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Improvement of liver preservation by the calcium channel blocker nisoldipine. An experimental study applying intravital microscopy to transplanted rat livers

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Abstract. It has been shown recently that inclusion of the calcium channel blocker nisoldipine to University of Wisconsin (UW) solution significantly improves survival after rat liver transplantation. To further elucidate the mechanisms involved, rat livers were stored for 1 h in UW solution with or without the addition of 1.4 μM nisoldipine (Miles, West Haven, Conn., USA). The liver grafts were investigated in vivo 90 min after transplantation by intravital fluorescence microscopy. Sinusoidal perfusion was reduced in all sublobular regions of the liver in the UW group (e.g. portal area: 76.7 ± 2.1%) and in the nisoldipine group (85.8 ± 1.5%). Diameters of liver sinusoids were comparably reduced in the UW and nisoldipine groups indicating that nisoldipine did not cause vasodilatation. Adhesion of leukocytes, however, rose significantly after liver transplantation particularly in periportal regions (25.8 ± 2.5%) compared to controls (17.1 ± 2.8%; $P < 0.05$). Adhesion of leukocytes was reduced when nisoldipine was included in UW solution (13.3 ± 1.7%; $P < 0.05$). Administration of latex particles, which were given in additional experimental groups, demonstrated an impressive increase in phagocytic activity of Kupffer cells after liver transplantation in periportal and pericentral areas (161 ± 14% and 184 ± 19% of controls). Phagocytosis was significantly reduced by nisoldipine in the periportal region (101 ± 10%; $P < 0.01$). Thus, the beneficial effect of nisoldipine was most pronounced in periportal regions, where the majority of Kupffer cells are located and leukocyte adhesion was at a maximum.

Key words: Liver preservation – Microcirculation – Leukocyte adhesion – Liver transplantation – Macrophage activation – Calcium channel blocker

Liver preservation and outcome of liver transplantation has been improved dramatically in the past years. With the introduction of new preservation media, such as the University of Wisconsin cold storage solution (UW) [1], preservation time could be extended thus allowing better organization and distribution of donor organs. However, primary nonfunction of liver grafts, which is the most serious complication of the transplantation procedure, still remains a critical problem [5]. Reperfusion injury to the transplanted organ has been suggested as a reason for primary nonfunction as well as for the poor quality of liver grafts [21].

Experimental studies in perfused and transplanted livers have shown a particular pattern of reperfusion injury after cold ischemia to the individual cell populations of the liver. Sinusoidal endothelial cells loose viability immediately after reperfusion, depending on the time of cold storage [4, 13, 15] while resident liver macrophages become activated under identical conditions [3, 20]. On the other hand, hepatic parenchymal cells tolerate cold ischemia significantly longer without morphological or functional losses [13]. Destruction of parenchymal cells most likely takes place subsequently, when their nutrition is disrupted, e.g. by microcirculatory failure [21]. There is evidence that oxygen free radicals contribute to reperfusion injury after liver transplantation [10, 16]. Furthermore, a rise in intracellular calcium during reperfusion has been proposed to contribute to cell injury [8] and to be involved in the activation of macrophages and the release of inflammatory mediators [17, 18]. Indeed, inclusion of the calcium channel blocker, nisoldipine, in a preservation solution improved survival rates significantly after rat liver transplantation. Part of this effect has been attributed to the prevention of Kupffer cell activation by nisoldipine [19].

The aim of our study was to evaluate the effect of nisoldipine on hepatic microcirculation, leukocyte adhesion and activity of Kupffer cells. Therefore, transplanted rat livers were investigated in vivo by fluorescence microscopy after cold storage in UW solution containing nisoldipine.

Materials and Methods

Liver transplantations were performed in Lewis rats (HAN, Hannover, Germany) weighing 200–230 g according to the technique described by Kamada [6]. The livers were stored for 60 min in UW sol-

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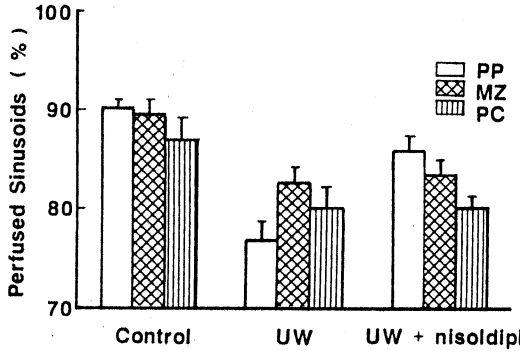

Fig. 1. Percentage of perfused sinusoids in sublobular regions of the liver. Discrimination of portal *(PP)*, midzonal *(MZ)*, and pericentral *(PC)* area was at a third of the distance between the centers of the portal and central field. Mean ± SEM

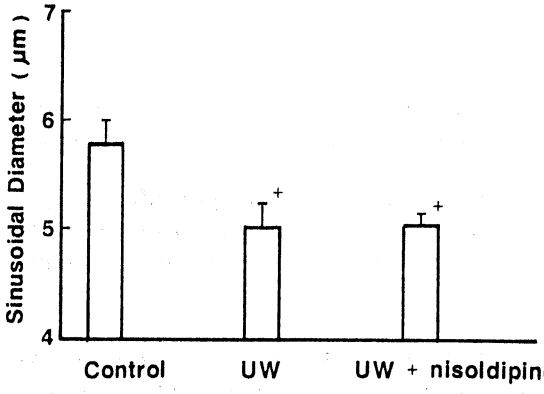

Fig. 2. Diameters of liver sinusoids. A circle with a radius of 150 µm was drawn around the central veins and diameters of all crossing sinusoids were measured. Mean ± SEM. * $P < 0.05$ versus control group

ution at 4 °C with or without the addition of nisoldipine (1.4 µM; Miles, New Haven, Conn.) prior to transplantation.

