Regional Vascular Responses to Thromboxane A₂ Analogue and Their Blockade with Vapiprost, a Selective Thromboxane Receptor Blocking Drug, in Anesthetized Dogs

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ABSTRACT — Regional vascular responses to the thromboxane A₂ analogue U46619 and effects of the selective thromboxane receptor blocking drug vapiprost on these responses were examined in anesthetized dogs. Hemodynamic responses to U46619 (0.5 µg/kg into the left atrium), norepinephrine (NE, 0.3 µg/kg, i.v.) and angiotensin II (All, 30 or 60 ng/kg, i.v.) were periodically tested before and after administration of vapiprost (10, 30 or 100 µg/kg, i.v.) or its vehicle. In the absence of vapiprost, U46619 increased total peripheral (TPR), vertebral (VR), coronary (CR) and renal (RR) vascular resistance by 60.1 ± 4.7%, 33.6 ± 4.9%, 15.3 ± 1.3% and 120.8 ± 17.4%, respectively, indicating that vasoconstrictor responses to U46619 were most prominent in the renal vascular bed as compared to those in the vertebral or coronary vasculatures. Vapiprost as well as the vehicle did not affect the baseline hemodynamics. However, vapiprost apparently inhibited the U46619-induced vasoconstriction in all measured vascular beds in a dose-related manner without attenuating vasoconstrictor responses to NE and All, although significantly larger inhibition of U46619-induced increases in RR was observed as compared to the inhibitions of VR and CR. These results demonstrate that there was a regional difference both in the vasoconstrictor responses to U46619 and in the blocking effects of vapiprost, and indicate that vapiprost is a potent and selective antagonist for thromboxane receptors in vivo.

Keywords: U46619, Vapiprost, Regional vascular resistance, Vasoconstriction

Thromboxane A₂ (TXA₂) is a potent platelet aggregatory and vasoconstrictor autacoid, which has been implicated in various pathophysiological conditions such as myocardial infarction, transient ischemic attacks, Raynaud's disease and lupus nephritis (1, 2). The biological effects of TXA₂/prostaglandin endoperoxide are mediated through a specific receptor termed a TP-receptor (3), whose molecular structure has recently been reported (4). Thus, much effort has been expended to search for selective antagonists of TP-receptors in order to prevent the deleterious effects of TXA₂, and a few of these drugs are now available for clinical studies (5).

Vapiprost ([1R-[1α(Z),2β,3β,5α]](-)^{(+)}-7-[5-[(1,1'-biphenyl)-4-yl]methoxy]-3-hydroxy-2-(1-piperidinyl)cyclopentyl]-4-heptenoic acid hydrochloride, SN-309; GR32191B), a recently synthesized agent, is a highly potent and specific TP-receptor antagonist in platelets and isolated vascular and airway smooth muscles without partial agonist activities (2, 6, 7). Preliminary in vivo studies have also demonstrated that systemically administered vapiprost appears to effectively block both platelet aggregation and vasoconstriction induced by stimulation via TP-receptors in animals (8) and humans (9). However, hemodynamic effects of this drug and its in vivo TP-receptor blocking actions, especially in terms of the specificity and potency, have not been fully investigated in whole animals. Therefore, the aim of this study was, first, to elucidate the cardiovascular properties of the specific TP-receptor agonist U46619 and, second, to evaluate the acute effect of vapiprost on the systemic hemodynamics and regional hemodynamic responses of important organs such as the brain, heart and kidney to the TXA₂ mimetic U46619, norepinephrine (NE) or angiotensin II (All) in anesthetized dogs. For this purpose, aortic, vertebral, coronary and
renal arterial blood flows were continuously and simultaneously measured in the same dog by electromagnetic flowmeters along with aortic pressure (10).

