Sex Difference in the Inhibitory Effect of Aspirin on Prostacyclin Production of Rat Aortae

Masako MORIKAWA, Takashi KOJIMA, Michiko INOUE
and Minoru TSUBOI

Department of Pharmacology, Tokyo College of Pharmacy,
Hachioji, Tokyo 192-03, Japan

Accepted January 5, 1984

Abstract—The effect of aspirin on the prostacyclin (PGI₂) production of rat aorta was investigated, and the influence of sex hormones on the effect of aspirin was studied by the treatments of hormone administration, ovariectomy and castration. There was no significant sex difference in the arterial production of PGI₂ between male and female rats. However, the PGI₂ production was decreased with aspirin treatment, and the effect of aspirin was more efficient in male rats. The inhibitory effect of aspirin was reduced in the rats treated with estradiol and the castrated male rats, but it was potentiated in the rats treated with testosterone and the ovariectomized female rats. These results suggest that sex hormones may regulate the effect of aspirin on the PGI₂ production in the aorta.

Aspirin has been reported to be an effective antithrombotic agent in clinical trials (1-3) and in animal experiments (4). However, the antithrombotic effect of aspirin was found to be more effective in males than in females (2, 4).

That effect of aspirin is attributed to its inhibitory effect on PGI₂ synthesis; aspirin is considered to acetylate cyclooxygenase, an enzyme involved in PGs synthesis (5, 6). PGI₂ is the main product of the PGs in aorta (7). Chang et al. (8, 9) reported that estradiol stimulated the PGI₂ production in cultured smooth muscle cells of rat aorta and promoted the PGI₂ production in intact rats. Nakao et al. (10) showed that testosterone suppressed the PGI₂ production in cultured rat aortic smooth muscle cells.

In this report, the sex difference in the effect of aspirin and the influence of sex hormones in the inhibitory effect of aspirin on PGI₂ production were investigated.

Materials and Methods

Materials: Aspirin was obtained from Sanko Seiyaku Kogyo Co. Ltd., testosterone (4-androsten-17 β -ol-3-on) and estradiol (1, 3,5-Oestratrien-3,17 β -diol) from Fluka AG,

ADP and arachidonic acid from Sigma, and [³H]-arachidonic acid (78 Ci/mmol) and [³H]-6-keto-prostaglandin F_{1 α} (120 Ci/mmol) from New England Nuclear. PGI₂ and PGH₂ were given by Ono Yakuhin Kogyo Co. Ltd.

Animals: Wistar strain rats of both sexes were used for this study. At 6 weeks of age, the female rats were anesthetized with ether and then bilaterally ovariectomized via the back route. The male rats were anesthetized with ether and then bilaterally castrated via the ventral route. All rats were housed in a temperature-controlled room with a fixed lighting schedule (a.m. 7:00-p.m. 7:00) and allowed free access to standard laboratory food (Oriental Yeast Co. Ltd.) and water.

Administration of sex hormones: Testosterone or estradiol was dissolved in corn oil. Three days after the operation, the rats were treated with either 5 mg/kg testosterone or 10 μ g/kg estradiol at intervals of three days from 6 weeks to 10 weeks. Control rats were given 1.0 ml/kg corn oil at the same interval from 6 weeks to 10 weeks.

Drug: Aspirin was suspended in 0.1% carboxymethylcellulose sodium salt solution for the p.o. administration. The dose levels

were 20, 50 and 100 mg/kg.

Preparation of aortic tissues: At an hour after administration of aspirin, the animals were sacrificed by bleeding from common carotid arteries, and the thoracic aortae were quickly isolated and kept in Krebs-Ringer Bicarbonate buffer (pH 7.4).

