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RENAL EFFECTS INDUCED BY PROLONGED mPGES1 INHIBITION

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Running Title: Renal Function and Prolonged mPGES1 inhibition

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ABSTRACT

The importance of mPGES1 in regulating renal function has been examined in mPGES1 deficient mice or by evaluating changes in its expression. However, it is unknown whether the prolonged mPGES1 inhibition induces significant changes of renal function when sodium intake is normal or low. This study examines the renal effects elicited by a selective mPGES1 inhibitor (PF-458) during 7 days in conscious chronically instrumented dogs with normal (NSI) or low (LSI) sodium intake. Results obtained in vitro and in vivo studies strongly suggest that PF-458 is a selective mPGES1 inhibitor. The administration of 2.4 mg/Kg/day of PF-458 to dogs with LSI did not induce significant changes in RBF and GFR. A greater dose of PF-458 (9.6 mg/kg/day) 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 PGE_2 and an increase ($P<0.05$) in 6 KetoPGF$_{1\alpha}$. The administration of PF-458 did not induce significant changes in renal excretory function, plasma renin activity, and aldosterone and TXB_2 plasma concentrations in dogs with LSI or NSI. The results obtained suggest that mPGES1 is involved in the regulation of RBF when sodium intake is low and that the renal effects elicited by mPGES1 inhibition are modulated by a compensatory increment in PGI_2. These results may have some therapeutical implications since it has been shown that prolonged mPGES1 inhibition has lower renal effects than those elicited by NSAIDs or selective COX2 inhibitors.

Key words: Renal hemodynamics, PGE_2, mPGES1, sodium diet, PGI_2

INTRODUCTION

Prostaglandin E_2 (PGE_2) synthesis is produced by one cytosolic (cPGES) and two membrane-bound (mPGES1 and mPGES2) PGE isomerases. The mPGES1 isomerase is inducible and up-regulated in response to proinflammatory stimuli, with a concomitant increased expression of COX2 (3). This isomerase seems to be also involved in regulating blood pressure (BP) and renal function since it is constitutively expressed in aortic and mesenteric arteries, macula densa, 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 sodium intake is low or normal. The importance of mPGES1 has been 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 sodium intake is low than when it is normal. This inhibition would also induce a transitory decrease in sodium excretion when sodium intake is normal. These hypotheses were based on studies showing that mPGES1 and COX2 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 the prolonged COX2 inhibition elicits both a renal vasoconstriction, that is significantly enhanced when sodium intake is low, and a transitory decrease in sodium excretion with sodium intake is normal (20,22). A decrease in plasma renin activity (PRA) and plasma aldosterone concentration (PAC) and an increase in plasma potassium concentrations (pK) could also occur in dogs treated with the mPGES1 inhibitor since it has been proposed that the mPGES isomerase is involved in the regulation of renin release (10). It has been also demonstrated that COX2 inhibition induces a significant decrease in PRA and PAC and an increase in pK (20,22). The results obtained during the prolonged administration of a mPGES-1 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 non selective COX or selective COX2 inhibitors elicits important changes in renal function (8,20,22).

METHODS

Experiments in conscious Hound dogs (17 to 24 kg) were performed in accordance with the rules of European Union, and approved by the University of Murcia Institutional Animal Care and Use Committee. 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 measurement 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 (Transonic, No. T208) and data 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. Previous studies performed by our group have shown that arterial pressure and renal function remained unaltered for more than 10 consecutive days when conscious chronically instrumented dogs were only treated with vehicle (8). Male and female dogs were included in each group and the results obtained were pooled because no sex-dependent differences were found.

At 9:00 AM, dogs were fed a diet (HD, Hill Pet Products), which provided 4-8 mmol sodium/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 days 1 and 7 of PF-458 administration, and at the end of day 3 of recovery period to measure plasma renin activity (PRA) and TXB_2, aldosterone, sodium (pNa) and potassium

