

## SHORT COMMUNICATION

### EFFECT OF INTRAPORTAL VERAPAMIL INFUSION ON HEPATIC ISCHEMIA-REPERFUSION INJURY

*Okan Erdoğan\*<sup>#</sup>, Sait Yıldız\*, Abdulkadir Başaran\*, Alper Demirbaş\*,  
Akin Yeşilkaya\*\**

\*Department of General Surgery, \*\*Department of Biochemistry, Akdeniz University School of Medicine,  
Antalya, Turkey

*Effect of intraportal verapamil infusion on hepatic ischemia-reperfusion injury.* O. ERDOĞAN, S. YILDIZ, A. BAŞARAN, A. DEMİRBAŞ, A. YEŞILKAYA. Pol. J. Pharmacol., 2001, 53, 137–141.

Removal of free oxygen radicals, generated during reperfusion of an ischemic organ by scavengers protects the tissue from reperfusion injury. The calcium channel blocker verapamil is an effective cytoprotective agent, preventing against reperfusion injury. The effects of verapamil were investigated previously using hepatic, renal or cardiac ischemia-reperfusion injury models. We investigated the effects of intravenous and intraportal administration of verapamil in prevention from the injury caused by free oxygen radicals generated during hepatic ischemia-reperfusion in rats. Thirty six male Sprague-Dawley rats after laparotomy were subjected to hepatic ischemia for 30 and 45 min followed by 60 min of reperfusion. Two minutes before ischemia the rats were pretreated by intravenous or intraportal administration of verapamil. The levels of glutathione and thiobarbituric acid reacting substances (TBARs) referred to as malonyldialdehyde (MDA) and the serum levels of transaminases were measured in liver tissue 1 and 24 h after the onset of reperfusion. Statistical analysis of the data by Student's *t*-test showed statistically significant differences between the group pretreated intraportally with verapamil and the other groups. Verapamil given intraportally exerted more beneficial effect. Therefore, we conclude that intraportal verapamil administration reduces the ischemia-reperfusion injury caused by free oxygen radicals.

**Key words:** *verapamil, reperfusion injury, liver*

---

<sup>#</sup> *correspondence*; e-mail: okaner7@yahoo.com

## INTRODUCTION

In ischemic states, elevated levels of calcium in cells or tissues have been shown in rat kidneys models [1–3, 11]. During reflow the mitochondrial calcium increased progressively so that a mitochondrial calcium loading occurred despite improvement in respiratory function within 1 to 4 h of reperfusion. Moreover, it has been believed that the elevated cytosolic calcium concentration during ischemia activates a protease capable of converting xanthine dehydrogenase to xanthine oxidase which is the main source of the superoxide radical, and calcium potentiates the damaging effect of oxygen-derived free radicals on the mitochondrial electron transport chain due to impairment of NADH-coenzyme Q-reductase activity. Calcium activates phospholipases A1, A2, C and some proteases. This activation results in an increase in the activity of membrane phospholipid hydrolases which causes the formation of oxygen free radicals which produces cellular damage [4–6].

A calcium channel blocker, verapamil, has been shown to be effective in preventing cellular damage during ischemia-reperfusion injury by acting on calcium influx [2, 5, 6, 11]. Pretreatment with verapamil has been demonstrated to be beneficial to preventing ischemia-reperfusion injury [1, 2]. The effects of verapamil and other scavengers ( -tocopherol, allopurinol, superoxide dismutase, N-acetylcysteine) as an adduct to preservation solutions [4, 8], or the effects achieved by intravenous administration of these agents have been investigated but little is known about intraportal pretreatment. In this study, we investigated the effects of intraportal administration of verapamil on hepatic ischemia-reperfusion injury and compared it with intravenous administration. Serum glutamyl oxaloacetyl transaminase (SGOT) and serum glutamyl pyruvic transaminase (SGPT) were used as indicators of hepatocellular injury, hepatic tissue malonyldialdehyde (MDA) level was a marker of oxidative membrane injury and the level of reduced glutathione was used to show free oxygen radical generation.