Measurement of PGI₂ production: The aortae were incubated in 50 mM Tris HCl buffer (pH 7.4) at 22°C for 2, 5, 10, 15, 20 and 30 min. The production of endogenous PGI₂ was determined by the modified method of Harada et al. (11, 12). The aorta was mechanically stimulated before each incubation by holding the strip with two forceps and gently stretching the strip ten times within 20 sec. The amounts of PGI₂ released in the medium were immediately bioassayed as the inhibitory activity against ADP-induced human platelet aggregation (13). The amount of PGI₂ increased time-dependently for the first 10 min and then decreased, so the aortae were incubated for 10 min. The content of PGI₂ was expressed as ng/mg of wet tissue, using synthetic PGI₂ as the standard.

Measurement of PGI₂ production from exogenous arachidonic acid: The aortae were incubated in Tris HCl buffer containing 1.0 µg/ml arachidonic acid at 37°C for 2 min. The PGI₂ production was immediately determined as described under "Measurement of PGI₂ production".

Measurement of PGI₂ production with addition of PGI₂ as a substrate: The aortae were incubated in Tris HCl buffer containing 0.5 µg/ml PGH₂ at 37°C for 2 min. The PGI₂ production was determined by the above-described method of bioassay.

Determination of 6-keto-PGF_{1α} production from [³H]-arachidonic acid: The aortae were incubated in Tris HCl buffer containing 2 µCi [³H]-arachidonic acid at 37°C for 20 min. PGI₂ was assayed as its hydrolysis product, 6-keto-PGF_{1α}. The radioactivity of 6-keto-PGF_{1α} was determined by the modified method of Sun et al. (14).

Measurement of PGI₂ production after addition of aspirin in vitro: The aortae were preincubated with aspirin (2.0, 4.0, 6.0 and 10.0 µg/ml) in Tris HCl buffer (1.0 ml/2.5 mg tissue) at 22°C for 10 min and rinsed

with Tris HCl buffer. Then they were incubated in Tris HCl buffer at 22°C for 10 min, and this incubation medium was measured for the PGI₂ production as described under "Measurement of PGI₂ production". In the study of interaction of aspirin with sex hormones, 1.0 µg/ml testosterone and 1.0 ng/ml estradiol were used. The aortae were preincubated with aspirin (6 µg/ml) and testosterone or estradiol in Tris HCl buffer (1.0 ml/2.5 mg tissue) at 22°C for 10 min.

Results

Effect of aspirin on PGI₂ production from endogenous arachidonic acid

Figure 1 shows the inhibitory effect of aspirin on the PGI₂ production. In the control group, no sex difference was found in the PGI₂ production. In aspirin-treated group, the PGI₂ production was decreased in a dose-dependent manner. Aspirin at a dose 20 mg/kg did not efficiently decrease the PGI₂ production in female rats. Aspirin at a dose 50 mg/kg significantly decreased the PGI₂ production and more significantly decreased it in male rats. Furthermore, aspirin at 100 mg/kg completely inhibited the PGI₂ production

Fig. 1. Effect of aspirin on prostacyclin production from male and female rat aortae. Thoracic aortae were obtained from rats an hour after treatment with aspirin. The PGI₂ after stretch-stimulation of the aortae was determined by the platelet aggregation method. Mean±S.D. (n=8). *P<0.05: Significant difference from the control value for each sex. ●: male, ▲: female.

in both sexes of rats (data not shown).

Effect of aspirin on PGI₂ production from exogenous arachidonic acid

Figure 2 shows that aspirin inhibited the PGI₂ production with the addition of arachidonic acid as a substrate. In the control group, there was no significant sex difference in the PGI₂ production, but the PGI₂ production in females was slightly larger than in males. In the aspirin-treated group, aspirin dose-dependently decreased the PGI₂ production. These results agree with those on the PGI₂ production from endogenous arachidonic acid. The effect of aspirin was more

efficient in males than in females.

Figure 3 shows that aspirin inhibited the 6-keto-PGF_{1 α} release from [³H]-arachidonic acid used as a substrate. The results correspond with those from endogenous and exogenous arachidonic acid. Aspirin affected the 6-keto-PGF_{1 α} release in a dose-dependent manner. This effect of aspirin was more effective in males than in females.