131 (pK) concentrations. Urinary excretion rates of PGE_2 ($uPGE_2$) and 6-keto-$PGF_{1\alpha}$ (u6 keto-
132 $PGF_{1\alpha}$) were also determined during the control period, at the end of days 1 and 7 of PF-458
133 administration, and at the end of day 3 of recovery period.
134 **Selectivity of PF-458 for mPGES1**. PF-458 [1-(5-chloro-6-(4-
135 chlorophenyl)benzo[d]oxazol-2-yl)-N-((1s,4s)-4-(hydroxymethyl)cyclohexyl)piperidine-4-
136 carboxamide] was prepared from cis-(4-aminocyclohexyl)methanol and the carboxylic acid
137 of methyl 1-(5-chloro-6-(4-chlorophenyl)benzo[d]oxazol-2-yl)piperidine-4-carboxylate
138 (compound 40, Ar = 4-chlorophenyl) as described by Arhancet et al (2). Selectivity was
139 examined in experiments performed in accordance with protocols approved by the Ethics
140 Committee of Pfizer. It was examined by measuring prostanoid levels in conditioned media
141 treated with a nonselective COX inhibitor (indomethacin), a selective COX2 inhibitor (SC-
142 236) and PF-458. Synovial fibroblasts derived from patients with rheumatoid arthritis
143 (RASF), fibroblasts from normal patients (NF), and modified human whole blood assay
144 (mHWB, a co-culture system of the human head and neck squamous cell carcinoma 1483
145 cells and human whole blood) were used as described previously (17). Rat basophilic
146 leukemia (RBL) cell line was purchased from ATCC, and used according to the supplier's
147 instructions. Prostanoid levels were also measured in the synovial fluid 3 hours after
148 intraarticular injection of LPS (1,5 ml, 100 ng/ml) to dogs pretreated with vehicle or a single
149 dose of PF-458 (14,6 mg/kg). This dose of PF-458 was orally administered 1 hour prior to
150 the intraarticular injection of LPS.
151 **Experimental Groups**.
152 **Group 1** (n=6). After a control period of three days, PF-458 was given orally during seven
153 consecutive days (2.4 mg/kg/day), giving half of the dose at 9:00 AM and the second half of
154 the dose at 7:00 PM. After PF-458 administration was finished, a recovery period of three
155 days was allowed.
156 **Group 2** (n=7). The protocol was similar to that described for group 1 with the exception
157 that the mPGES1 inhibitor was given daily at a dose four times higher (9.6 mg/kg/day).

Group 3 (n=6). The protocol was similar to that described for group 1 with the exceptions that PF-458 was given at the dose of 9.6 mg/kg/day and that total sodium load was increased to 70 mEq/d by continuously infusing isotonic saline at a rate of 425 mL/d.

Analytic Methods

Sodium and potassium levels were measured by flame photometry, and GFR was estimated by clearance of endogenous creatinine as in previous studies (8,20,22). PRA and PAC were measured using commercial RIA (Diasorin). All eicosanoids were measured using 2D LC/MS/MS as previously described (29) with slight modification 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 Fischer test (GB Stat, Dynamic Microsystems, 1996). $P<0.05$ was considered significant.

RESULTS

Selectivity of PF-458 for mPGES1. Table 1 show that indomethacin inhibited the biosynthesis of PGE_2 and other prostanoids with equal potency in protein-free cellular assays or in the mHWB assay. Although selective COX2 inhibition by SC-236 also inhibited the production of prostanoids in cytokine-stimulated assays with comparable potency as expected, it only inhibited TXB_2 release at concentrations known to crossing over COX1. It can be observed in table 1 and figure 1 that the selective mPGES1 inhibitor PF-458 induced a significant reduction of PGE_2, 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 $PGF_{1\alpha}$, PGD_2 and $PGF_{2\alpha}$, but not TXB_2 were significantly higher in PF-458-treated dogs compared to controls. 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 mmHg and did not change throughout the experiment (110 ± 5 mmHg during PF-458 administration, and 109 ± 5 mmHg during the recovery period). The prolonged mPGES1 inhibition did not elicit significant changes in

RBF and GFR (figures 2 and 3) with respect to the values found during the control period (191 ± 14 and 47 ± 4 mL/min, respectively). Renal hemodynamic remained within control values during recovery period. Table 2 shows that UNaV, UKV 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. Table 3 shows that PRA and plasma concentrations of aldosterone and TXB_2 also remained within control values during PF-458 administration and last day of recovery period. However, the prolonged administration of the mPGES1 inhibitor led to significant changes in $uPGE_2$ and u6keto-$PGF_{1\alpha}$. It can be observed in figure 4 that $uPGE_2$ decreased the first day and remained significantly reduced during the seven days that PF-458 was administered. During recovery period there was an increase in $uPGE_2$ but the urinary excretion rate of this PG remained reduced ($P<0.05$) with respect to the values found during control period. Contrary to the changes in $uPGE_2$, the administration of PF-458 elicited an elevation in u6keto-$PGF_{1\alpha}$ that was only significant the first day that this mPGES1 inhibitor was given. The u6keto-$PGF_{1\alpha}$ returned to control values during recovery period (figure 4).

