coronary artery ligation has been found to exert a prophylactic effect against ventricular arrhythmias induced by acute coronary artery ligation. This antiarrhythmic activity is most likely attributed to a quinidine-like membrane stabilizing effect with a possible contribution of α-adrenoceptor blocking activity. The study confirms the potential utility of imipramine as an antiarrhythmic agent.

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In-vivo and in-vitro hepatoprotective effect of 4-thia-prostaglandin E_1 and 7-fluoroprostacyclin in rats

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Abstract—4-Thia-prostaglandin E_1 and to a lesser extent 7-fluoroprostacyclin showed a potent protective effect against carbon tetrachloride-induced liver injury in-vivo and in-vitro in rat.

The cytoprotective action of prostaglandins has been intensively studied in gastric mucosa damaged by aspirin, ethanol, and taurocholate (Robert et al 1979; Teramoto et al 1987). Stachura et al (1981) showed protection by prostaglandins in the liver in which acute damage induced by carbon tetrachloride was reduced by 16,16-dimethyl prostaglandin E_2. Several other prostaglandins have a hepatoprotective effect (Arai et al 1986) and we now report studies of two prostaglandin derivatives, 4-thia-prostaglandin E_1 (4-thia-PGE_1) and 7-fluoroprostacyclin (7-fluoro-PGI_2) given orally and examined in-vitro.

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Methods

In-vitro experiment. Male Sprague–Dawley rats (158–192 g, Shizuoka Laboratory Animal Corp., Hamamatsu, Japan) were given sodium phenobarbitone 80 mg kg^{-1} d^{-1} i.p. for 3 days and then fasted overnight before use. Isolated hepatocytes were prepared by collagenase perfusion (Nakamura et al 1981). The hepatocytes were plated in a 9 cm^2 plastic dish (Corning) at a density of 1×10^5 cells cm^{-2} in William's E medium (Flow Laboratories) containing 10% foetal calf serum heat-inactivated at 56°C for 10 min (Flow Laboratories), 100 μg mL^{-1} benzylpenicillin potassium (Meiji, Tokyo, Japan), 100 μg mL^{-1} streptomycin sulphate (Meiji, Tokyo, Japan), and 0·25 μg mL^{-1} amphotericin B (Flow Laboratories). After incubation at 37°C in a humidified atmosphere of air/CO_2 (95:5) for 3 h to allow the cells to attach, the cultures were rinsed with Hanks BSS and incubated for 1 h in complete Williams E medium with 0·08% ethanol (vehicle), 4-thia-PGE_1 or 7-fluoro-PGI_2 at final concen-

Table 1. In-vivo and in-vitro hepatoprotective effect of 4-thia-prostaglandin E_1 and 7-fluoro-prostacyclin in CCl_4-induced liver cell injury.

In-vitro	Treatment	Cell viability (%)
—	—	$87{\cdot}8 \pm 3{\cdot}7^{d}$
Ethanol	0·08%	$89{\cdot}1 \pm 10{\cdot}0^{d}$
CCl_4	400 $\mu g\ mL^{-1}$	$41{\cdot}7 \pm 6{\cdot}1$
CCl_4 + 4-thia-PGE_1	5×10^{-13} M	$51{\cdot}2 \pm 4{\cdot}1^{b}$
	5×10^{-11} M	$60{\cdot}7 \pm 7{\cdot}2^{d}$
	5×10^{-9} M	$70{\cdot}6 \pm 6{\cdot}9^{d}$
	5×10^{-7} M	$77{\cdot}2 \pm 8{\cdot}8^{d}$
CCl_4 + 7-fluoro-PGI_2	5×10^{-13} M	$48{\cdot}6 \pm 8{\cdot}1$
	5×10^{-11} M	$55{\cdot}7 \pm 10{\cdot}7^{c}$
	5×10^{-9} M	$69{\cdot}1 \pm 2{\cdot}4^{d}$
	5×10^{-7} M	$68{\cdot}9 \pm 9{\cdot}9^{d}$
4-Thia-PGE_1	5×10^{-13} M	$81{\cdot}5 \pm 6{\cdot}8^{d}$
	5×10^{-11} M	$83{\cdot}3 \pm 10{\cdot}5^{d}$
	5×10^{-9} M	$80{\cdot}9 \pm 12{\cdot}3^{d}$
	5×10^{-7} M	$78{\cdot}4 \pm 11{\cdot}3^{d}$
7-Fluoro-PGI_2	5×10^{-13} M	$83{\cdot}5 \pm 4{\cdot}3^{d}$
	5×10^{-11} M	$84{\cdot}5 \pm 6{\cdot}8^{d}$
	5×10^{-9} M	$85{\cdot}2 \pm 10{\cdot}9^{d}$
	5×10^{-7} M	$83{\cdot}6 \pm 6{\cdot}1^{d}$
In-vivo	Treatment	SGPT (units L^{-1})
—	—	36 ± 23^{c}
CCl_4	6670 mg kg^{-1}	1652 ± 951
CCl_4 + 4-thia-PGE_1	0.035 mg kg^{-1}	1496 ± 739
	0·1 mg kg^{-1}	1138 ± 515^{b}
CCl_4 + 7-fluoro-PGI_2	0·1 mg kg^{-1}	1756 ± 925
	0·3 mg kg^{-1}	1140 ± 581^{a}
CCl_4 + GSH	100 mg kg^{-1}	1188 ± 562^{b}

