probe was placed in segment II or III for dome segments; segment VIII or V for central segments or



Separation of the liver segments according to their anatomical localizations: Hepatic segments I, VI and VII were considered as hilar segments, hepatic segments V and VIII were considered as central segments, and hepatic lobules II, III and IV were considered as dome segments.

Fig 1. Basic explanation of rat liver division.