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The Effect of OP 2507, a Stable Analogue of Prostacyclin, on Hep G2 Exposed to Hypoxia

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IRITA, K., SAKAI, H., ISobe, H., YAMAKAWA, M., NAWATA, H. and YOSHITAKE, J. The Effect of OP 2507, a Stable Analogue of Prostacyclin, on Hep G2 Exposed to Hypoxia. Tohoku J. Exp. Med., 1990, 162(2), 177-182 — We developed a model for screening drugs to reduce ischemic liver damage using Hep G2, a hepatoblastoma cell line, and examined the effect of OP 2507, a stable analogue of prostacyclin, on hypoxic cell damage. Hypoxic exposure of the cells was done for 16 hr in an air-tight chamber which was placed inside an incubator and was purged with 5% CO2/95% N2. Adding OP 2507 (0.01-10 ng/ml) to the incubation medium during hypoxic exposure reduced mitochondrial damage estimated by MTT-reducing activity, while it failed to inhibit lactate accumulation in the medium. OP 2507 seems to be a good candidate for improving the preservation of liver allografts. —— hypoxia; Hep G2; prostaglandin I2 analogue

Although the introduction of University of Wisconsin solution has prolonged the preservation of liver allografts, ischemic liver damage is still a major cause of primary non-function grafts (Belzer and Southard 1988). Ischemic liver damage is primarily caused by mitochondrial dysfunction, resulting in ATP depletion followed by intracellular acidosis, cell swelling, calcium accumulation, activated proteolysis and, finally, cell death (Hochachka 1986). A pharmacological intervention to maintain mitochondrial function, therefore, seems to be an essential for the preservation of liver allografts (Nishida et al. 1987). Prostacyclin has been shown to have a cytoprotective effect on ischemia-induced hepatic cell injury, an effect probably due to the maintenance of mitochondrial function (Sikujara et al. 1983; Toledo-Pereyra 1984; Okabe et al. 1986). Viebahn et al. (1990) reported the usefulness of primary hepatocyte culture to screen methods for liver preservation. We examined the effect of OP 2507, [15-cis-(4-propylcyclohexyl)-16, 17, 18,

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19, 20-pentanor-9-deoxy-6, 9\-nitrilo-PGF\(_1\) methyl ester (Terawaki et al. 1988), a newly-developed stable analogue of prostacyclin, on hypoxic cell damage using Hep G2 culture.

**MATERIALS AND METHODS**

Hepatoblastoma cell line, Hep G2, was seeded at about 1 x 10\(^5\) cells/well in 16 mm, 24-well plates (Sumitomo, Tokyo) and grown in Eagle's minimal essential medium (MEM) containing 10\% FCS. After 4–5 days of seeding, the culture reached confluence, and was used for the experiment. The culture was incubated in 0.4 ml of serum-free MEM supplemented with glutamine in the presence and in the absence of OP 2507. OP 2507 was supplied by Ono Pharmaceutical Co., Osaka, and was dissolved in distilled water.

Hypoxic exposure of the cultures was done following the method by Yu et al. (1989). Briefly, cultures were put in an airtight incubator chamber (Model 1336; Belco, Vineland, NJ, USA), which was placed inside an incubator and maintained at 37°C. The chamber (11 liter) was purged with 5\% CO\(_2\)/95\% N\(_2\) for 15 min at a rate of 10 liter/min. The atmospheric oxygen content was monitored by an oxygen monitor (Model OX-227; Rikakens-Keiki, Tokyo), and the partial pressure of oxygen in the culture medium was determined by a Ciba Corning blood-gas analyzer (Model 168; Medfield, MA, USA). The changes in the atmospheric oxygen content and the partial pressure of oxygen in the culture medium were exactly the same as reported by Yu et al. (1989). The atmospheric oxygen content became zero after 10 min of purging. The partial pressure of oxygen in the culture medium decreased gradually and reached 17–23 mmHg after 16 hr. The control (normal oxygen tension) cultures were maintained at 37°C in 95\% atmospheric air and 5\% CO\(_2\) with 95\% humidity.

OP 2507 was added to the medium from the beginning of hypoxic exposure. After 16 hr of normal or hypoxic exposure, the medium was collected. Released lactate dehydrogenase (LDH) activity was measured by an Ektachem (Eastman Kodak, Rochester, NY, USA). Lactate concentration in the medium was measured by an Analox GM7 (Baxter, Deerfield, IL, USA). Mitochondrial function was measured by the reduction of 3(4, 5-dimethyl-thiazoyl-2-yl)2, 5 diphenyltetrazolium bromide (MTT) dye according to Carmichael's method with modifications (1987). MTT reducing activity is thought to represent electron transport chain activity. The cultures were further incubated with 0.2 ml of MEM containing 0.5 mg/ml MTT dye at 37°C under normal condition. After 1 hr of incubation, 750 \(\mu\)l of dimethylsulfoxide was added to each well to solubilize the MTT formazan. Complete solubilization of the dye was achieved by repeated pipetting of the solution. A hundred \(\mu\)l of each resultant solution was transferred to a 94-well microtiter plate, and the optical density was measured immediately with an enzyme-linked immunosorbent assay reader NJ-2000 (Japan Inter Med, Tokyo) equipped with a 570 nm filter. The spectrophotometer was calibrated to zero absorbance using wells that contained MEM, MTT dye and DMSO. The experiments were repeated four times, and typical results are shown.