Effect of aspirin on PGI₂ production with addition of PGH₂ used as a substrate

Table 1 shows the effect of aspirin on the PGI₂ production from exogenous PGH₂. When arachidonic acid was used as the substrate, aspirin at a dose 50 mg/kg com-

Fig. 2. Effect of aspirin on prostacyclin production from male and female rat aortae. Thoracic aortae were obtained from rats an hour after treatment with aspirin. PGI₂ after incubation with arachidonic acid (1.0 μ g/ml) was determined by the platelet aggregation method. Mean $\pm$ S.D. (n=8). *P<0.05: Significant difference from the control value for each sex, ■: male, □: female.

Fig. 3. Effect of aspirin on [³H]-6-keto prostaglandin F_{1 α} production from [³H]-arachidonic acid. The production of [³H]-6-keto PGF_{1 α} from [³H]-arachidonic acid by male and female aortae was measured. Mean $\pm$ S.D. (n=5). *P<0.05: Significant difference from the control value for each sex, ■: male, □: female.

Table 1. Effect of aspirin on prostacyclin production with addition of prostaglandin H₂ as a substrate

	PGI ₂ production (ng/mg tissue)*	
	Control	Aspirin (50 mg/kg)
Male	0.23 $\pm$ 0.07	0.26 $\pm$ 0.09
Female	0.25 $\pm$ 0.03	0.26 $\pm$ 0.05

*Mean $\pm$ S.D. (n=6). PGI₂ was measured after incubation with PGH₂ (0.5 μ g/ml) by the platelet aggregation method.

pletely inhibited the PGI₂ production of rat aorta in both sexes. However, when PGH₂ was added as the substrate, aspirin had no influence on the PGI₂ production.

Effect of aspirin in vitro

Figure 4 shows that the PGI₂ production is inhibited by aspirin in vitro. In male rats, aspirin inhibited the PGI₂ production at 4.0 μ g/ml, 61.0 $\pm$ 16.7%; at 6.0 μ g/ml, 32.6 $\pm$ 13.7%; and at 10.0 μ g/ml, 19.4 $\pm$ 10.5%. In female rats, aspirin at 4.0 μ g/ml did not inhibit the PGI₂ production, and aspirin at 6.0 μ g/ml inhibited the PGI₂ production (56.8 $\pm$ 15.0%). There was a sex difference in the effect of aspirin at 4.0, 6.0 and 10.0 μ g/ml on the PGI₂ production in vitro.

Influence of gonadectomy and sex hormones

Table 2 shows the influence hormones and gonadectomy on PGI₂ production and the effect of aspirin.

i) **Change in PGI₂ production by gonadectomy:** In castrated male rats, aspirin showed a tendency to increase the PGI₂ production. The inhibitory effect of aspirin on the PGI₂ production was weaker in castrated male rats than in intact male rats. In ovariectomized female rats, the effect of aspirin was enhanced, and the amount of the produced PGI₂ in ovariectomized female rats was the same as that in intact male rats.

Fig. 4. Effect of aspirin on prostacyclin production from male and female rat aortae in vitro. Thoracic aortae were preincubated with aspirin and then were incubated in Tris HCl buffer. PGI₂ was measured as described under "Materials and Methods". Mean $\pm$ S.D. (n=5). *1: Significant difference from the control value for each sex ($P<0.05$), *2: Significant difference from female value ($P<0.05$). Each value is expressed as the relative rate with respect to the control for each sex. ●: male, ▲: female.

