Renal effects induced by prolonged mPGES1 inhibition

Francisco Salazar,1 Michael L. Vazquez,2* Jaime L. Masferrer,2 Gabriel Mbalaviele,2 Maria T. Llinas,1 Fara Saez,1 Grace Arhancet,2 and F. Javier Salazar1*

1Department of Physiology, School of Medicine, Campus Mare Nostrum of Excellence, University of Murcia, Murcia, Spain; and 2Pfizer, Incorporated, St. Louis, Missouri

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Salazar F, Vazquez ML, Masferrer JL, Mbalaviele G, Llinas MT, Saez F, Arhancet G, Salazar FJ. Renal effects induced by prolonged mPGES1 inhibition. Am J Physiol Renal Physiol 306: F68–F74, 2014. First published November 6, 2013; doi:10.1152/ajprenal.00492.2013.—The importance of membrane-bound PGE synthase 1 (mPGES1) in the regulation of renal function has been examined in mPGES1-deficient mice or by evaluating changes in its expression. However, it is unknown whether prolonged mPGES1 inhibition induces significant changes of renal function when Na+ intake is normal or low. This study examined the renal effects elicited by a selective mPGES1 inhibitor (PF-458) during 7 days in conscious chronically instrumented dogs with normal Na+ intake (NSI) or low Na+ intake (LSI). Results obtained in both in vitro and in vivo studies have strongly suggested that PF-458 is a selective mPGES1 inhibitor. The administration of 2.4 mg·kg−1·day−1 PF-458 to dogs with LSI did not induce significant changes in renal blood flow (RBF) and glomerular filtration rate (GFR). A larger dose of PF-458 (9.6 mg·kg−1·day−1) reduced RBF (P < 0.05) but not GFR in dogs with LSI and did not induce changes of renal hemodynamic in dogs with NSI. Both doses of PF-458 elicited a decrease (P < 0.05) in PGE2 and an increase (P < 0.05) in 6-keto-PGF1α. The administration of PF-458 did not induce significant changes in renal excretory function, plasma renin activity, and plasma aldosterone and thromboxane B2 concentrations in dogs with LSI or NSI. The results obtained suggest that mPGES1 is involved in the regulation of RBF when Na+ intake is low and that the renal effects elicited by mPGES1 inhibition are modulated by a compensatory increment in PGI2. These results may have some therapeutic implications since it has been shown that prolonged mPGES1 inhibition has lower renal effects than those elicited by nonsteroidal anti-inflammatory drugs or selective cyclooxygenase-2 inhibitors.

METHODS

Experiments in conscious hound dogs (17–24 kg) were performed in accordance with the rules of the European Union and were approved by the Institutional Animal Care and Use Committee of the University of Murcia. Dogs were surgically instrumented under anesthesia (8, 20) for mean arterial pressure (MAP) and renal blood flow (RBF) measurements. Briefly, tygon catheters were inserted into the abdominal aorta for arterial pressure measurements and blood sample collection and into the inferior vena cava for infusions. A transit-time flow probe (4R, Transonic Systems) was implanted on the left renal artery for the measurement of RBF. The catheters and cable connected to the probe were tunneled subcutaneously, exteriorized between the scapulae, and placed in neck collars. The arterial pressure and flow lines were connected to an analog-to-digital collection system (T208, Transonic Systems), and data were analyzed using an IBM personal computer. MAP and RBF data were obtained every second and subsequently averaged over a 15-min period (12:00 to 12:15 PM) as in a previous study (22). These measurements were made each day in the three experimental groups. A previous study (8) performed by our group has shown that arterial pressure and renal function remained unaltered for >10 consecutive days when conscious chronically instrumented dogs were only treated with vehicle. Male and female dogs were included in each group, and the results obtained were pooled because no sex-dependent differences were found.

PROSTAGLANDIN (PG)E2 synthesis is produced by one cytosolic PGE synthase (PGES) and two membrane-bound PGE isomerase (mPGES1 and mPGES2). mPGES1 is inducible and upregulated in response to proinflammatory stimuli, with a concomitant increased expression of cyclooxygenase (COX)-2 (3). This isomerase also seems to be involved in the regulation of blood pressure (BP) and renal function since it is constitutively expressed in aortic and mesenteric arteries, macula densa, the collecting duct system, and medullary interstitial cells (7, 19, 26, 28). However, the physiological importance of mPGES1 has only been examined in mPGES1-deficient mice or by evaluating changes in its expression (5, 6, 11, 13–15, 28).

