Enhancement of Endogenous Prostaglandin I₂ (Prostacyclin) Generation In Vivo by a Phenolic Anti-Inflammatory Agent in the Rabbit

Michiko Kawamura, Yoshiteru Harada and Makoto Katori

Department of Pharmacology, Kitasato University School of Medicine, Sagamihara, Kanagawa 228, Japan

Received June 10, 1991 Accepted July 9, 1991

ABSTRACT — Angiotensin II (i.v.) increased the plasma level of 6-keto-PGF₁α from 241 ± 60 pg/ml (n = 8) to 612 ± 108 pg/ml (n = 8) in anesthetized rabbits, which was further significantly increased by pretreatment with a phenolic anti-inflammatory agent, MK-447, to 1007 ± 218 pg/ml (n = 8). MK-447 alone did not affect the level. The level of thromboxane B₂ was not affected by these treatments. These results suggest that MK-447 may selectively enhance the PGI₂ level by acting as a tryptophan-like cofactor of PG endoperoxide synthase.

Vascular endothelial cells generate prosta
glandin (PG) I₂, which has a potent anti-aggregatory activity and a relaxing activity against vascular smooth muscles. On the con
try, blood platelets generate thromboxane (TX) A₂, which causes platelet aggregation and vasconstriction. Both active metabolites of arachidonic acid are generated through the common intermediates, PG endoperoxides, PGG₂ and PGH₂ (1). It has been reported that PG endoperoxide synthase requires heme compounds and tryptophan-like compounds as cofactors (2, 3). We have reported that MK-447 (2-aminomethyl-4-t-butyl-6-iodophenol · HCl) acts as a tryptophan-like cofactor in PG endoperoxide generation by microsomal frac
tions (4), and this agent stimulates PGI₂ gen
eration from endogenous and exogenous arachidonic acid in isolated rat aortae (5, 6). In this paper, it is shown that MK-447 selec
tively increases the plasma level of 6-keto-
PGF₁α, a stable metabolite of PGI₂, after the angiotensin II (A II)-induced hypertensive re
sponse in anesthetized rabbits.

Male rabbits (2.5 – 3.5 kg) were anesthetized with pentobarbital (40 mg/kg, s.c.; Dainippon Pharmaceut. Co., Ltd., Osaka), and the systemic blood pressure was measured by a pres
ture transducer (MPU-0.5, Nihon Kohden, Tokyo) through a polyethylene cannula inserted into the femoral artery and recorded on a polygraph (RM-85, Nihon Kohden, Tokyo). A II (Peptide Institute Inc., Osaka) was injected intravenously before and 30 min after intra-peritoneal injection of MK-447 and/or indomethacin (10 mg/kg). Blood (5 ml) was collected from the carotid artery into plastic tubes containing EDTA and indomethacin (77 mM and 10 µM, respectively) at the max
ginal level of blood pressure after injection of A II. Blood plasma was obtained by centri
fugation at 2,000 × g for 20 min. Ethanol (80% at final concentration) was added to the plasma and then it was centrifuged again at 2,000 × g for 20 min. The supernatant was di
luted with distilled water (diluting ethanol to less than 10%), acidified to pH 3.0 with 1 N-
HCl and then applied to a Sep-Pak C₁₈ col
um. 6-Keto-PGF₁α and TXB₂ were separated by high performance liquid chromatography
and were measured by radioimmunoassay according to the previously described method (7, 8). Results were expressed as the mean ± S.E.M. Statistical analysis was performed by Student's t-test.

MK-447 and indomethacin were kindly provided from Merck, Sharp and Dohme Res. Lab. (West Point, U.S.A). Antibodies for radioimmunoassay of 6-keto-PGF1α and TXB2 were kindly supplied by Ono Pharmaceutical Co., Ltd. (Osaka).