Table 2. Change in prostacyclin production by gonadectomy and sex hormone treatment

		PGI ₂ production (ng/mg tissue)		
		Control	ASA-20	ASA-50
Male	I	0.71 $\pm$ 0.18	0.38 $\pm$ 0.12* ¹	0.19 $\pm$ 0.06* ¹
	I+E	0.92 $\pm$ 0.15	0.67 $\pm$ 0.19	0.30 $\pm$ 0.15* ¹
	I+T	0.64 $\pm$ 0.09	0.22 $\pm$ 0.15* ¹	—
	C	0.77 $\pm$ 0.13	0.65 $\pm$ 0.15	0.32 $\pm$ 0.14* ¹
	C+E	0.97 $\pm$ 0.20* ²	0.92 $\pm$ 0.11	0.98 $\pm$ 0.17
	C+T	0.64 $\pm$ 0.22	0.43 $\pm$ 0.12	—
Female	I	0.88 $\pm$ 0.25	0.63 $\pm$ 0.22	0.36 $\pm$ 0.16* ¹
	I+E	0.97 $\pm$ 0.25	0.80 $\pm$ 0.20	—
	I+T	0.80 $\pm$ 0.17	0.34 $\pm$ 0.14* ¹	—
	O	0.79 $\pm$ 0.23	0.39 $\pm$ 0.27	0.18 $\pm$ 0.10* ¹
	O+E	0.96 $\pm$ 0.20	0.82 $\pm$ 0.22	0.81 $\pm$ 0.19
	O+T	0.79 $\pm$ 0.17	0.39 $\pm$ 0.19* ¹	—

I, Intact; C, Castration; O, Ovariectomy; E, Estradiol (10 μ g/kg); T, Testosterone (5.0 mg/kg); —, Not tested; ASA, Aspirin. The values of the mean $\pm$ S.D. (n=5) are given. *¹: Significant difference from the control value for each sex ($P<0.05$). *²: Significant difference from the intact male control value ($P<0.05$).

ii) Change in PGI₂ production by treatment with estradiol: To study the influence of sex hormones on PGI₂ production, estradiol was administered. Doses of 10, 50 and 100 µg/kg were chosen. The influence of estradiol was similar among these three doses.

In the intact and castrated male rats treated with estradiol, the PGI₂ production tended to increase. In the castrated male rats treated with estradiol, aspirin did not decrease the PGI₂ production, but in the intact male rats with estradiol, aspirin showed a tendency to decrease the PGI₂ production. In the intact and ovariectomized female rats treated with estradiol, the PGI₂ production tended to increase, and the effect of aspirin was reduced.

iii) Change in PGI₂ production by treatment with testosterone: To study the influence of testosterone on PGI₂ production, rats were administered with testosterone (2.0, 5.0 and 10.0 mg/kg). The influence of testosterone was similar at 5.0 and 10.0 mg/kg, but it was weaker at 2.0 mg/kg.

In the intact male rats treated with testosterone, the effect of aspirin on the PGI₂ production was similar to that in intact male rats. However, in the castrated male rats with testosterone, aspirin did not significantly decrease the PGI₂ production. In the intact and ovariectomized female rats treated with testosterone, the effect of aspirin on the PGI₂ production was enhanced and similar to that in intact male rats.

Interaction of aspirin with sex hormones in vitro

Interaction of aspirin with sex hormones was examined in vitro. It is shown that testosterone and estradiol did not influence the effect of aspirin directly.

Discussion

It is known that PGI₂ is the main product in rat aorta and an important material in thrombosis. Some investigators have studied the PGI₂ production in rat aorta. Pomerantz et al. (15) reported the sex hormonal modification of 6-keto-PGF_{1α} release by intact and gonadectomized rats. Their results showed that more 6-keto-PGF_{1α} was released by male rat aorta than by female rat aorta,

estradiol decreased 6-keto-PGF_{1α}, and testosterone had no influence on 6-keto-PGF_{1α} release by rat aorta. On the other hand, Chang et al. and Nakao et al. (8–10) reported that estradiol increased the PGI₂ production in rat aorta, and testosterone decreased the PGI₂ production in the cultured smooth muscle cells of rat aorta. These two conclusions contradicted each other. However, sex hormones must have some influence on the PGI₂ production in rat aorta. In this study, the effect of aspirin on the PGI₂ production of rat aorta was examined in intact, gonadectomized and sex hormone treated rats.