The present study was performed to examine the role of mPGES1 in the prolonged regulation of renal hemodynamic and excretory function when Na+ intake is low or normal. The importance of mPGES1 was examined by administering a selective inhibitor of this isomerase to conscious dogs, and the hypothesis was that the prolonged administration of this inhibitor induces a greater renal vasoconstriction when Na+ intake is low than when it is normal. This inhibition could also induce a transitory decrease in Na+ excretion when Na+ intake is normal. These hypotheses were based on studies showing that mPGES1 and COX-2 are colocalized in the kidney (21) and seem to be functionally linked in the macula densa during a low-salt diet (7, 18). Our hypotheses were also supported by studies showing that prolonged COX-2 inhibition elicits both renal vasoconstriction that is significantly enhanced when Na+ intake is low and a transitory decrease in Na+ excretion when Na+ intake is normal (20, 22). A decrease in plasma renin activity (PRA) and plasma aldosterone concentration (PAC) as well as an increase in plasma K+ concentrations (pK) could also occur in dogs treated with the mPGES1 inhibitor since it has been proposed that mPGES1 is involved in the regulation of renin release (10). It has been also demonstrated that COX-2 inhibition induces a significant decrease in PRA and PAC as well as an increase in pK (20, 22). The results obtained during the prolonged administration of a mPGES1 inhibitor may have important clinical implications since this enzyme is involved in the generation of the PG involved in the inflammatory process (3) and the administration of nonselective COX or selective COX-2 inhibitors elicits important changes in renal function (8, 20, 22).

* M. L. Vazquez and F. J. Salazar share senior authorship.

Address for reprint requests and other correspondence: F. J. Salazar, Dept. of Physiology, School of Medicine, Univ. of Murcia, Murcia 30100, Spain (e-mail: salazar@um.es).
At 9:00 AM, dogs were fed a diet (HD, Hill Pet Products), which provided 4–8 mmol Na+/day, and were allowed free access to tap water. Twenty-four-hour urine samples were collected between 9:00 and 9:30 AM. Plasma samples for creatinine measurement were drawn daily, at 11:30 AM. Plasma samples were also obtained during the control period, at the end of \( d \) and 7 of PF-458 administration, and at the end of day 3 of the recovery period to measure PRA and thromboxane (TX)B2, aldosterone, Na+ concentration (pNa), and pK. Urinary excretion rates of PGE2 and 6-keto-PGF1α were also determined during the control period, at the end of \( d \) and 7 of PF-458 administration, and at the end of day 3 of the recovery period.

**Selectivity of PF-458 for mPGES1**

PF-458 \(^{1}\)-[5-(5-chloro-6-(4-chlorophenyl)benzo[d]oxazol-2-yl)-N-((1S,4S)-4-(hydroxymethyl)cyclohexyl)piperidine-4-carboxamide] was prepared from \( \text{cis}(4\text{-aminocyclohexyl})\text{methanol and the carboxylic acid of methyl 1-[5-chloro-6-(4-chlorophenyl)benzo[d]oxazol-2-yl]} \) piperidine-4-carboxylate (compound 40, Ar = 4-chlorophenyl) as previously described by Arhancet et al. (2). Selectivity was examined in experiments performed in accordance with protocols approved by the Ethics Committee of Pfizer. Selectivity was examined by measuring prostanoid levels in conditioned media treated with a nonselective COX inhibitor (indomethacin), a selective COX-2 inhibitor (SC-236), and PF-458. Synovial fibroblasts derived from patients with rheumatoid arthritis, fibroblasts from normal patients, and a modified human whole blood (mHWB) assay (a coculture system of the human head and neck squamous cell carcinoma 1483 cells and human whole blood) were used as previously described (17). The rat basophilic leukemia cell line was purchased from the American Type Culture Collection and used according to the supplier’s instructions. Prostanoid levels were also measured in the synovial fluid 3 h after an intraarticular injection of LPS (1.5 ml, 100 ng/ml) to dogs pretreated with vehicle or a single dose of PF-458 (14.6 mg/kg). This dose of PF-458 was orally administered 1 h before the intraarticular injection of LPS.