Group 2. No changes in MAP were found during PF-458 administration (108 ± 3 mmHg) and recovery period (110 ± 3 mmHg), with respect to the MAP values found during 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. Figure 2 shows that the greater dose of PF-458 led to a decrease of RBF that was significant only the 6^{th} day of treatment, and that RBF returned to control values during recovery period. The mPGES1 inhibition with the greater dose of PF-458 did not modify GFR (figure 3). Tables 2 and 3 show that this dose of PF-458 did not elicit significant changes in UNaV, UKV, PRA and in the plasma concentrations of K, aldosterone and TXB_2. It can be observed in figure 4 that the administration of 9.6 mg/kg/day of PF-458 induced a decrease in $uPGE_2$ and an elevation in u6 keto-$PGF_{1\alpha}$ that remained significant until the last day of this administration. During recovery period, the urinary excretion of both PGs returned to control levels (figure 4).

Group 3. Arterial pressure did not change throughout the experiment in this group of dogs with normal sodium intake since MAP was 114 ± 2 mmHg during control period, 114 ± 3 mmHg during PF-458 administration, and 112 ± 3 mmHg during recovery period. Renal hemodynamic did not change in response to the prolonged mPGES1 inhibition since RBF and GFR remained within control values (254 ± 30 ml/min and 52 ± 3 ml/min, respectively) throughout the experiment (figure 5). As occurred in both groups of dogs with low sodium intake, the administration of the mPGES1 inhibitor to dogs with normal sodium intake did not elicit significant changes in UNaV, UKV, pK (table 2), PRA, and in the plasma concentrations of aldosterone and TXB_2 (table 3). Figure 6 shows that the administration of the mPGES1 inhibitor to dogs with normal sodium intake was also effective in reducing $uPGE_2$ ($P<0.05$) and that induced an increase in u6 keto-$PGF_{1\alpha}$ that was only significant the first day of treatment. The urinary excretion rate of both PGs returned to control levels during the recovery period (figure 6).

DISCUSSION

The present work is the first study evaluating the role of mPGES-1 in the prolonged regulation of renal function by administrating a selective mPGES1 inhibitor to conscious animals with low or normal sodium intake. The results suggest that PGE_2 derived from mPGES1 activity is involved in the prolonged regulation of renal hemodynamic and that the renal 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 the 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 the 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 sodium intake. Figure 4 shows that $uPGE_2$ decreased by 41% and 61% the first day that the lower

and higher doses of PF-458 were administered. The falls in $uPGE_2$ were of 53% and 60% the last day that these doses of PF-458 were given. These reductions in $uPGE_2$ were similar to those found in mPGES-1 knockout mice (50%) (6) and in conscious dogs treated with a selective COX2 inhibitor (58%) (20), but slightly lower than in conscious dogs treated with a nonselective COX inhibitor (70%) (8). Partial reduction in $uPGE_2$ 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 occurs. The COX2-derived PGH_2 seems to be shunted towards $PGF_{2\alpha}$ (figure 1), which in turn can be converted to PGE_2 by PGE 9-ketoreductase (27), and thereby contribute to PGE_2 synthesis. However, it cannot be excluded that the higher dose of PF-458 used in this study is still not high enough to achieve a complete inhibition of mPGES1 in the macula densa or vascular smooth muscle cells.