## MATERIALS and METHODS

Thirty six male Sprague-Dawley rats weighing 200–250 g were used in this study. All the animals had free access to food pellets and tap water before

the experiments. Experiments were carried out on rats receiving intraperitoneally urethane at 1 g/kg (Sigma Chemicals Co.). Polyethylene cannulas were inserted to left femoral artery for blood pressure monitoring and blood sampling and to left femoral vein for drug and saline administration. All animals received intravenously 80 U of heparin. A midline laparotomy was performed and liver hilum was exposed. During the experiments 0.5–1.0 ml of saline was given intravenously at 15 min intervals to maintain hemodynamic stability. The entire hepatic pedicle was clamped with a vascular clamp. Animals were randomized into three groups (Tab. 1). In the control group (Group 1) no free-radical scavenger was used. In the groups of rats receiving pretreatment 2 min before the onset of warm ischemia, verapamil at 0.2 mg/kg was administered either by intravenous (Group II) or intraportal (Group III) route. Half of the animals in each group was subjected to ischemia for 30 min and the other half for 45 min. Sixty minutes after the beginning of reperfusion by removal of the clamps, blood samples were taken for the measurement of SGOT and SGPT levels and tissue samples were taken to measure the levels of reduced glutathione and MDA. Twenty four hours later relaparotomy was performed and tissue and blood samples were taken for the same measurements as mentioned before.

Table 1. Experimental groups

Group	Warm ischemia duration	
	30 min	45 min
I Control (no pretreatment)	n = 6	n = 6
II Intravenous verapamil pretreatment	n = 6	n = 6
III Intraportal verapamil pretreatment	n = 6	n = 6

**Measurements of SGOT and SGPT activities.** The levels of SGOT and SGPT were measured with autoanalyzer (Hitachi-911, Hitachi Co., Japan). The results were expressed as U/L.

**Measurements of reduced glutathione levels.** Fairbanks and Klee's method with 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) was used [3]. Glutathione levels were expressed as U GSH/mg of protein.

**Measurements of thiobarbituric acid reacting substances (TBARs).** After homogenization of the tissue, Stocks and Dormandy's method was

used to determine TBARs [13] and Lowry's method was applied for protein measurement [7]. Lipid peroxide levels were expressed as nmol MDA/mg of protein.

**Statistical analysis.** The results are given in the text as means  $\pm$  SE. Comparisons of the means were made using Student's *t*-test.

This study was approved by the Ethics Committee of Akdeniz University, Antalya, Turkey.

## RESULTS

SGOT and SGPT levels after 30 min of warm ischemia were significantly better in the group pretreated intraportally with verapamil ( $p < 0.05$ ). The group administered the drug intravenously had lower SGOT and SGPT levels compared with the control group but this difference was not statistically significant. Forty five minutes of prolonged warm ischemia resulted with a rise in the activities of both transaminases in all the groups. The group receiving intraportal verapamil pretreatment had the lowest levels and the difference was statistically significant ( $p < 0.05$ ), whereas the lower levels observed in Group II compared with Group I were not significantly different (Tab. 2, 3).

Table 2. Mean  $\pm$  SE SGOT levels (U/L)

Group	30 min of warm ischemia		45 min of warm ischemia	
	1 h	24 h	1 h	24 h
I	508 $\pm$ 62	932 $\pm$ 444	1299 $\pm$ 201	1422 $\pm$ 148
II	510 $\pm$ 189	889 $\pm$ 235	819 $\pm$ 159	915 $\pm$ 318
III	305 $\pm$ 133	533 $\pm$ 115	467 $\pm$ 88	525 $\pm$ 85

Table 3. Mean  $\pm$  SE SGPT levels (U/L)

Group	30 min of warm ischemia		45 min of warm ischemia	
	1 h	24 h	1 h	24 h
I	349 $\pm$ 42	685 $\pm$ 226	859 $\pm$ 98	1116 $\pm$ 154
II	377 $\pm$ 70	637 $\pm$ 123	653 $\pm$ 115	811 $\pm$ 277
III	196 $\pm$ 52	397 $\pm$ 22	297 $\pm$ 18	363 $\pm$ 84

Intraportal verapamil pretreatment caused a decrease in the formation of MDA in comparison with the other groups (Tab. 4). This decrease was

statistically significant both after 30 and 45 min of ischemia ( $p < 0.05$ ). Forty five minutes of warm ischemia produced a rise in MDA formation in all the groups. The MDA levels observed in the group pretreated with verapamil intravenously was lower than in the control group but this difference was also not statistically significant.