Plasma levels of 6-keto-PGF1α and TXB2 before injection of A II (control) were 241 ± 60 and 185 ± 64 pg/ml (n = 8), respectively (Fig. 1). Injection of A II (5 µg/kg) caused the blood pressure to increase to 172 ± 5 mmHg (n = 5), which was followed by a hypotensive response of −33 ± 6 mmHg (n = 5). The plasma level of 6-keto-PGF1α was dose-dependently increased by A II injection. At the dose of 5 µg/kg of A II, the level of 6-keto-PGF1α was 612 ± 108 pg/ml (n = 8, P < 0.05). Pretreatment with MK-447 (1 mg/kg) caused a further significant increase in the level (1007 ± 218 pg/ml, n = 8). The increase in the level of 6-keto-PGF1α after A II alone and MK-447 + A II was suppressed by pretreatment with indomethacin below the control level (164 ± 30 pg/ml and 119 ± 25 pg/ml, n = 4). Furthermore, the hypotensive response following the hypertensive response induced by A II injection was also completely inhibited by indomethacin (data not shown).

MK-447 alone did not affect the level of 6-keto-PGF1α (Fig. 1). The level of TXB2 was not significantly affected by any of these treatments (Fig. 1).

The dose-dependent effect of MK-447 on the level of 6-keto-PGF1α is shown in Fig. 2. MK-447 (0.1 and 1 mg/kg) induced a dose-dependent increase in the level of 6-keto-PGF1α after injection of A II (5 µg/kg). However, further increase in the dose of MK-447 (3 mg/kg) did not increase the level of 6-keto-PGF1α. Practically no change in the level of TXB2 was observed after these treatments.

The results presented here confirmed the report by Rowe and Nasjletti showing that PGI2 is released during the hypertensive response to A II (9). Mullen and Moncada have reported that A II stimulates PGI2 release without enhancement of TXA2 generation and suggested that A II activates phospholipase A2 and stimulates the release of arachidonic acid from vascular endothelial cells (10). In the present study, MK-447 induced the enhancement of the A II-induced increase in the plasma level of 6-keto-PGF1α as shown in Fig. 1. MK-447 itself, however, did not significantly affect the level of either 6-keto-PGF1α or TXB2.
observations seem to support the hypothesis that the stimulatory action of MK-447 on PGI$_2$ generation is not due to an enhancement of the release of arachidonic acid from vascular endothelial cells or platelets.

Figure 2 shows that lower doses of MK-447 (0.1 and 1 mg/kg) enhanced the increase in plasma 6-keto-PGF$_{1A}$ level after A II, but the enhancing activity decreased at a higher dose (3 mg/kg). This result is consistent with our previous findings that MK-447 exhibits dual effects, stimulation and inhibition, on PGH$_2$ biosynthesis by microsomal fractions from bovine seminal vesicles (4) and on PGI$_2$ formation in isolated rat aortae (6). Originally MK-447 was reported to enhance PGH$_2$ formation by acting as a scavenger of oxygen-derived free radicals, which are generated in the conversion of PGG$_2$ to PGH$_2$ (11). In experiments using purified enzyme preparations, it was shown that PG endoperoxide synthase requires tryptophan or other aromatic compounds as cofactors for scavenging oxygen-derived free radicals (2, 3). The authors reported that MK-447 acts as a cofactor similar to phenol or tryptophan in the PG endoperoxide formation and suggested that MK-447 enhances the PG biosynthesis by supplementing tryptophan-like scavenger to the cells. On the other hand, Lands (12, 13) proposed an importance of maintaining the level of ambient lipid peroxides, which are necessary to initiate the PG endoperoxide synthase catalyzed reaction. Excess amounts of phenol compounds such as MK-447 rather seems to inhibit formation of PG endoperoxide by deprivation of the peroxide. Thus, the present results indicate that MK-447 may exhibit dual effects, stimulation and inhibition, on the conversion of arachidonic acid to PG endoperoxide after the release induced by A II.

In conclusion, it is suggested that MK-447 enhances the PGI$_2$ formation induced by A II in vivo by acting as a tryptophan-like cofactor of PG endoperoxide synthase.

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