**RESULTS**

Hypoxic exposure of Hep G2 for 16 hr produced a mild hypoxic cell damage; e.g., mitochondrial MTT reducing activity was moderately decreased, and lactate accumulated in the medium, while the leakage of LDH was minimum. Under the conditions used, 3–8\% of total cellular LDH leaked into the medium both in the normal and in the hypoxic cultures. Mitochondrial function estimated by MTT-formazan formation decreased by about 25\% after hypoxic exposure. OP 2507 preserved MTT-reducing activity (Fig. 1). Hypoxia induced lactate accu-
Fig. 1. Effect of OP 2507 on the mitochondrial MTT reducing activity in the normal and in the hypoxic cultures of Hep G2. Hep G2 was incubated with indicated concentrations of OP 2507 under normal or hypoxic conditions for 16 hr in MEM, and the mitochondrial MTT reducing activity of the cells was measured. Values are means of duplicate examinations. The results are reproducible.

Fig. 2. Effect of OP 2507 on the lactate accumulation in the normal and in the hypoxic cultures of Hep G2. Hep G2 was incubated as in Fig. 1, and the lactate concentration of the medium was measured. Values are means of duplicate examinations. The results are reproducible.
mulation in the medium, which was not affected by adding OP 2507 (Fig. 2). OP 2507 had no effect on cultures maintained in normal conditions.

**Discussion**

We developed a model for screening drugs to reduce ischemic liver damage. We used a hepatoblastoma cell line, Hep G2, and introduced the MTT method to evaluate hypoxic cell damage. Hep G2 has been shown to resemble normal hepatocytes biochemically and morphologically (Javitt 1990). Hep G2 produces and secretes serum proteins, complements, clotting factors and lipoproteins. Hep G2 has tight junctions and microvilli. Although Hep G2 is not a normal hepatocyte, and its glucose metabolism has not been clarified, Hep G2 is a useful analogue of the human hepatocyte. Hypoxic exposure of Hep G2, instead of the primary hepatocyte culture, seems to be a convenient method for screening drugs for better preservation of liver allografts. The MTT method has been developed to assess the chemosensitivity of tumor cell lines or biopsied tissue to anticancer drugs. Carmichael et al. (1987) showed that formazan crystals were solubilized more easily by dimethylsulfoxide than by isopropyl alcohol, when adherent cells were used. We confirmed this observation using Hep G2. The sensitivity of the MTT method in evaluating cell damage is not clear. In our model, cell damage was detected more efficiently by a decrease in MTT-reducing activity than by LDH release. Although the exact correlation between the damage to mitochondria and the decrease in MTT-reducing activity should be elucidated, the simplicity and the rapidity make the MTT method suitable for semiautomatic large scale screening of drugs.

OP 2507 is stable over 24 hr in an aqueous solution, and has a smaller hypotensive effect than PGI2 (Terawaki et al. 1988). Terawaki et al. (1988) reported that OP 2507 had a cytoprotective effect against ischemic cerebral damage in cats. We demonstrated here that OP 2507 had a direct effect on ameliorating mitochondrial dysfunction in Hep G2 exposed to hypoxia. Prostaglandin E1, I2 and their derivatives have been shown to reduce hepatic damage induced by carbon tetrachloride (Bursch et al. 1989), galactosamine (Lapis et al. 1986; Noda et al. 1986), lipopolysaccharide (Mizoguchi et al. 1987) and ischemia (Sikujara et al. 1983; Toledo-Pereyra 1984; Okabe et al. 1986). Furthermore, the administration of prostaglandin E1 was postulated to benefit patients with fulminant viral hepatitis (Sinclair et al. 1989) and with primary non-functioning grafts of the liver (Greig et al. 1989). Although mechanisms for cytoprotective activity of these prostaglandins are unclear, they have been thought to stabilize lysosomal membrane and to increase intracellular cAMP levels, which in turn inhibit massive influxes of calcium (Hochachka 1986; Lapis et al. 1986; Noda et al. 1986). Sikujara et al. (1983) reported that pretreatment of rats with an intravenous infusion of PGI2 lessened a decrease in hepatic ATP levels after 75 min of ischemia followed by 60 min of reperfusion. PGI2 also increased cGMP
levels in the liver, but failed to increase cAMP levels during and after ischemia. Okabe et al. (1986) showed that PGI₂ inhibited a decrease in oxygen consumption and in ATP synthesis of isolated rat liver which was preserved in Collin’s solution, whether or not the portal triad was clamped for 30 min before isolating the liver. They concluded that PGI₂ preserved oxidative phosphorylation in mitochondria, which coincides with our present observation.

Pyruvate dehydrogenase, which determines the rate of oxidation of glucose, and therefore that of lactate, is present in mitochondria (Randle et al. 1988). Although the improvement of mitochondrial function by OP 2507 was expected to be associated with a lower lactate level, OP 2507 failed to reduce lactate accumulation. There are several possible explanations. Lactate accumulation may have been due to continued glucose supply and anaerobic glycolysis (Belzer and Southard 1988). Improvement of mitochondrial function by OP 2507 may not have been sufficient to oxidize large amount of lactate (Mazer et al. 1990). The activity of pyruvate dehydrogenase may have been suppressed even in the presence of OP 2507. Because acidosis is thought to exacerbate ischemic cell damage (Belzer and Southard 1988), reducing lactate levels is also important. Dichloroacetate, which activates pyruvate dehydrogenase, has been shown to decrease blood lactate levels in patients who had lactic acidosis due to tissue hypoxia (Stacpoole 1989). Using the same experimental methods described in this paper, we observed that dichloroacetate suppressed lactate accumulation during hypoxic exposure of Hep G2 (submitted). Dichloroacetate alone, however, failed to improve MTT-reducing activity.

In summary, we developed a model for screening drugs to reduce ischemic liver damage. OP 2507 seems to be a good candidate for maintaining mitochondrial function during ischemia.

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References