Each value represents the mean (±s.d.). Significant difference between the treated groups and the CCl_4-treated group were determined by Student's *t*-test (unpaired, 2-tailed method). (a, $P < 0{\cdot}10$; b, $P < 0{\cdot}05$; c, $P < 0{\cdot}01$; d, $P < 0{\cdot}001$).

trations indicated in Table 1. Carbon tetrachloride (CCl_4, Wako, Tokyo, Japan) was then added to the experimental dish to a final concentration of 400 $\mu g\ mL^{-1}$. After a 3 h incubation, cell viability was assayed by trypan blue exclusion as described previously (Schanne et al 1979), and expressed as the percentage of the total number of unstained, attached cells. All measurements were made by counting six fields from 4 to 20 cultures.

In-vivo experiment. Male Sprague–Dawley rats (159–193 g, Shizuoka Laboratory Animal Corp., Hamamatsu, Japan) on a standard diet and with free access to water were divided into groups (Table 1) containing 11–36 animals fasted for 18 h before s.c. administration of 50% CCl_4 in corn oil (6670 mg kg^{-1}). Prostaglandins were dissolved in 154 mM NaCl. Glutathione, reduced form (GSH, Wako, Tokyo, Japan) was dissolved in distilled water. 4-Thia-PGE_1, 7-fluoro-PGI_2 and GSH were administered p.o. 30 min before, and at 1, 2, 8 and 18 h after CCl_4 administration at the doses shown in Table 1. The GSH-treated group was used as a positive control. Serum glutamic pyruvic transaminase (SGPT) activity was measured 24 h after CCl_4 administration by the UV-rate method (Henry et al 1960).

Results

The results are shown in Table 1. CCl_4 400 $\mu g\ mL^{-1}$, reduced the viability of cultured rat liver cells. 4-Thia-PGE_1 or 7-fluoro-PGI_2, 5×10^{-11}, 5×10^{-9} and 5×10^{-7} M, added to the culture 60 min before CCl_4 increased the cell viability by 34%–85% above that in the CCl_4-treated culture after 3 h incubation. 4-Thia-PGE_1, 5×10^{-13}, also showed a significant increase in cell viability (23%). Neither prostaglandin had an effect on the viability in the absence of CCl_4. CCl_4 s.c., increased SGPT activity 46 times above the control value at 24 h. 7-Fluoro-PGI_2, $5 \times 0{\cdot}3$ mg kg^{-1} p.o., decreased the rise in SGPT activity after CCl_4 administration. 4-Thia-PGE_1, 0·1 mg kg^{-1} p.o., showed a similar hepatoprotective effect on CCl_4-induced liver injury of rats.

Discussion

Several studies have shown that prostaglandins can protect liver cells from damage by hepatotoxins (Arai et al 1986; Stachura et al 1980, 1981; Ruwart et al 1981). Inhibition of cyclo-oxygenase during the initial stage of the injury increases the hepatotoxicity of CCl_4 (Guarner et al 1983). 4-Thia-PGE_1 and 7-fluoro-PGI_2 protect against CCl_4-induced liver cell injuries in-vitro and in-vivo. Guarner et al (1985) discussed several mechanisms for protection against CCl_4-induced liver injury by prostacyclin and 16,16-dimethyl-prostaglandin E_2 including the possibility of cell membrane preservation. Our in-vitro studies show a direct protective effect on the hepatocytes.

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