MATERIALS AND METHODS

Experimental setup
Twenty-three mongrel dogs of either sex weighing 10–18 kg were anesthetized with sodium pentobarbital (25 mg/kg, i.v.), and were ventilated by a Harvard respirator (model 607). A catheter filled with heparinized saline (0.9% NaCl) was inserted through the left carotid artery into the aortic root and connected to a pressure transducer (Nihon Kohden, TP-200T) for the measurement of aortic pressure (AoP). The left vertebral artery was dissected free just before entering the transverse foramen of the sixth cervical vertebrae. The left renal artery was exposed by a retroperitoneal approach through a flank incision. A left thoracotomy was performed through the fifth intercostal space. The pericardium was opened, and the heart was suspended in a pericardial cradle. The ascending aorta and the proximal portion of the left circumflex coronary artery (LCX) were dissected free from surrounding tissues. Electromagnetic flow probes (Statham, SP7515 and SP7518) of appropriate sizes were placed around the vertebral artery, the renal artery, the LCX and the ascending aorta, and were connected to electromagnetic flowmeters (Statham, SP2204) for the measurement of vertebral (VBF), renal (RBF), coronary (CBF) arterial and aortic blood flow (AoF), respectively. The four flowmeters were synchronized with each other via connectors to avoid any electromagnetic interaction among the flow probes. A catheter-tip manometer (Millar, PC-350) was introduced through the left atrial appendage into the left ventricular cavity to measure left ventricular pressure (LVP). A polyvinyl tube (10 cm long) filled with heparinized saline was inserted through the left atrial appendage into the left atrium for administration of U46619.

Mean blood flow was obtained by using an electronic resistance-capacitance filter with a 5-sec time constant. The first derivative of LVP (LVdP/dt) was derived from differentiating the LVP signal with an electronic differentiator (Nihon Kohden, ED-601G). Heart rate was continuously counted with a cardiotachometer (Nihon Kohden, AT-600G) triggered by the LVP pulse. Data were continuously recorded on an eight-channel pen oscillograph (NEC San-ei, 8K23-L) and simultaneously stored on the magnetic tape of an FM data recorder (Sony, A47).

Experiments were started after a stabilization period of at least 30 min. The dogs were divided into four groups. In the first group (n = 8), hemodynamic responses to U46619 (0.5 µg/kg into the left atrium), NE (0.3 µg/kg, i.v.) and AII (30 or 60 ng/kg, i.v.) were recorded in this order as control responses, and then the vehicle for vapiprost (0.3 ml/kg) was given i.v. Responses of hemodynamic variables to these three vasoconstricting agents were tested at 5, 30, 60, 90 and 120 min after administration of the vehicle. In the second, third and fourth groups, the same experimental protocol was performed as in the first group, but vapiprost at doses of 10 µg/kg, i.v. (n = 4), 30 µg/kg, i.v. (n = 4) and 100 µg/kg, i.v. (n = 7) instead of the vehicle were given, respectively.

Drugs
The compounds employed in this study were vapiprost (SN-309, Nippon Glaxo, Ltd., Tokyo), U46619 ((15S)-hydroxy-11α,9α-(epoxymethano)prosta-5Z,13E-dienoic acid, Funakoshi Co., Ltd., Tokyo), NE (Sankyo Co., Ltd., Tokyo) and AII (Peptide Institute, Inc., Osaka). Stock solutions of vapiprost (500 µg/ml) were made up of vapiprost (0.05%), α-cyclodextrin (0.166%), mannitol (5.0%), NaH2PO4·2H2O (0.093%), Na2HPO4 (0.011%) and distilled water, and the pH of the solution was adjusted with NaOH to pH 6.0. The composition of the vehicle for vapiprost was the same as that of the stock solution of vapiprost with the exception of the lack of vapiprost and NaOH. The stock solution was diluted with an appropriate volume of vehicle just before use, and vapiprost or the vehicle was given i.v. in a bolus. U46619 was dissolved in ethanol in advance and diluted with 0.2% sodium carbonate, followed by further dilution with saline to the final concentration (10 µg/ml). U46619 containing the final concentration of 0.1% ethanol was administered into the left atrium in a bolus. The vehicle for U46619 (0.05 ml/kg) had virtually no hemodynamic effect. NE and AII were dissolved in saline.

