Biochemical Pharmacology, Vol. 46, No. 4, pp. 767–769, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

Orally administered prostaglandin E_1 derivative can enhance liver regeneration in partially hepatectomized rats

(*Received* 4 *December* 1992; *accepted* 5 *May* 1993)

Abstract—The effect of orally administered 17(*S*),20-dimethyl-6-oxo prostaglandin E_1 (PGE_1) methyl ester (OP) on liver regeneration was examined in 66% hepatectomized rats. Administration of OP increased the mitotic index of the hepatocyte 3 days after hepatectomy, and the mitotic index response due to OP was dose dependent. The OP administration had no effect on food intake, but reduced water intake. The serum scores relative to nutrition and hepatic function showed transient change after OP administration, whereas the serum blood urea nitrogen level indicated a slight renal dysfunction with OP. The fat store in the body was transiently reduced. These observations lead us to conclude that orally administered OP is capable of stimulating liver regeneration without serious systemic effects.

Enhanced liver regeneration can contribute to good prognosis in liver disease, particularly when the liver has been partially necrotized [1]. Prostaglandin E_1 (PGE*) has been considered to be involved in the liver regenerative process; there was an increase in the PGE concentration in the liver that undergoes compensatory growth in response to surgical removal of part of the liver mass [2]. Moreover, it was also found that non-orally administered PGE and its derivatives stimulated rat liver regeneration after partial hepatectomy [3, 4]. However, the effect of the oral administration of PGE on liver regeneration has not yet been examined. Recently a PGE derivative of OP has been developed which is active when administered orally.

The present study was designed to investigate whether orally administered OP influences liver regeneration in partially hepatectomized rats.

Materials and Methods

Forty-five male Wistar rats were used. They were housed individually (12:12; light–dark cycle) in a room with the temperature kept at 22 ± 1°, and given free access to tap water [5]. A standard 66% hepatectomy was carried out under ether anesthesia when the rat body weight reached about 180 g [6]. Surgery, food and water intake, and body weight estimations took place between 10:00 and 12:00 to eliminate the effect of diurnal variations.

The rats were killed 0, 1, 3 and 5 days after hepatectomy. The remainder of the liver was removed about 30 min after hepatectomy when the rats were killed on day 0. The specimens obtained from the caudate lobe were stained with hematoxylin and eosin, and the mitotic figure for hepatocytes per 1000 counts was expressed as the mitotic index [7, 8]. The Lee index [body wt $(g)^{0.33}$/Nasoanal length (mm) × 10^4] and retroperitoneal white adipose tissue weight were estimated. Immediately before the rats were killed blood samples were taken from the tail vein for chemical analysis, and serum concentrations of substances indicating nutritional, hepatic and renal conditions (total protein; albumin; GPT, alanin aminotransferase; blood urea nitrogen) were measured with an auto-analyzer (Hitachi, Tokyo, Japan) [7].

OP (Ono Pharmaceutical Co., Ltd, Osaka, Japan) was administered orally three times a day (07:00, 14:00 and

* Abbreviations: PGE, prostaglandin E_1; OP, 20-dimethyl-6-oxo-PGE_1 methyl ester; GPT, glutamic-pyruvic transaminase.

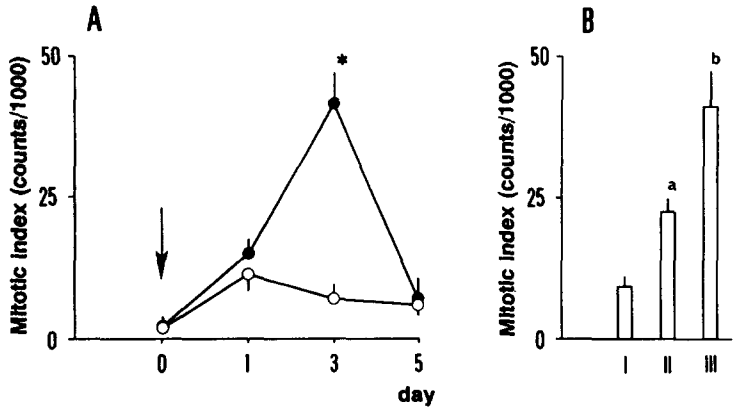

Fig. 1. (A) Time courses for mitotic index following OP (1.6 μg/kg three times daily) (●) or saline (○) administration. Arrow shows the day of hepatectomy. Values are means ± SE (N = 5). *$P < 0.01$ vs saline. (B) Responses in the mitotic index 3 days after hepatectomy. Different doses of OP (II, 0.8 μg/kg three times daily; III, 1.6 μg/kg three times daily) and saline (I) were orally administered. Values are means ± SE (N = 5). [a] $P < 0.01$ vs I. [b] $P < 0.01$ vs II.

Table 1. Food and water intake during the first 3 and 5 days following saline (I) or OP (1.6 μg/kg three times daily) (II) administration

	Days after hepatectomy 3	5
Food intake (g)		
I	28.8 ± 2.9	59.4 ± 4.1
II	33.6 ± 3.4	60.2 ± 4.9
Water intake (mL)		
I	39.8 ± 2.2	73.8 ± 2.5
II	35.6 ± 2.1	63.4 ± 2.4*

Values are means ± SE (N = 5). * $P < 0.05$ vs I.