In intact rats, there was no significant sex difference in the PGI₂ production in rat aorta when endogenous and exogenous arachidonic acid were used as substrates, but the PGI₂ production in females was slightly more than in males. Aspirin decreased the PGI₂ production dose-dependently, and the effect was more pronounced in males than in females.

Neither the significant sex difference nor the inhibitory effect of aspirin on PGI₂ production was found in control rat aorta added with PGH₂ as a substrate. These results show that aspirin does not affect the PGI₂ synthesis process from PGH₂. The site for the effect of aspirin seems to be in the course of the conversion of arachidonic acid to PGH₂.

It is doubtful that a difference of the pharmacokinetics of aspirin causes the sex difference in PGI₂ production. After 20 or 50 mg/kg of aspirin was administered to male and female rats, there was no sex difference in the maximum serum concentration, the time of the maximum serum concentration and the AUC of salicylic acid.

On the other hand, when the effect of aspirin was examined in vitro, aspirin significantly inhibited the PGI₂ production at a lower concentration in male rats, but in female rats, high concentrations of aspirin were necessary to inhibit the PGI₂ production. A significant sex difference was found in the effect of aspirin on PGI₂ production. Aspirin affected the PGI₂ production not only in vivo but also in vitro. Therefore, it is suggested that the sex difference in the effect of aspirin

is not due to a process involving the pharmacokinetics of aspirin, but is a process involving the PGI₂ production.

In sex hormone treated rats, estradiol tended to increase the PGI₂ production in rat aorta, but testosterone tended to decrease it. In the male rats treated with estradiol, the effect of aspirin was weaker in castrated rats than in intact rats. It seems that in intact rats, endogenous testosterone may influence the action of exogenous estradiol in the process of PGI₂ production. Accordingly, it seems that aspirin strongly inhibited the PGI₂ production in the intact male rats treated with estradiol. Estradiol tended to increase the PGI₂ production and suppress the effect of aspirin in intact and ovariectomized female rats. Moreover, it was proven by Chang et al. (8) that estradiol stimulated cyclooxygenase in the cultured smooth muscle of rat aorta. Their results agree with the results of this report. It is suggested from the results in this study that the low concentration of estradiol (10 µg/kg) must have affected the process of PGI₂ production and reduced the effect of aspirin.

In the male rats treated with testosterone, the PGI₂ production tended to decrease, but the effect of aspirin was different between castrated rats and intact rats. Castration weakened the effect of aspirin on the process of PGI₂ production. So, it is suggested that endogenous testosterone plays an important role in the regulatory system for PGI₂ production. Nakao et al. (10) also reported that testosterone suppressed the PGI₂ production in cultured smooth muscle cells of rat aorta. This effect of testosterone is contrary to that of estradiol, and these two sex hormones must regulate the PGI₂ production in the body.

There is a possibility that aspirin may interact with sex hormones directly, but in vitro, the effect of aspirin on the PGI₂ production did not differ significantly in the presence or absence of hormone. So, it seems that sex hormones do not directly influence the effect of aspirin on the PGI₂ production.

The inhibitory effect of aspirin on the PGI₂ production was enhanced by testosterone treatment, but weakened by estradiol treat-

ment. In female rats, it is likely that the capacity for PGI₂ production is latently elevated by estradiol and the effect of aspirin consequently weakened. However, in male rats, it is likely that the capacity for PGI₂ production is reduced or not influenced by testosterone, and the effect of aspirin is strongly enhanced. It has not yet been reported whether testosterone suppresses cyclooxygenase.

These results suggest that the sex difference in the effect of aspirin is regulated by sex hormones. This must be one of the main reasons that the effect of aspirin on the PGI₂ production in rat aorta is stronger in males than in females. Further studies on the enzyme level are to be carried out by the authors.