Data analyses
Total peripheral (TPR), vertebral (VR), coronary (CR) and renal (RR) vascular resistances were calculated as the quotients of the mean AoP and mean AoF, mean VBF, mean CBF and mean RBF, respectively. TPR, thus, in this study does not involve the resistance of coronary vascular beds. Time sequence data were analyzed by two-way analysis of variance, and unpaired data were analyzed with the t-test. The level for statistical significance was P < 0.05. All results are expressed as means ± S.E.
RESULTS

Systemic and regional vascular responses to U46619, NE and All

Doses of U46619, NE and All employed in this study were selected to cause a similar degree of pressor response. In fact, comparable increases in diastolic aortic pressure induced by U46619 (0.5 μg/kg), NE (0.3 μg/kg) and All (30 or 60 ng/kg) in the vehicle group were observed to be 25.1 ± 3.0 mmHg, 26.0 ± 2.6 mmHg and 19.1 ± 2.5 mmHg, respectively. Responses to these agonists disappeared within 5 min after the injection in most cases. Regional hemodynamic responses to U46619, NE and All obtained before treatment with vapiprost or vehicle are summarized in Fig. 1. U46619 caused significant decreases in AoF, VBF and RBF, and a small but significant increase in CBF. Only VBF and RBF significantly decreased with NE, while all four flows significantly decreased with All. Significantly larger decreases in RBF when compared to the response of AoF were observed with each of these three agonists (Fig. 1), whereas the U46619-induced change in CBF and All-induced changes in VBF and CBF were significantly less potent than those in AoF. All vascular resistances calculated from each blood flow and mean AoP significantly increased in response to each agonist (Fig. 1), although the pattern of the responsiveness of these flows was different among these three vasoconstrictors. U46619 elicited significant increases in TPR, VR, CR and RR by 60.1 ± 4.7%, 33.6 ± 4.9%, 15.3 ± 1.3% and 120.8 ± 17.4% above the respective basal value obtained before treatment. Injections of NE and All also increased TPR by 20.8 ± 2.7% and 48.2 ± 9.0%, VR by 29.5 ± 4.9% and 21.5 ± 3.4%, CR by 14.1 ± 1.4% and 23.3 ± 1.8%, and RR by 44.6 ± 3.8% and 139.1 ± 17.5%, respectively. These results indicate that vasoconstrictor responses to U46619 as well as All or NE were the most prominent in the renal vascular bed as compared to those in the vertebral or coronary vasculatures.

Effects of vapiprost on hemodynamic variables

The highest dose of vapiprost (100 μg/kg, i.v.) in this study as well as the lower doses (10 and 30 μg/kg, i.v.) exerted no effect on the time course of systemic hemodynamics at least until 120 min after the injection of the antagonist when compared to that in dogs receiving the vehicle (Fig. 2). As shown in Table 1, baseline values for this group were comparable to those for the vehicle group.

Effects of vapiprost on regional vascular responses to U46619, NE and All

As shown in Fig. 3, administration of vehicle did not affect the responses to any of these vasoconstrictors with the exception that a small increase in the U46619-
Fig. 2. Time courses of hemodynamic variables before and after intravenous administration of vehicle (open circles, \(n = 8\)) and 100 \(\mu\)g/kg of vapiprost (closed circles, \(n = 7\)). \(\text{mAoP} = \text{mean aortic pressure, AoF} = \text{aortic blood flow, VBF} = \text{vertebral blood flow, CBF} = \text{coronary blood flow, RBF} = \text{renal blood flow, HR} = \text{heart rate, LVEDP} = \text{left ventricular end-diastolic pressure.} (+)\text{LVdP/dt} = \text{peak positive left ventricular dP/dt.} \) All points showing percent changes from the respective predrug value are expressed as means ± S.E. Administration of vehicle or vapiprost is shown by an arrow.