**Experimental Groups**

**Group 1** \((n = 6)\). After a control period of 3 days, PF-458 was given orally during 7 consecutive days \((2.4 \text{ mg·kg}^{-1} \text{·day}^{-1})\), with half of the dose given at 9:00 AM and the second half of the dose given at 7:00 PM. After PF-458 administration was finished, a recovery period of 3 days was allowed.

**Group 2** \((n = 5)\). The protocol for group 2 was similar to that described above for group 1 with the exception that the mPGES1 inhibitor was given daily at a dose four times higher \((9.6 \text{ mg·kg}^{-1} \text{·day}^{-1})\).

**Group 3** \((n = 6)\). The protocol for group 3 was similar to that described above for group 1 with the exception that PF-458 was given at a dose of 9.6 mg·kg\(^{-1}\)·day\(^{-1}\) and the total Na+ load was increased to 70 meq/day by a continuous infusion isotonic saline at a rate of 425 ml/day.

**Analytic Methods**

Na+ and K+ levels were measured by flame photometry, and GFR was estimated by the clearance of endogenous creatinine as in previous studies (8, 20, 22). PRA and PAC were measured using commercial radioimmunoassay (Diasorin). All eicosanoids were measured using two-dimension liquid chromatography tandem mass spectrometry as previously described (29) with slight modifications to the chromatography.

**Statistical Analysis**

Data are expressed as means ± SE. Significance of differences between values in the same group was evaluated by one-way ANOVA and a Fisher test (GB Stat, Dynamic Microsystems, 1996). \( P \) values of <0.05 were considered significant.

**RESULTS**

**Selectivity of PF-458 for mPGES1**

The results shown in Table 1 demonstrate that indomethacin inhibited the biosynthesis of PGE2 and other prostanooids with equal potency in protein-free cellular assays or in the mHWB assay. Although selective COX-2 inhibition by SC-236 also inhibited the production of prostanooids in cytokine-stimulated assays with comparable potency, as expected, it only inhibited TXB2 release at concentrations known to cross over to COX-1. As shown in Table 1 and Fig. 1, the selective mPGES1 inhibitor PF-458 induced a significant reduction of PGF2α, but not of other eicosanoids in cells in vitro and in dogs. Consistent with the shunting mechanism of eicosanoid biosynthesis upon mPGES-1 inhibition (2), the levels of PGF1α, PGD2, and PGF2β, but not TXB2, were significantly higher in PF-458-treated dogs compared with control dogs. Collectively, these data strongly suggest that PF-458 is a selective inhibitor of mPGES-1 in vitro and in vivo.

**Group 1.** MAP had a basal value of \(110 ± 6 \text{ mmHg}\) and did not change throughout the experiment \((110 ± 5 \text{ mmHg}\) during PF-458 administration and \(109 ± 5 \text{ mmHg}\) during the recovery period). Prolonged mPGES1 inhibition did not elicit significant changes in RBF and GFR (Figs. 2 and 3) with respect to the values found during the control period \((191 ± 14 \text{ and } 47 ± 4 \text{ ml/min}, \text{respectively}). \) Renal hemodynamics remained within control values during the recovery period. As shown in Table 2, urinary Na+ excretion, urinary K+ excretion, and pK did not change throughout the experiment. As occurred in the other two experimental groups, urine flow rate did not change significantly during mPGES1 inhibition. As shown in Table 3, PRA and plasma concentrations of aldosterone and TXB2 also remained within control values during PF-458 administration.

**Table 1.** \( IC_{50} \) values of synovial fibroblasts derived from patients with rheumatoid arthritis, fibroblasts from normal patients, and a modified human whole blood assay as well as rat basophilic leukemia cells treated with a nonselective COX inhibitor (indomethacin), a selective COX-2 inhibitor (SC-236), or a selective membrane-bound PGE synthase 1 inhibitor (PF-458).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>PGE2 (normal fibroblasts)</th>
<th>6-keto-PGF1α (fibroblasts derived from patients with rheumatoid arthritis)</th>
<th>PGE2 (normal fibroblasts)</th>
<th>PGE2 (modified human whole blood assay)</th>
<th>TXB2 (modified human whole blood assay)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indomethacin</td>
<td>0.01</td>
<td>0.02</td>
<td>0.02</td>
<td>0.014</td>
<td>10</td>
</tr>
<tr>
<td>SC-236</td>
<td>0.10</td>
<td>0.10</td>
<td>0.40</td>
<td>ND</td>
<td>0.2</td>
</tr>
<tr>
<td>PF-548</td>
<td>0.03</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;10</td>
<td>1.9</td>
</tr>
</tbody>
</table>

