Beneficial Effect of Tetrahydrobiopterin on the Survival of Rats Exposed to Hepatic Ischemia-Reperfusion Injury


ABSTRACT

Introduction. The protective role of nitric oxide (NO) against hepatic ischemia-reperfusion (I/R) injury remains controversial. In this study we investigated the effect of tetrahydrobiopterin (BH4) on the survival of rats exposed to an hepatic I/R injury.

Methods. The rats were subjected to 100 minutes of 70% hepatic ischemia after administration of BH4 or saline. A specific inducible NO synthase (iNOS) blocker, 1400W, was used to evaluate endogenous iNOS. NOS protein measured the histological appearance of the liver by Western blotting, and survival was evaluated after reperfusion.

Results. The 1-week survival rate was 60% among the BH4 group and 10% for the saline group. The serum ALT and bilirubin values in the BH4 group were significantly lower than the saline group. Histological examination of the liver revealed only a small necrotic area in the BH4 group as opposed to massive necrosis and cell infiltration in the saline group. Injection of 1400W significantly decreased the prolongation of survival produced by BH4.

Conclusions. BH4 significantly improved the survival rate, the histological findings, and the liver function, thereby reducing liver failure. Western blotting showed a higher level of iNOS protein in the BH4 group than the saline group, 1400W suppressed this effect of BH4. Taken together, these observations suggest that NO derived from reactions driven by BH4-induced iNOS exerts a protective effect against reperfusion injury.

SOME STUDIES have reported that suppression of inducible nitric oxide (NO) synthase (iNOS) improves ischemia reperfusion (I/R) hepatic injury.1,2 In contrast other workers have observed that iNOS inhibition dose neither affected or improved I/R hepatic injury.3,4 Thus the protective role of NO against hepatic reperfusion injury remains controversial.

Tetrahydrobiopterin (BH4) is an important factor in NO synthesis that enhances iNOS gene expression. Exogenous BH4 administration has been reported to restore impaired endothelium-derived NO synthesis in acute endothelial dysfunction. In animal models it seems to improve I/R injuries in the stomach, kidney, and lung.5–7

In this study we investigated the effect of BH4 on the survival of rats exposed to an hepatic I/R injury. A specific iNOS blocker, 1400W, was used to evaluate the impact of endogenous iNOS.

METHODS

The rats were subjected to 100 minutes of 70% hepatic warm ischemia. More specifically, laparotomy was performed under phenobarbital anesthesia and the blood supply to 70% of the liver lobes interrupted with a vascular clamp. The remaining lobes were intact to prevent blockage of intestinal venous outflow. After reperfusing the ischemic lobes, the shunted lobes were resected to eliminate any effects from the non I/R lobes.

BH4 or saline was administered 30 minutes before reperfusion. For some hosts, 1400W was injected at the start of the hepatic ischemia, and BH4 or saline administered 30 minutes before reperfusion. Survival; liver enzyme levels; serum bilirubin levels as indicators of liver failure; histological examination; and iNOS protein measurements by Western blotting were performed after reperfusion. Blood and liver samples were collected 12 hours after the start of reperfusion. Quantification of Western blotting for iNOS was performed using gel analysis software.

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RESULTS

The 1-week survival rate was 60% among the BH4 group and 10% for the saline group. Injection of 1400W significantly decreased the prolonged survival obtained by BH4. None of rats injected with 1400W survived beyond 7 days after reperfusion. The serum alanine transaminase and bilirubin values at 12 hours after reperfusion in the BH4 group were significantly lower than those in the saline group (Fig 1). The iNOS protein level in the BH4 group was about eight times higher than in the saline group, a difference that was statistically significant (Fig 1). Histological examination of the liver revealed only a small necrotic area in the BH4 group as opposed to massive necrosis and cell infiltration in the saline group (Figs 2A and 2B).

DISCUSSION

BH4 prolonged the survival of the rats subjected to hepatic I/R injury. Biochemical parameters were better in the BH4 than the saline group. Histological examination of the liver revealed only a few necrotic areas in the BH4 group, as opposed to massive necrosis in the saline group. The serum ALT and bilirubin values were significantly lower in the BH4 than the saline group. BH4 clearly protected the rat liver from an I/R injury. BH4 increased the level of iNOS protein, probably resulting in increased NO synthesis. 1400W, a specific inhibitor of iNOS, abrogated the beneficial effect of BH4. Taken together, these observations indicate that NO derived from reactions by BH4-induced iNOS exerts a protective effect against I/R injury. We speculate that I/R causes deficiency of endogenous BH4, resulting in the production of superoxide and nitroxyl anions, which in turn participate in the I/R hepatic injury. Exogenous delivery of BH4 seems to activate NO synthesis and the microcirculation to the I/R injured liver thereby producing improvement.

In conclusion, the results of this study suggest that, as a prerequisite cofactor of the NOS reaction, BH4 prevented liver damage in our model of severe I/R injury, probably by increasing NO production via iNOS synthesis. Since the specific iNOS inhibitor 1400W impaired survival, the effectiveness of BH4 may depend on the function of iNOS. Thus, exogenous BH4 application may be useful to prevent I/R injury. Further study is needed to confirm these findings and investigate the mechanism in detail.

REFERENCES


Fig 2. Histology of the reperfused livers. (A) Histology revealed only a small necrotic area in the BH4 group. (B) Massive necrosis and cell infiltration were recognized in the saline group.