This study examines whether the prolonged administration of a selective mPGES1 inhibitor leads to an elevation in BP when sodium intake is normal or reduced. An increase in BP during the prolonged mPGES1 inhibition could be expected since this enzyme is constitutively expressed in aortic and mesenteric arteries (28) and in several structures of the kidney (7,19,26). However, the prolonged administration of the mPGES1 inhibitor did not elicit BP changes in dogs with low or normal sodium intake. The absence of changes in BP during mPGES1 inhibition is consistent with the results obtained in mPGES-1 knockout mice with low or normal sodium intake (4,6,11) and those showing that the prolonged administration of nonselective or selective COX2 inhibitors do not induce BP changes in dogs with low or normal 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 sodium intake is low or normal. Future studies are needed to examine whether selective mPGES1 inhibition induces an elevation in BP when sodium intake and/or angiotensin II (Ang II) levels are elevated since previous studies have reported contradictory findings with respect to the BP response to increments in sodium intake or Ang II in mPGES1 knockout

mice (6,11,15,28). 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 regulating renal hemodynamic and excretory function when sodium 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, the prolonged mPGES1 inhibition may induce a renal vasoconstriction since the PGE_2 produced in adjacent tubular segments may elicit a paracrine effect on renal vasculature. The reason why the 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 PGE_2 induced by the lower dose. One possibility is an elevation in PGI_2 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 regulating renal function, future studies are needed to examine whether the renal effects elicited by the prolonged mPGES1 inhibition are compensated by a change in PGI_2 or other arachidonic acid metabolites.

The renal hemodynamic effect elicited by mPGES1 inhibition when sodium intake is normal or low is clearly lower than that found during nonselective COX or selective COX2 inhibition since the administration of meclofenamate or nimesulide elicits a significant and continuous decrease of RBF and GFR in dogs with low sodium intake and only a prolonged decrease in RBF when sodium intake is normal (8,20,22). The different renal hemodynamic response to the inhibition of one or both COX isoforms with that observed when mPGES1 activity is reduced may be partly explained by the fact that COX inhibition reduces the production of several PGs, and mPGES1 inhibition reduces PGE_2 production and most probably induces an increase in PGI_2 synthesis. This hypothesis is supported by the results of this study showing that the administration of PF-458 reduces $uPGE_2$ and increases u6 keto-

294 $PGF_{1\alpha}$ (Figures 4 and 6). The previous hypothesis is also supported by studies showing that
295 deletion of mPGES1 reduced PGE_2 expression, augmented PGI_2 expression, and had no
296 effect on TXA_2 biosynthesis (4).

297 Renal excretory response to mPGES1 inhibition was also examined to evaluate whether
298 the PGE_2 derived from mPGES1 activity is involved in regulating renal excretory function in
299 dogs with normal or low sodium intake. The hypothesis was that renal excretory function
300 would be affected by mPGES1 inhibition since it has been reported that mPGES1 knockout
301 mice have a reduced renal excretory ability under different experimental situations (13,15).
302 However, contrary to the transitory decrease in UNaV and UV found during the prolonged
303 administration of nonselective COX or selective COX2 inhibitors to dogs with normal
304 sodium intake (8,20,22), the prolonged inhibition of mPGES1 did not elicit significant
305 changes in renal excretory function. The absence of changes in the urinary sodium and water
306 excretion during mPGES1 inhibition may be partly secondary to the compensatory effects
307 elicited by other mechanisms involved in regulating renal excretory function. New studies
308 are needed to further examine the role of mPGES1 in regulating renal hemodynamic and
309 excretory function and to evaluate whether changes in other regulatory mechanisms modulate
310 the effects elicited by the prolonged reduction of mPGES1 activity.

311 The prolonged inhibition of mPGES1 did not elicit significant changes of PRA in dogs
312 with normal or low sodium intake despite it has been proposed that PGE_2 derived from
313 mPGES1 activity could be involved in the regulation of renin release (10,18). This absence
314 of changes in PRA during mPGES1 inhibition may be explained by an elevation in PGI_2
315 since this PG is also involved in the regulation of renin release (16) and we found that
316 mPGES1 inhibition elicits an increment in u6-keto$PGF_{1\alpha}$. The increment in PGI_2 may result
317 from shunting of endoperoxides toward other prostanoids synthetic pathways (4,17).
318 Compensatory changes in other mechanisms involved in the regulation of renin release (16)
319 may also be involved in the absence of changes in PRA during the prolonged inhibition of
320 mPGES1. That mPGES1 does not play an important role in the elevation of PRA that occurs

during low sodium intake is supported by studies showing that stimulation of renin by furosemide is not affected by mPGES1 deletion (6). The absence of changes in pK during the 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 COX2 inhibitors (8,20,22). The results of this study may have clinical implications since the increase in pK is considered an important complication of NSAIDs drugs (1,23).