Table 4. Mean  $\pm$  SE hepatic malonyldialdehyde levels (nmol MDA/mg of protein)

Group	30 min of warm ischemia		45 min of warm ischemia	
	1 h	24 h	1 h	24 h
I	3.95 $\pm$ 0.58	4.38 $\pm$ 0.84	3.65 $\pm$ 0.58	5.58 $\pm$ 2.27
II	2.54 $\pm$ 0.23	3.53 $\pm$ 0.92	3.23 $\pm$ 0.24	4.05 $\pm$ 0.77
III	1.68 $\pm$ 0.26	2.36 $\pm$ 0.74	2.32 $\pm$ 0.48	3.22 $\pm$ 0.59

Table 5 shows reduced glutathione levels. There is no obvious change in reduced glutathione levels with the duration of warm ischemia. The group receiving intraportal verapamil had higher levels of this compound compared with other groups. The difference was statistically significant ( $p < 0.05$ ). The results in the group administered verapamil intravenously were better but not significant in comparison with the control group.

Table 5. Mean  $\pm$  SE reduced glutathione levels (U GSH/mg of protein)

Group	30 min of warm ischemia		45 min of warm ischemia	
	1 h	24 h	1 h	24 h
I	1.41 $\pm$ 0.84	1.36 $\pm$ 0.59	1.48 $\pm$ 0.56	1.35 $\pm$ 0.41
II	1.72 $\pm$ 0.78	1.81 $\pm$ 0.38	1.90 $\pm$ 0.26	1.87 $\pm$ 0.47
III	2.73 $\pm$ 0.67	2.31 $\pm$ 0.22	2.70 $\pm$ 0.37	2.69 $\pm$ 0.71

## DISCUSSION

Much attention has been focused on the role of calcium channel blockers in the prevention of ischemia-reperfusion injury. Either the addition of verapamil to perfusate, or intravenous pretreatment with verapamil has been shown to be beneficial in ischemia-reperfusion injury [2, 6, 11, 12]. In accordance with these reports, our study shows that pretreatment with verapamil reduces ischemia-reperfusion injury, and the route of administration plays

an important role. To the best of our knowledge, this is the first study concerning intraportal administration of verapamil or other scavengers. Currently, some agents are administered intraportally for chemotherapy.

The molecular interaction between calcium channel blockers and free oxygen radical injury is not clear. Verapamil can block the conversion of the intracellular enzyme xanthine dehydrogenase to xanthine oxidase and by this mechanism it can prevent free radical generation as Ishii et al. have demonstrated in ischemic rat livers [5]. Vasodilator effects of calcium channel blockers may also play a role. It has been reported that intravenous verapamil pretreatment increased bile secretion, and a decrease in SGPT and lactic dehydrogenase levels compared to the control were obtained at the end of 24 h [6, 9, 10]. The same report suggesting the minimal protective influence of verapamil on early effects of liver ischemia-reperfusion demonstrates that verapamil does not prevent the early generation of oxygen radicals upon reperfusion, and calcium plays a role in late hepatocyte injury. It has also been suggested that studies investigating the protective action of verapamil on the effects of warm liver ischemia-reperfusion should be carried out for at least 24 h post reperfusion [9, 10, 12]. In our study, intraportal verapamil pretreatment resulted in protection from warm ischemia-reperfusion injury at 1 and 24 h after reperfusion. Stein et al. have suggested that a beneficial effect of verapamil occurs only in rats sensitized to oxidative injury by chemical depletion of hepatic glutathione with diethyl maleate [12]. In another report it has been shown that intravenous verapamil pretreatment gave better but not significant results. Adding a prostacycline analogue made the results significant [2]. In our study, the group pretreated intravenously with verapamil had better SGOT and SGPT levels than the control group but this difference was not found to be statistically significant as it is the case also for MDA and GSH. Our results are in line with those presented by Stein and Dosluoglu. Intraportal route by direct access to hepatic microcirculation may be the key for obtaining a higher drug concentration in the hepatocellular membranes, hence giving rise to significant results that could not be obtained by the intravenous route. In the study of Totsuka et al. it has been indicated that intraportal administration of PGE1 has a greater protective effect than intravenous administration

against ischemic liver injury. The results of this study are similar to our study [14].