Table 2. Changes in serum indicative of hepatic or renal disfunction in rats given saline (I) or OP (1.6 μg/kg three times daily) (II)

	Days after hepatectomy 0	1	3	5
Total protein (g/dL)				
I	5.1 ± 0.0	4.7 ± 0.1	4.2 ± 0.2	5.1 ± 0.1
II	5.1 ± 0.0	4.7 ± 0.0	4.8 ± 0.1*	5.3 ± 0.2
Albumin (g/dL)				
I	2.1 ± 0.0	1.7 ± 0.0	1.5 ± 0.1	1.6 ± 0.0
II	2.1 ± 0.0	1.6 ± 0.0*	1.6 ± 0.0	1.9 ± 0.0*
GPT (U)				
I	19.6 ± 0.8	81.6 ± 11.2	25.6 ± 2.4	24.6 ± 2.3
II	20.0 ± 0.6	142.6 ± 17.7*	39.4 ± 3.1	23.0 ± 1.0
Blood urea nitrogen (mg/dL)				
I	12.8 ± 0.9	12.4 ± 0.5	11.9 ± 1.5	15.9 ± 0.7
II	11.9 ± 0.9	18.2 ± 0.9*	16.1 ± 1.7*	22.2 ± 1.6*

Values are means ± SE (N = 5). *$P < 0.01$ vs I.

20:00) for 3 days, starting on the day of hepatectomy. OP was administered twice on the day of hepatectomy. Oral administration was carried out with an intragastric feeding tube, and each administration was 0.8 or 1.6 μg/kg.

The data were ANOVA analysed, and specific values were evaluated by Duncan's multiple range test.

Results

The mitotic index increased significantly at 3 days after hepatectomy in the OP treated rats compared to the control rats (Fig. 1A). The increase in mitotic index in response to OP was dose dependent (Fig. 1B).

Food intake of the OP treated and control rats was not appreciably different throughout the period of the experimental day while the water intake was reduced by OP administration 5 days after hepatectomy (Table 1). In the serum (Table 2), OP treatment increased total protein 3 days after hepatectomy, but returned to normal in the next 2 days. Albumin was reduced 2 days after hepatectomy but increased 3 days later. Glutamic-pyruvic transaminase (GPT) had increased 1 day after hepatectomy in the OP treated rats, but recovered in the next 4 days. Blood urea increased from 1 to 5 days after hepatectomy in the OP treated rats. The Lee index and retroperitoneal white adipose tissue weight were reduced 3 days after hepatectomy, but recovered 2 days later (Table 3).

Discussion

The present study demonstrated that liver regeneration after partial hepatectomy was effectively stimulated even when OP was administered orally; the mitotic index was increased 3 days after hepatectomy (Fig. 1A). Our results are compatible with the view that PGE and its derivatives enhance rat liver regeneration [3, 4]. The action of oral OP on the regeneration may be specific to OP, because the mitotic index response due to OP was dose dependent (Fig. 1B).

OP administration for 3 days was effective in increasing the mitotic index 3 days after hepatectomy (Fig. 1A), indicating a cumulative effect of OP.

Although PGE has been shown to provoke hypotension and uterus contraction, there was a 100–1000-fold difference between the oral and non-oral administration in the effective concentration [9, 10]. Indeed, in rats no observable hypotension or uterus contraction was induced by oral administration of 100 μg/kg/day OP (Ohtake and Sakaguchi, unpublished data). Considering these results together with the finding that oral administration of 4.8 μg/kg/day OP increased the mitotic index, consequent to liver regeneration (Fig. 1A), it is likely that promotion of liver regeneration by oral OP is independent of the pharmacological actions of OP.

As PGE has been shown to produce directly an increase in the portal venous flow in rats and dogs [11, 12], the mitotic index effect produced by OP may originate from the increased blood flow. However, the 4.8 μg/kg/day OP used in this study failed to increase portal venous and hepatic arterial blood flow in conscious rats (Aono and Sakaguchi, unpublished data) and the increased mitosis may stem from the direct action of OP on hepatocytes. Although water intake was reduced by OP (Table 1) this cannot have been responsible for the increase in mitotic index, changes in water intake have no influence on liver regeneration [8].

As total protein and albumin concentrations fluctuated within the normal range when OP was administered (Table

Table 3. Fat metabolism after saline (I) or OP (1.6 μg/kg three times daily) (II) administration

	Days after hepatectomy 0	1	3	5
Lee index				
I	314.7 ± 1.2	305.1 ± 0.8	318.3 ± 2.3	311.3 ± 2.3
II	312.1 ± 2.0	305.9 ± 0.3	305.2 ± 2.1*	312.8 ± 1.6
Retroperitoneal white adipose tissue (mg)				
I	1631.8 ± 145.5	1286.0 ± 35.7	1558.0 ± 89.0	1203.4 ± 163.0
II	1527.0 ± 194.9	1093.2 ± 69.3	828.2 ± 61.0*	1324.8 ± 130.5

Values are means ± SE (N = 5). *$P < 0.01$ vs I.

2), the nutritional condition of the animals seemed to be unaffected. OP transiently enhanced GPT, showing damage to hepatocytes (Table 2), and stimulation of the mitotic index was induced 2 days later (Fig. 1). This could mean that the hepatotrophic action of OP is followed by a hepatotoxic action. Although blood urea was substantially enhanced during OP administration (Table 2), a liver regenerative response was obtained (Fig. 1). It is likely that renal dysfunction shown by the increase in blood urea did not affect liver regeneration. Effects on fat metabolism are negligible as the Lee index and retroperitoneal white adipose tissue weight were restored during OP treatment (Table 3). These results lead us to conclude that orally administered OP can stimulate liver regeneration without serious systemic effects.

Acknowledgements—The authors are greatly indebted to Mr K. Kunihara for his assistance. The test agents used in this study were supplied by Ono Pharmaceutical Co., Ltd, Osaka, Japan.

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