The results of this study may have important pathophysiological implications since they demonstrate that the renal hemodynamic and excretory effects, elicited by the prolonged inhibition of mPGES1, are significantly lower than those reported during nonselective or selective COX2 inhibition when sodium intake is normal or low (8,20,22). Further studies are clearly needed to evaluate whether mPGES1 inhibition induce changes in BP and renal function when sodium intake is enhanced. It has been reported that a high sodium intake induces an elevation of BP in mPGES1 knockout mice (15). The different effects elicited by PF-458 and COX inhibitors when sodium intake is normal or low may be partly due to the different effect on PGI_2 synthesis since the reduction of PGE_2 induced by mPGES1 inhibition is accompanied by an elevation in u6-keto$PGF_{1\alpha}$. Our results strongly suggest that pharmacological targeting of mPGES1 may represent an alternative for avoiding the undesirable renal effects observed with NSAIDs or COX2 inhibitors when sodium 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 COX2 but not that of mPGES1 accelerates the response to thrombogenic stimuli (4). However, no clinical studies have examined whether the 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. It would be especially important for aged patients because they have a higher predisposition to NSAIDs renal adverse effects (9) and are frequently asked to take a low sodium diet because of the cardiovascular and renal changes associated to aging.

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Disclosures:

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454

Table 1. Synovial fibroblasts derived from patients with rheumatoid arthritis (RASF), fibroblasts from normal patients (NF) and modified human whole blood assay (mHWB) and rat basophilic leukemia (RBL) cells treated with a nonselective COX inhibitor (indomethacin), a selective COX2 inhibitor (SC-236) or a selective mPGES1 inhibitor (PF-458).

Compounds	IC50 (μM)					
	Protein-free				Presence of proteins	
	PGE_2 (NF)	6 keto$PGF_{1\alpha}$ (RASF)	$PGF_{2\alpha}$ (NF)	PGD_2 (RBL)	PGE_2 (mHWB)	TXB_2 (mHWB)
Indomethacin	0.01	0.02	0.02	0.014	10	1
SC-236	0.10	0.10	0.40	ND	0.2	10
PF-548	0.03	>100	>100	>10	1.9	ND

ND: not determined

Table 2. Urinary sodium excretion (UNaV, mEq/day), urinary potassium excretion (UKV, mEq/day) and plasma potassium concentration (PK, mEq/L) in conscious dogs with low or normal sodium intake during control period, days 1, 4 and 7 of PF458 administration and days 1 and 3 of recovery period

		CONTROL	E1	E4	E7	R1	R3
PF-458 (2.4 mg/Kg/day) Low Sodium	UNaV	5.6 ± 0.2	7.3 ± 2.1	4.2 ± 1.2	7.3 ± 1.8	6.5 ± 1.7	4.8 ± 1.2
	UKV	37 ± 2	35 ± 4	34 ± 3	38 ± 3	34 ± 4	36 ± 1
	PK	4.2 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	4.2 ± 0.1	4.1 ± 0.1	4.2 ± 0.1
PF-458 (9.6 mg/Kg/day) Low Sodium	UNaV	4.7 ± 0.7	3.8 ± 1.0	3.7 ± 0.9	5.0 ± 1.6	3.3 ± 1.0	5.7 ± 1.3
	UKV	32 ± 1	29 ± 4	31 ± 1	35 ± 2	34 ± 1	35 ± 1
	PK	4.2 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1
PF-458 (9.6 mg/Kg/day) Normal Sodium	UNaV	62 ± 2	63 ± 5	70 ± 5	60 ± 5	78 ± 6	64 ± 3
	UKV	34 ± 2	35 ± 1	36 ± 2	42 ± 5	37 ± 2	39 ± 3
	PK	4.3 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	4.4 ± 0.1	4.4 ± 0.1

Table 3. Plasma renin activity (PRA, ng AngI/ml/hr), plasma aldosterone concentration (PAC, pg/ml), and plasma concentration of TXB_2 (ng/ml) in conscious dogs with low or normal sodium intake during control period, days 1 (E1), and 7 (E7) of PF458 administration and day 3 of recovery period (R3).