Acknowledgement: The authors wish to thank Ono Yakuhin Kogyo Co. Ltd., Osaka, Japan for the generous gifts of standard PGI₂ and PGH₂.

References

- 1 Harris, W.H., Saizman, E.W., Athanasoulis, C.A., Waltman, A.C. and Desanctis, R.W.: Aspirin prophylaxis or venous thromboembolism after total hip replacement. *N. Engl. J. Med.* **297**, 1246–1249 (1977)
- 2 The Canadian Cooperative Study Group: A randomized trial of aspirin and sulfinpyrasone in threatened stroke. *N. Engl. J. Med.* **299**, 53–59 (1978)
- 3 Harter, H.R., Burch, J.W., Majerus, P.W., Stanfird, N., Delez, J.A., Anderson, C.B. and Weerts, C.A.: Prevention of thrombosis in patients on hemodialysis by low-dose aspirin. *N. Engl. J. Med.* **301**, 577–579 (1979)
- 4 Kelton, J.G., Hirsh, J., Carter, C.J. and Buchanan, M.R.: Sex differences in the antithrombotic effects of aspirin. *Blood* **52**, 1072–1076 (1978)
- 5 Vane, J.R.: Possible action through inhibition of synthesis of prostaglandins. *Nature* **231**, 232–235 (1971)
- 6 Roth, G.J. and Maferus, P.W.: The mechanism of the effect of aspirin on human platelets. *J. Clin. Invest.* **56**, 624–632 (1975)
- 7 Moncada, S., Higgs, E.A. and Vane, J.R.: Human arterial and venous tissue prostacyclin (prostaglandin X), a potent inhibitor of platelet aggregation. *Lancet* **i**, 18–20 (1977)
- 8 Chang, W.C., Nakao, J., Orimo, H. and Murota, S.: Stimulation of prostaglandin cyclooxygenase and prostacyclin synthetase activities by estradiol in rat aortic smooth muscle cells. *Biochim.*

- Biophys. Acta **620**, 472–482 (1980)
- 9 Chang, V.C., Nakao, J., Neichi, T., Orimo, H. and Murota, S.: Effect of estradiol on the metabolism of arachidonic acid by aortas and platelets in rats. *Biochim. Biophys. Acta* **664**, 291–297 (1981)
- 10 Nakao, J., Chang, W.C., Murota, S. and Orimo, S.: Testosterone inhibits prostacyclin production by rat aortic smooth muscle cells in culture. *Atherosclerosis* **39**, 203–209 (1981)
- 11 Harada, Y., Tanaka, K. and Katori, M.: Acceleration of endogeneous PGI₂ generation from isolated rat aorta by MK-447. *Japan. J. Pharmacol.* **31**, 845–848 (1981)
- 12 Harada, Y., Tanaka, K. and Katori, M.: Decreased activity of prostaglandin endoperoxide synthetase in prostaglandin I₂ (prostacyclin) biosynthesis from arachidonic acid in isolated rat aortae. *Proc. Japan. Acad.* **56B**, 394–398 (1980)
- 13 Ellis, E.F., Wright, K.F., Jones, P.S., Richardson, D.W. and Ellis, C.K.: Effect of oral aspirin dose on platelet aggregation and vascular prostacyclin (PGI₂) synthesis in human and rabbits. *J. Cardiovasc. Pharmacol.* **2**, 387–397 (1980)
- 14 Sun, F.F., Chapman, J.P. and McGuire, J.C.: Metabolism of prostaglandin endoperoxide in animal tissues. *Prostaglandins* **14**, 1055–1074 (1977)
- 15 Pomerantz, K., Maddox, Y., Maggi, F., Ramey, E. and Ramwell, P.: Sex and hormonal modification of 6-keto-PGF_{1α} release by rat aorta. *Life Sci.* **27**, 1233–1236 (1980)