**COX, cyclooxygenase; TXB2, thromboxane B2; ND, not determined.**

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and the last day of the recovery period. However, prolonged administration of the mPGES1 inhibitor led to significant changes in urinary PGE2 and urinary 6-keto-PGF1α. As shown in Fig. 4, urinary PGE2 decreased the first day and remained significantly reduced during the 7 days that PF-458 was administered. During the recovery period, there was an increase in urinary PGE2, but the urinary excretion rate of this PG remained reduced (P < 0.05) with respect to the values found during the control period. Contrary to the changes in urinary PGE2, the administration of PF-458 elicited an elevation in urinary 6-keto-PGF1α that was only significant the first day that this mPGES1 inhibitor was given. Urinary 6keto-PGF1α returned to control values during the recovery period (Fig. 4).

**Group 2.** No changes in MAP were found during PF-458 administration (108 ± 3 mmHg) and the recovery period (110 ± 3 mmHg) with respect to MAP values found during the control period (109 ± 4 mmHg). Control values of RBF and GFR (221 ± 23 ml/min and 47 ± 3 ml/min, respectively) were similar to those found in *group 1*. As shown in Fig. 2, the larger dose of PF-458 led to a decrease of RBF that was significant only the sixth day of treatment and RBF returned to control values during the recovery period. mPGES1 inhibition with the larger dose of PF-458 did not modify GFR (Fig. 3). As shown in Tables 2 and 3, this dose of PF-458 did not elicit significant changes in sodium excretion, urinary K+ excretion, PRA, PAC, pK, and TXB2. As shown in Fig. 4, the administration of 9.6 mg·kg⁻¹·day⁻¹ PF-458 induced a decrease in urinary PGE2 and an elevation in urinary 6keto-PGF1α that remained significant until the last day of this administration. During the recovery period, the urinary excretion of both PGs returned to control levels (Fig. 4).

**Group 3.** Arterial pressure did not change throughout the experiment in this group of dogs with normal Na⁺ intake since MAP was 114 ± 2 mmHg during the control period, 114 ± 3 mmHg during PF-458 administration, and 112 ± 3 mmHg during the recovery period. Renal hemodynamics did not change in response to prolonged mPGES1 inhibition since RBF and GFR remained within control values (254 ± 30 ml/min and 52 ± 3 ml/min, respectively) throughout the experiment (Fig. 5). As occurred in both groups of dogs with low Na⁺ intake, the administration of the mPGES1 inhibitor to dogs with normal Na⁺ intake did not elicit significant changes in urinary Na⁺ excretion, urinary K⁺ excretion, and pK (Table 2) as well as PRA, PAC, and TXB2 (Table 3). As shown in Fig. 6, the administration of the mPGES1 inhibitor to dogs with normal Na⁺ intake was also effective in reducing urinary PGE2 (P < 0.05) and induced an increase in urinary 6keto-PGF1α that was only significant the first day of treatment. The urinary excretion rate of both PGs returned to control levels during the recovery period (Fig. 6).

**DISCUSSION**

The present work is the first study to evaluate the role of mPGES-1 in the prolonged regulation of renal function by administration of a selective mPGES1 inhibitor to conscious animals with low or normal Na⁺ intake. The results suggest that PGE2 derived from mPGES1 activity is involved in the prolonged regulation of renal hemodynamics and that the renal
RENAL FUNCTION AND PROLONGED MPGES1 INHIBITION

Table 2. UNaV, UKV, and pK in conscious dogs with low or normal Na\(^+\) intake during the control period, days 1, 4, and 7 of PF-458 administration, and days 1 and 3 of the recovery period