Table 1. Baseline values of hemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>Vehicle ((\mu)g/kg, i.v.)</th>
<th>Vapiprost ((\mu)g/kg, i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Number of animals</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>(\text{mAoP (mmHg)})</td>
<td>120 ± 7</td>
<td>88 ± 9</td>
</tr>
<tr>
<td>(\text{AoF (ml/min)})</td>
<td>1159 ± 68</td>
<td>1128 ± 75</td>
</tr>
<tr>
<td>(\text{VBF (ml/min)})</td>
<td>23 ± 4</td>
<td>33 ± 4</td>
</tr>
<tr>
<td>(\text{CBF (ml/min)})</td>
<td>23 ± 3</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>(\text{RBF (ml/min)})</td>
<td>125 ± 14</td>
<td>135 ± 11</td>
</tr>
<tr>
<td>(\text{HR (beats/min)})</td>
<td>161 ± 6</td>
<td>109 ± 7*</td>
</tr>
<tr>
<td>(\text{LVEDP (mmHg)})</td>
<td>6.6 ± 1.1</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>(\text{(+)}\text{LVdP/dt (mmHg/sec)})</td>
<td>1931 ± 200</td>
<td>1725 ± 206</td>
</tr>
</tbody>
</table>

\(\text{mAoP}: \text{mean aortic pressure, AoF}: \text{aortic blood flow, VBF}: \text{vertebral blood flow, CBF}: \text{coronary blood flow, RBF}: \text{renal blood flow, HR}: \text{heart rate, LVEDP}: \text{left ventricular end-diastolic pressure, (})\text{LVdP/dt}: \text{peak positive left ventricular dP/dt.} \text{*P < 0.05 vs. vehicle. Data represent means ± S.E.} \)
induced pressor response was seen 120 min after the administration (Fig. 3A), whereas vapiprost (100 μg/kg, i.v.) significantly inhibited the response to U46619 but not those to NE or AII (Fig. 3B). Furthermore, Fig. 4 demonstrates that vapiprost at 10, 30 and 100 μg/kg, i.v. apparently inhibited the U46619-induced vasoconstriction in all vascular beds measured in the present experiments in a dose-dependent manner, but the vehicle did not. Vasoconstrictor responses to NE and AII during the observation period (120 min) were not significantly reduced by vapiprost or the vehicle except that AII-induced increases in RR decreased (P < 0.05) from 162 ± 42% to 94 ± 24% at 120 min after administration of vapiprost (100 μg/kg, i.v.). Durations of the inhibitory effect of vapiprost on the vascular responses to U46619 also appear to be dose-related in each of the vasculatures. When percentage inhibitions of U46619-induced increases in each vascular resistance by vapiprost at 100 μg/kg, i.v. were averaged out during the observation period, the increases in TPR, VR, CR and RR were inhibited by 70.8 ± 5.3%, 49.3 ± 9.5%, 49.4 ± 6.0% and 86.9 ± 2.6%, respectively. Thus, the inhibitory degree in RR by vapiprost at this dose was significantly (P < 0.01) larger than that in VR or CR.

Fig. 3. Effects of vehicle (A, n = 8) and vapiprost (B, n = 7) on pressor responses to U46619 (0.5 μg/kg into the left atrium), norepinephrine (NE: 0.3 μg/kg, i.v.) and angiotensin II (AII: 30 or 60 ng/kg, i.v.). PRE (closed column) represents the response obtained before treatment with vehicle or vapiprost. All columns are expressed as means ± S.E. *P < 0.05, **p < 0.01 vs. PRE.
DISCUSSION