		CONTROL	E1	E7	R3
PF-458 (2.4 mg/Kg/day) Low Sodium	PRA	1.3 ± 0.2	1.4 ± 0.1	1.2 ± 0.2	1.0 ± 0.1
	PAC	110 ± 10	122 ± 21	105 ± 30	81 ± 15
	TXB_2	3.5 ± 1.6	2.2 ± 0.3	2.9 ± 0.8	3.4 ± 1.1
PF-458 (9.6 mg/Kg/day) Low Sodium	PRA	1.7 ± 0.3	1.5 ± 0.4	1.8 ± 0.3	1.3 ± 0.3
	PAC	198 ± 63	177 ± 33	164 ± 34	144 ± 37
	TXB_2	4.5 ± 1.4	2.9 ± 0.8	3.0 ± 0.6	3.6 ± 0.5
PF-458 (9.6 mg/Kg/day) Normal Sodium	PRA	0.4 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
	PAC	29 ± 15	22 ± 13	33 ± 10	43 ± 14
	TXB_2	1.8 ± 0.5	2.4 ± 1.1	2.6 ± 0.3	3.6 ± 1.4

* $P<0.05$ vs control period

FIGURE LEGENDS

Figure 1. PGE_2, 6 ketoPGF$_{1\alpha}$, PGD_2, $PGF_{2\alpha}$ and TXB_2 concentrations in synovial fluids after a single intraarticular injection of LPS in dogs pretreated with vehicle or PF-458. * P<0.05 vs. vehicle

Figure 2. Changes in renal blood flow (RBF) during the seven days of PF-458 administration at two different doses and during the 3-day recovery period in dogs with low sodium intake. * P<0.05 vs. average of the 3-day control period.

Figure 3. Changes in glomerular filtration rate (GFR) during the seven days of PF-458 administration at two different doses and during the 3-day recovery period in dogs with low sodium intake.

Figure 4. Changes in urinary PGE_2 and 6 ketoPGF1α excretion during days 1 (E1) and 7 (E7) of PF-458 administration (2.4 and 9.6 mg/Kg/day) and during day 3 (R-3) of recovery period in dogs with low sodium intake (n=6). * P<0.05 vs. control value.

Figure 5. Changes in renal blood flow (RBF) and glomerular filtration rate (GFR) during the seven days of PF-458 administration (9.6 mg/Kg/day) and during the 3-day recovery period in dogs with normal sodium intake.

Figure 6. Changes in urinary PGE_2 and 6 ketoPGF1α excretion during days 1 (E1) and 7 (E7) of PF-458 administration (9.6 mg/Kg/day) and during day 3 (R-3) of recovery period in dogs with normal sodium intake (n=6). * P<0.05 vs. control value.