<table>
<thead>
<tr>
<th></th>
<th>Control Period</th>
<th>PF-458 Administration</th>
<th>Recovery Period</th>
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<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 4</td>
<td>Day 7</td>
</tr>
<tr>
<td>PF-458 (2.4 mg·kg(^{-1})·day(^{-1})) and low Na(^+) intake</td>
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<tr>
<td>UNaV</td>
<td>5.6 ± 0.2</td>
<td>7.3 ± 2.1</td>
<td>4.2 ± 1.2</td>
</tr>
<tr>
<td>UKV</td>
<td>37 ± 2</td>
<td>35 ± 4</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>pK</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
<td>4.4 ± 0.1</td>
</tr>
<tr>
<td>PF-458 (9.6 mg·kg(^{-1})·day(^{-1})) and low Na(^+) intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNaV</td>
<td>4.7 ± 0.7</td>
<td>3.8 ± 1.0</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>UKV</td>
<td>32 ± 1</td>
<td>29 ± 4</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>pK</td>
<td>4.2 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
<tr>
<td>PF-458 (9.6 mg·kg(^{-1})·day(^{-1})) and normal Na(^+) intake</td>
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<td></td>
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</tr>
<tr>
<td>UNaV</td>
<td>62 ± 2</td>
<td>63 ± 5</td>
<td>70 ± 5</td>
</tr>
<tr>
<td>UKV</td>
<td>34 ± 2</td>
<td>35 ± 1</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>pK</td>
<td>4.3 ± 0.1</td>
<td>4.2 ± 0.1</td>
<td>4.3 ± 0.1</td>
</tr>
</tbody>
</table>

UNaV, urinary Na\(^+\) excretion (in meq/day); UKV, urinary K\(^+\) excretion (in meq/day); pK, plasma K\(^+\) concentration (in meq/l).

**Effects elicited by mPGES1 inhibition seem to be compensated by an increase in other prostanoids, such as PGI\(_2\). This study also provides new findings showing that prolonged inhibition of mPGES1 does not induce changes in PRA, PAC, and pK.**

The role of mPGES1 in the prolonged regulation of renal function was examined by administration of two doses of a selective inhibitor that were highly effective in reducing PGE\(_2\) production. However, apparently, the higher dose induced a more significant decrease in PGE\(_2\) synthesis since only this dose elicited a decrease of RBF in dogs with low Na\(^+\) intake. As shown in Fig. 4, urinary PGE\(_2\) decreased by 41% and 61% the first day that the lower and higher doses of PF-458 were administered. The falls in urinary PGE\(_2\) were 53% and 60% the last day that these doses of PF-458 were given. These reductions in urinary PGE\(_2\) were similar to those found in mPGES1 knockout mice (50%) (6) and in conscious dogs treated with a selective COX-2 inhibitor (58%) (20) but were slightly lower than in conscious dogs treated with a nonselective COX inhibitor (70%) (8). The partial reduction in urinary PGE\(_2\) during PF-458 administration could be explained by the fact that mPGES1 is a major, but not exclusive, source of PGE\(_2\) and by the activation of a compensatory pathway by which the production of PGE\(_2\) is low or normal. As shown in this study and those reported previously, selective inhibition of mPGES1 is consistent with results obtained in mPGES-1 knockout mice with low or normal Na\(^+\) intake (4, 6, 11) and studies showing that prolonged administration of nonselective or selective COX-2 inhibitors do not induce BP changes in dogs with low or normal Na\(^+\) intake. The absence of changes in BP during mPGES1 inhibition is consistent with results obtained in mPGES-1 knockout mice with low or normal Na\(^+\) intake (4, 6, 11) and studies showing that prolonged administration of nonselective or selective COX-2 inhibitors do not induce BP changes in dogs with low or normal Na\(^+\) intake (8, 20, 22). Taken together, the results of this study and those reported previously suggest that mPGES1 is not involved in the regulation of BP when Na\(^+\) intake is low or normal. Future studies are needed to examine whether selective mPGES1 inhibition induces an elevation in BP when Na\(^+\) intake and/or ANG II levels are elevated, since

Table 3. PRA, PAC, and TXB\(_2\) in conscious dogs with low or normal Na\(^+\) intake during the control period, days 1 and 7 of PF-458 administration, and day 3 of the recovery period