The present experiments demonstrate that there was a regional difference in the vasoconstrictor response to systemically given TXA2 analogue U46619 as well as NE or AII in the anesthetized dog, being most prominent in the renal vascular beds as compared to the vertebral and coronary ones. It is probable that the increase in vascular resistance induced by U46619 is mainly due to its vasoconstrictor action rather than vascular obstruction resulting from platelet aggregation, since the effects of repeated injections of U46619, similar to the effects of the other two agonists NE and AII were short-lived and highly reproducible within an interval of 25−30 min in the vehicle group as recently reported by another laboratory (11). In support of this view, Kadowitz and Hyman (12) have suggested that obstruction of the vascular bed by platelet aggregates would contribute little if anything to the increased vascular resistance in response to U46619 in dogs. U46619 has been previously shown to have essentially the same receptor profile as natural TXA2 (13, 14). Thus, the present findings suggest that a difference in the population and/or sensitivity of TP-receptors may...
exist among different vessels, as reported in the case of
AII receptors (15). Additionally, the relatively weak
effect of U46619 in the vertebral and coronary vascula-
tures, as compared to other vasculatures, may be re-
lated to previous observations that U46619 stimulated
vascular prostacyclin synthesis in vitro (16, 17) and that
U46619 enhanced the release of systemic and coronary
prostacyclin in dogs (18, 19). Furthermore, possible in-
volvement of endothelium-derived relaxing factor in
cerebral (20) and coronary (19) arterial responses to
U46619 in vivo has been suggested. Such indirect
effects of U46619 in addition to the direct vasoconstric-
tor effect might operate to a relatively large extent in
these vasculatures. A facilitatory action of U46619 on
the vasoconstrictor response to renal nerve stimulation
has recently been reported (21), and this may also ex-
plain, at least partly, the effect of U46619 on the abun-
dantly innervated renal vasculature. The present in vivo
results, however, are inconsistent with those in previous
in vitro studies, in which TXA2 analogues had a similar
contractile potency in different isolated dog or cat
arteries such as basilar, coronary, mesenteric and renal
arteries (22, 23), although there is no comparative
study with respect to the regional vascular responsive-
to TXA2 or U46619 in vivo, as far as we know.
These additional mechanisms may be related to the dis-
crepancy between the in vivo and in vitro experimental
findings described above.

Results of this investigation clearly demonstrate that
vapiprost, at doses causing no direct hemodynamic
effect, potently blocks vasoconstrictor responses of ver-
tebral, coronary and renal vascular beds specifically to
the TXA2 analogue U46619 in a dose-dependent man-
ner in anesthetized dogs. These data further confirm
the previous findings showing that vapiprost represents
a highly selective and potent TP-receptor antagonism,
which is evaluated in platelets and vascular and airway
smooth muscles obtained from a range of species in-
cluding humans (6, 7, 9, 24, 25). Results that vapiprost
even at the highest dose employed in this study virtual-
ly had no effect on the baseline hemodynamics are also
consistent with the previous data that this drug has no
partial agonist activity (2, 8), and suggest that the
hemodynamic influence of tonic formation and release
of TXA2, if any, is presumably negligible in the anes-
thetized open-chest dog, in contrast to the case of cyclo-
oxigenase inhibitors such as indomethacin, which has
been found to cause an increase in vascular resistance
(26, 27). Therefore, these characteristics of vapiprost
indicate that this drug is not only a pharmacological
tool as a TP-receptor blocking agent but also may be
useful in the treatment of patients with various car-
diovascular diseases such as transient ischemic attacks,
unstable angina and Raynaud’s disease that are consi-
dered to involve TXA2-induced vasoconstriction (1, 2),
although it should be noted that species differences may
exist in the responsiveness of platelets and vessels to
TXA2 and endoperoxide analogues (28).

The potency and duration of the TP-receptor block-
ing activity of vapiprost in the renal vascular bed
appeared relatively prominent and long-lasting as com-
pared to those in the vertebral and coronary ones as
shown in Fig. 4. These present results suggest the pos-
sibility that blockade of the TP-receptor may exert a ben-
cificial action, particularly in some renal diseases, since
renal vasoconstriction due to intrarenal TXA2 produc-
tion has been found to participate in cyclosporine
nephrotoxicity (29–31) and lupus nephritis (32, 33).
Additionally, enhanced renal vascular reactivity to the
TXA2 analogue U46619 has recently been shown in
Goldblatt hypertensive rats (34, 35) and genetically
hypertensive rats (36). Thus, these findings imply that
the role of renal TP-receptors in regulation of perfusion
of the kidney may be important under pathophysio-
logical conditions in addition to normal conditions.

Acknowledgments

We thank Nippon Glaxo, Ltd. for kindly supplying vapiprost.

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