In this study, verapamil pretreatment *via* intraportal route resulted in better protection against ischemia-reperfusion injury than after the intravenous drug administration. This suggests that pretreatment with other scavengers *via* the intraportal route is worth studying. Further studies with the use of intraportal verapamil pretreatment may prove that it can be useful in hepatic resection and transplantation.

## REFERENCES

1. Demirbas A., Bozoklu S., Ozdemir A., Bilgin N., Haberal M.: The effect of alpha tocopherol on the preservation injury caused by free oxygen radicals in the canine kidney autotransplantation model. *Transplant. Proc.*, 1993, 25, 2274.
2. Dosluoğlu H.H., Aktan A.O., Yegen C., Okbay N., Yalçın A.S., Yalın R., Ercan S.: The cytoprotective effects of verapamil and iloprost (ZK 36374) on ischemia/reperfusion injury of kidneys. *Transplant Int.*, 1993, 6, 138–142.
3. Fairbanks V.F., Klee G.G.: Biochemical aspects of haematology. In: *Textbook of Clinical Chemistry*. Ed. Tietz N.W., W.B. Saunders Company, New York, 1986, 1495–1588.
4. Fukuzawa K., Emre S., Senyuz A., Acarlı K., Schwartz M.E., Miller C.M.: N-Acetylcysteine ameliorates reperfusion injury after warm hepatic ischemia. *Transplantation*, 1995, 59, 6–9.
5. Ishii K., Suita S., Sumimoto H.: Effects of verapamil on conversion of xanthine dehydrogenase to oxidase in ischemic rat liver. *Res. Exp. Med.*, 1990, 190, 389–399.
6. Karwinski W., Garcia R., Helton W.S.: Protective effects of the calcium channel blocker verapamil on hepatic function following warm ischemia. *J. Surg. Res.*, 1996, 64, 150–155.
7. Lowry O.H., Rosenbrough N.S., Far A.L., Randle R.J.: Protein measurement with Folin phenol reagent. *J. Biol. Chem.*, 1951, 193, 265–275.
8. Nauta R.J., Tsimoyiannis E., Uribe M., Walsh D.B., Miller D., Butterfield A.: The role of calcium ions and calcium channel entry blockers in experimental ischemia-reperfusion-induced liver injury. *Ann. Surg.*, 1991, 213, 137–142.
9. Sakr M.F., Abdel-Aal A.N.: Protective effect of cyclosporine A (Cy A) against the hepatic injury associated with ischemia and reperfusion. *Int. Surg.*, 1996, 81, 180–183.
10. Sakr M.F., Zetti G.M., Hassanein T.I., Farghali H., Nalesnik M.A., Gavaler J.S., Starzl T.E., Van Thiel D.H.: FK 506 ameliorates the hepatic injury associated with ischemia and reperfusion in rats. *Hepatology*, 1991, 13, 947–951.

11. Shapiro J.I., Cheung C., Habashi A., Chan L., Schrier R.W.: The effect of verapamil on renal function after warm and cold ischemia in the isolated perfused rat kidney. *Transplantation*, 1985, 40, 596–600.
12. Stein H.J., Mathys M.J., Hinder R.A., Lamprechts H.: Effect of verapamil on hepatic ischemia/reperfusion injury. *Amer. J. Surg.*, 1993, 165, 96–100.
13. Stocks J., Dormandy T.L.: The autooxidation of human red cell lipid induced by hydrogen peroxide. *Brit. J. Haematol.*, 1971, 20, 95–111.
14. Totsuka E., Sasaki M., Takashi K., Toyoki Y., Seino K., Chiba S., Narumi S., Hakamada K.: The effects of intraportal prostaglandin E1 administration on hepatic warm ischemia and reperfusion injury in dogs. *Surg. Today*, 1995, 25, 421–428.

*Received: April 27, 2000; in revised form: April 17, 2001.*