<table>
<thead>
<tr>
<th></th>
<th>Control Period</th>
<th>Administration of PF-458</th>
<th>Recovery Period</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 7</td>
<td>Day 1</td>
</tr>
<tr>
<td>PF-458 (2.4 mg·kg(^{-1})·day(^{-1})) and low Na(^+) intake</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>PRA</td>
<td>1.3 ± 0.2</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>PAC</td>
<td>110 ± 10</td>
<td>122 ± 21</td>
<td>105 ± 30</td>
</tr>
<tr>
<td>TXB(_2)</td>
<td>3.5 ± 1.6</td>
<td>2.2 ± 0.3</td>
<td>2.9 ± 0.8</td>
</tr>
<tr>
<td>PF-458 (9.6 mg·kg(^{-1})·day(^{-1})) and low Na(^+) intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA</td>
<td>1.7 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>PAC</td>
<td>198 ± 63</td>
<td>177 ± 33</td>
<td>164 ± 34</td>
</tr>
<tr>
<td>TXB(_2)</td>
<td>4.5 ± 1.4</td>
<td>2.9 ± 0.8</td>
<td>3.0 ± 0.6</td>
</tr>
<tr>
<td>PF-458 (9.6 mg·kg(^{-1})·day(^{-1})) and normal Na(^+) intake</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRA</td>
<td>0.4 ± 0.1</td>
<td>0.5 ± 0.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>PAC</td>
<td>29 ± 15</td>
<td>22 ± 13</td>
<td>33 ± 10</td>
</tr>
<tr>
<td>TXB(_2)</td>
<td>1.8 ± 0.5</td>
<td>2.4 ± 1.1</td>
<td>2.6 ± 0.3</td>
</tr>
</tbody>
</table>

PRA, plasma renin activity (in ng ANG I·ml\(^{-1}\)·h\(^{-1}\)); PAC, plasma aldosterone concentration (in pg/ml); TXB\(_2\), plasma concentration of TXB\(_2\) (in mg/ml).
previous studies (6, 11, 15, 28) have reported contradictory findings with respect to the BP response to increments in Na⁺ intake or ANG II in mPGES1 knockout mice. The different BP response to exogenous ANG II in mPGES1 knockout mice has been attributed to the genetic background of the mice used in these studies (5).

The main objective of this study was to examine the role of mPGES1 in the regulation of renal hemodynamic and excretory function when Na⁺ intake is normal or low. Contradictory data have been reported with respect to the presence of mPGES1 in vascular structures (7, 19, 24). However, even if this isomerase is not expressed in the renal vasculature, prolonged mPGES1 inhibition may induce renal vasoconstriction since PGE2 produced in adjacent tubular segments may elicit a paracrine effect on the renal vasculature. The reason why prolonged inhibition of mPGES1 only may elicit an increase in renal vascular resistance when the selective inhibitor is administered at a high dose could be that changes in other regulatory mechanisms compensate the possible renal vasoconstriction secondary to the decrease in PGE2 induced by the lower dose. One possibility is an elevation in PGI2 as a consequence of the shunting effect of arachidonic acid metabolism. Considering the complexity in the interactions among the pathways of the arachidonic acid metabolites in the regulation of renal function, future studies are needed to examine whether the renal effects elicited by prolonged mPGES1 inhibition are compensated by a change in PGI2 or other arachidonic acid metabolites.

The renal hemodynamic effect elicited by mPGES1 inhibition when Na⁺ intake is normal or low is clearly lower than that found during nonselective COX or selective COX-2 inhibition since the administration of meclofenamate or nimesulide elicits a significant and continuous decrease of RBF and GFR in dogs with low Na⁺ intake and only a prolonged decrease in RBF when Na⁺ intake is normal (8, 20, 22). The different renal

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**Fig. 4.** Changes in urinary PGE2 and 6-keto-PGF1α excretion during the control period, day 1 (E-1) and day 7 (E-7) of PF-458 administration (2.4 and 9.6 mg·kg⁻¹·day⁻¹), and day 3 of the recovery period (R-3) in dogs with low Na⁺ intake (n = 6). *P < 0.05 vs. the control value.

**Fig. 5.** Changes in RBF and GFR during 7 days of PF-458 administration (9.6 mg·kg⁻¹·day⁻¹) and during the 3-day recovery period in dogs with normal Na⁺ intake.