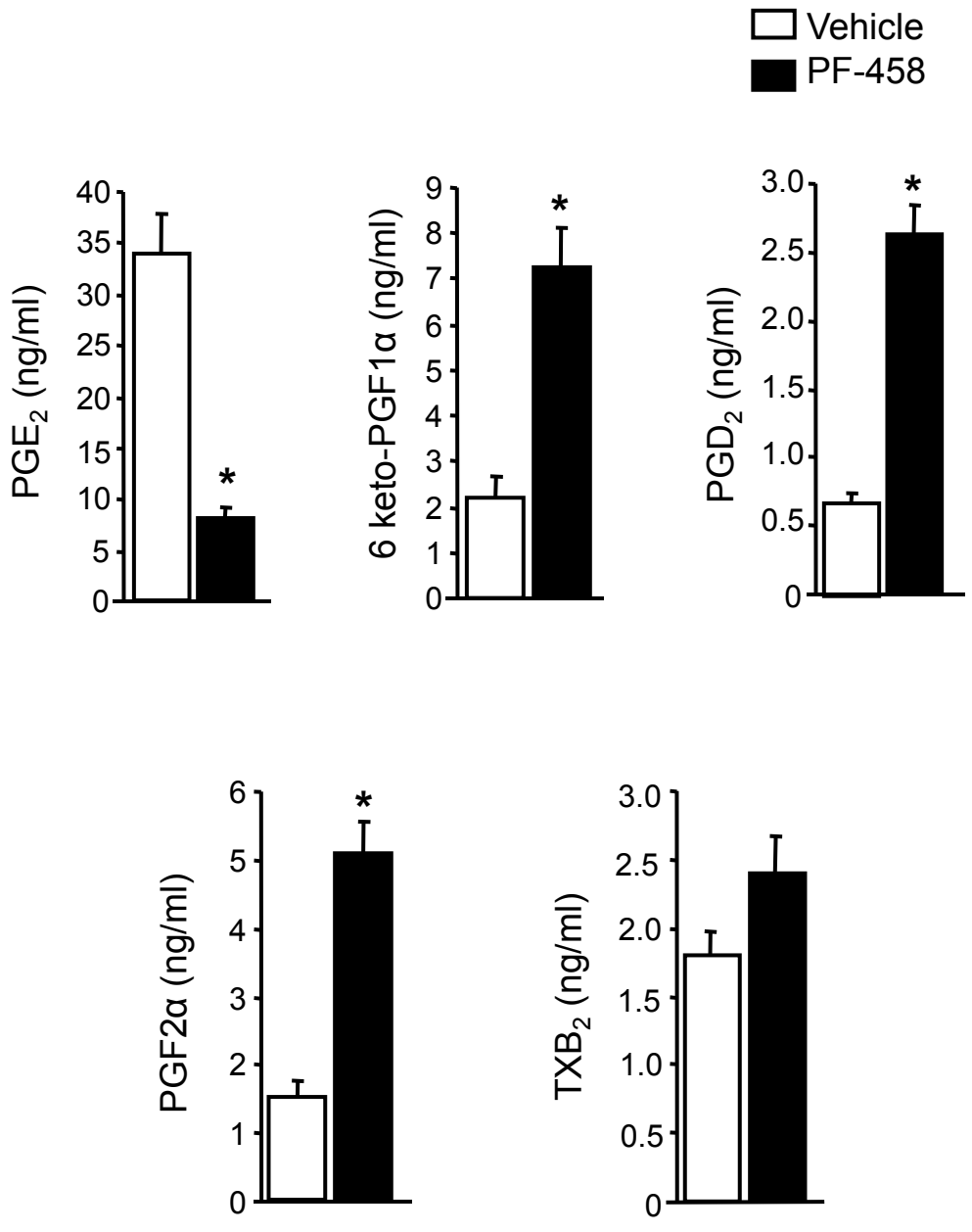
Vehicle
PF-458
PGE2 (ng/ml)
40
35
30
25
20
15
10
5
0
*
6 keto-PGF1α (ng/ml)
9
8
7
6
5
4
3
2
1
0
*
PGD2 (ng/ml)
3.0
2.5
2.0
1.5
1.0
0.5
0
*
PGF2α (ng/ml)
6
5
4
3
2
1
0
*
TXB2 (ng/ml)
3.0
2.5
2.0
1.5
1.0
0.5
0