Leukocyte adhesion and sinusoidal perfusion were studied by intravital fluorescence microscopy 90 min after reperfusion of the liver grafts and in sham-operated controls ($n = 6$ per group). Using pentobarbital anesthesia (30 mg/kg), the abdomen was opened and the left liver lobe exposed on a plexiglas stage for intravital microscopy (Leitz Orthoplan, Wetzlar, Germany; 545 nm filter; 12× eye piece, 10×/20× water immersion objectives, final magnification ×310 or ×815), as has been described recently [11, 12]. After injection of acridine orange (0.9 mg/ml; Sigma, Deisenhofen, Germany), a leukocyte marker, 5–6 liver lobules were studied for 30 s each at the sublobular level, e.g. periportal, midzonal, and pericentral areas. Off-line analysis of video-recorded experiments allowed the determination of perfused sinusoids and permanently adherent leukocytes, defined as leukocytes with an adhesion time longer than 20 s [12].

Macrophage activity was assessed by the injection of latex particles ($260*10^6$; 0.8 µm; Polysciences, St. Goar, Germany) in a separate experimental series containing similar groups ($n = 6$ per group). Latex particles taken up by macrophages were measured in 5–6 periportal and pericentral areas of the liver 15 min after injection.

Data were given as mean ± SEM. Statistical significance was evaluated using ANOVA and Students *t*-test as appropriate.

Results

Sinusoidal perfusion was reduced by about 10% after liver transplantation, particularly in the periportal area. The addition of nisoldipine to the UW solution attenuated sinusoidal perfusion slightly, as shown in Fig. 1. The diameters of sinusoids of 150 µm around the central veins were significantly reduced in both transplantation groups compared to controls (Fig. 2). There were no changes in sinusoidal diameters the UW + nisoldipine group. Adhesion of leukocytes rose after liver transplantation in all sublobular regions. This effect was significantly reduced in the periportal region when nisoldipine was added to UW solution (Table 1).

In an additional experimental series, phagocytosis of latex particles was higher in periportal ($316 \pm 22/mm^2$) than in pericentral regions ($166 \pm 13/mm^2$) in the control group. Compared to these values (= 100%), transplantation after cold storage in UW solution resulted in a substantial rise in phagocytozed latex beads in periportal ($161 \pm 14\%$) and pericentral ($184 \pm 19\%$) regions. After inclusion of nisoldipine in the UW solution, phagocytosis of latex beads was significantly reduced in periportal ($102 \pm 10\%$; $P < 0.01$) and in pericentral areas ($137 \pm 27\%$).

Discussion

As indicated by the number of perfused sinusoids and diameters of hepatic sinusoids, transplantation of livers 1 h after cold storage in UW solution led to a moderate reduction in sinusoidal perfusion, which was slightly improved by nisoldipine. However, it seems obvious that this effect cannot account for the improved survival under identical conditions as has been shown previously [19]. Therefore, the action of the calcium channel blocker must be different from a direct effect on the microvascular perfusion.

It is well known that tissue macrophages, such as the Kupffer cells, release a variety of inflammatory mediators with the potential of influencing leukocyte adhesion and the microcirculation [22]. Since it has been shown that Kupffer cells are activated after cold storage [20] and transplantation [9], one aim of this study was to evaluate the activity of Kupffer cells in vivo after transplantation. Therefore, we used fluorescence labelled latex particles, as described by McCuskey et al. [14]. Uptake of latex particles has been observed in a ratio of 2:1 in periportal and pericentral areas, reflecting the physiological distribution of tissue macrophages [2]. In our study, the significant increase in both regions after transplantation was completely blocked by nisoldipine in the portal area and reduced in the central area. This suggests, that the benefical effect of the calcium channel blocker was due to prevention of

Table 1. Adhesion of leukocytes in sublobular regions of the liver. Percentage of leukocytes adherent longer than 20 s to the sinusoidal wall are expressed as percentage of all observed leukocytes. Data are given as mean ± SEM

Group	Periportal	Midzonal	Pericentral
Control	17.1 ± 2.8	11.3 ± 1.3	5.5 ± 0.8
UW	25.8 ± 2.5	16.7 ± 2.5	13.8 ± 2.4
UW/nisoldipine	13.3 ± 1.7	16.7 ± 2.5	6.4 ± 0.8

* $P < 0.05$ UW versus UW plus nisoldipine group

macrophage activation thereby reducing locally or systemically acting mediators, e.g. leukotrienes or cytokines [17, 18].

The pattern of leukocyte adhesion after liver transplantation with maximal adhesion in the portal regions supports the idea that the release of adhesion promoting mediators by Kupffer cells induces leukocyte adhesion. Indeed, the decrease in adherent leukocytes was maximal periportally (Table 1), where most of the Kupffer cells are located [2]. Because firm adhesion of leukocytes is necessary for leukocyte emigration and tissue injury [7], this sequence of events may explain the protective action of nisoldipine.

The results of this study indicated that the benefical effect of the calcium channel blocker nisoldipine on survival as shown by Takei et al. [19] was not due to its action on the microvasculature, e.g. on vasodilatation. It seems, however, that prevention of macrophage activation results in a reduced adhesion of leukocytes with attenuation of the postischemic liver injury. Thus, inclusion of a calcium channel blocker in preservation solutions may be useful in the clinical situation.

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