**Fig. 6.** Changes in urinary PGE2 and 6-keto-PGF1α excretion during the control period, days 1 and 7 of PF-458 administration (9.6 mg·kg⁻¹·day⁻¹), and day 3 of the recovery period in dogs with normal Na⁺ intake (n = 6). *P < 0.05 vs. the control value.
Renal function and prolonged mPGES1 inhibition

The renal excretory response to mPGES1 inhibition was also examined to evaluate whether PGE₂ derived from mPGES1 activity is involved in the regulation of renal excretory function in dogs with normal or low Na⁺ intake. The hypothesis was that renal excretory function would be affected by mPGES1 inhibition since it has been reported that mPGES1 knockout mice have a reduced renal excretory ability under different experimental situations (13, 15). However, contrary to the transitory decrease in urinary Na⁺ excretion and urinary K⁺ excretion found during the prolonged administration of nonselective COX or selective COX-2 inhibitors to dogs with normal Na⁺ intake (8, 20, 22), prolonged inhibition of mPGES1 did not elicit significant changes in renal excretory function. The absence of changes in urinary Na⁺ and water excretion during mPGES1 inhibition may be partly secondary to the compensatory effects elicited by other mechanisms involved in the regulation of renal excretory function. New studies are needed to further examine the role of mPGES1 in the regulation of renal hemodynamic and excretory function and to evaluate whether changes in other regulatory mechanisms modulate the effects elicited by the prolonged reduction of mPGES1 activity.

Prolonged inhibition of mPGES1 did not elicit significant changes of PRA in dogs with normal or low Na⁺ intake despite the fact that it has been proposed that PGE₂ derived from mPGES1 activity could be involved in the regulation of renin release (10, 18). This absence of changes in PRA during mPGES1 inhibition may be explained by an elevation in PGL₂ since this PG is also involved in the regulation of renin release (16) and we found that mPGES1 inhibition elicits an increment in urinary 6-keto-PGF₁α. The increment in PGL₂ may result from shunting of endoperoxides toward other prostanoid synthetic pathways (4, 17). Compensatory changes in other mechanisms involved in the regulation of renin release (16) may also be involved in the absence of changes in PRA during prolonged inhibition of mPGES1. That mPGES1 does not play an important role in the elevation of PRA that occurs during low Na⁺ intake is supported by a study (6) showing that stimulation of renin by furosemide is not affected by mPGES1 deletion. The absence of changes in pK during prolonged administration of PF-458 during several days is in sharp contrast with the effect in pK elicited by the prolonged administration of nonselective COX or selective COX-2 inhibitors (8, 20, 22). The results of this study may have clinical implications since the increase in pK is considered an important complication of treatment with nonsteroidal anti-inflammatory drugs (NSAIDs) (1, 23).

The results of this study may have important pathophysiological implications since they demonstrate that the renal hemodynamic and excretory effects, elicited by prolonged inhibition of mPGES1, are significantly lower than those reported during nonselective or selective COX-2 inhibition when Na⁺ intake is normal or low (8, 20, 22). Further studies are clearly needed to evaluate whether mPGES1 inhibition induces changes in BP and renal function when Na⁺ intake is enhanced. It has been reported that high Na⁺ intake induces an elevation of BP in mPGES1 knockout mice (15). The different effects elicited by PF-458 and COX inhibitors when Na⁺ intake is normal or low may be partly due to the different effects on PGL₂ synthesis since the reduction of PGE₂ induced by mPGES1 inhibition is accompanied by an elevation in urinary 6-keto-PGF₁α. Our results strongly suggest that pharmacological targeting of mPGES1 may represent an alternative for avoiding the undesirable renal effects observed with NSAIDs or COX-2 inhibitors when Na⁺ intake is normal or low. In support of this hypothesis, it has been proposed that mPGES1 deletion is as effective as NSAIDs in models of pain and inflammation (25) and that deletion of COX-2 but not that of mPGES1 accelerates the response to thrombogenic stimuli (4). However, no clinical studies have examined whether mPGES1 inhibition is effective in the treatment of inflammatory processes (12). New experimental and clinical studies are needed to confirm that mPGES1 inhibition elicits lower effects on renal function than those elicited by COX inhibitors. This would be especially important for aged patients because they have a higher predisposition to the renal adverse effects of NSAIDs (9) and are frequently asked to follow a low-Na⁺ diet because of the cardiovascular and renal changes associated with aging.

Acknowledgments

Present address of J. L. Masferrer: Ironwood Pharmaceuticals, 301 Binney St., Cambridge, MA.

Present address of G. Mbalaviele: Div. of Bone and Mineral Diseases, Washington University School of Medicine, St. Louis, MO.

Present address of G. Arhancet: Novus International, 20 Research Park, St. Charles, MO.

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Author Contributions


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