Figure 1

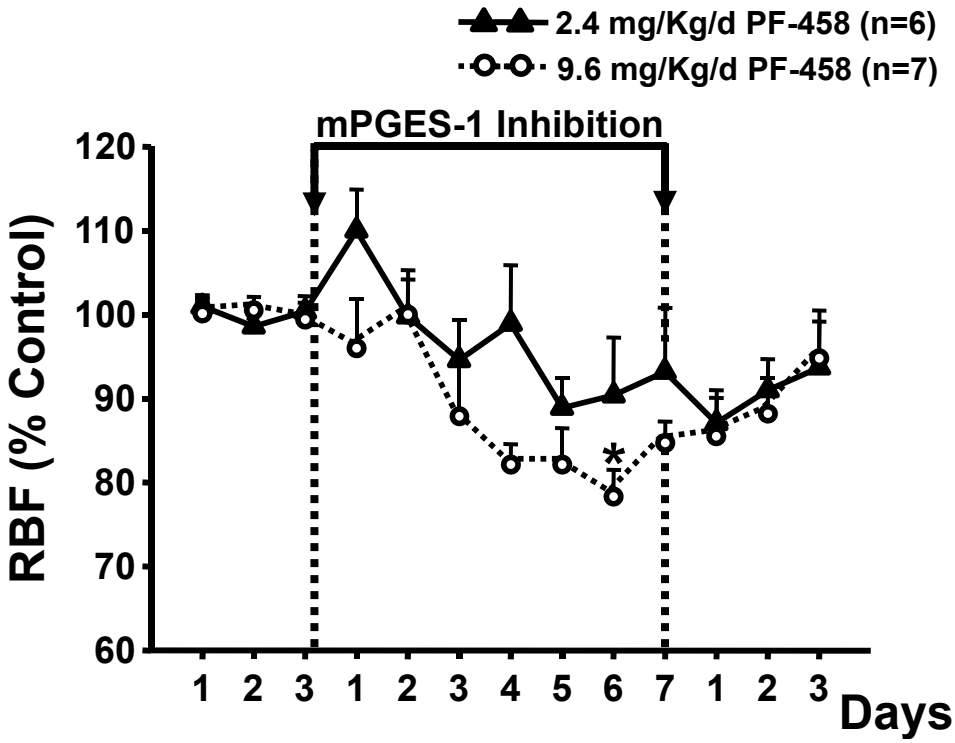
2.4 mg/Kg/d PF-458 (n=6)
9.6 mg/Kg/d PF-458 (n=7)
mPGES-1 Inhibition
RBF (% Control)
120
110
100
90
80
70
60
1 2 3 1 2 3 4 5 6 7 1 2 3
Days
*

Figure 2.

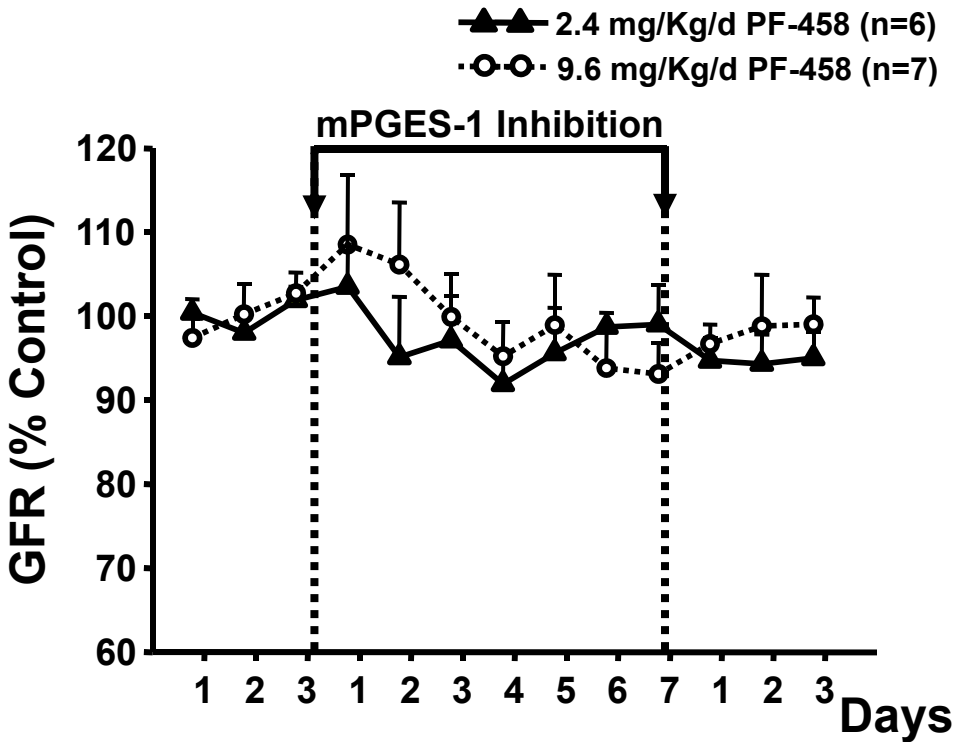
2.4 mg/Kg/d PF-458 (n=6)
9.6 mg/Kg/d PF-458 (n=7)
mPGES-1 Inhibition
GFR (% Control)
120
110
100
90
80
70
60
1 2 3 1 2 3 4 5 6 7 1 2 3
Days

Figure 3

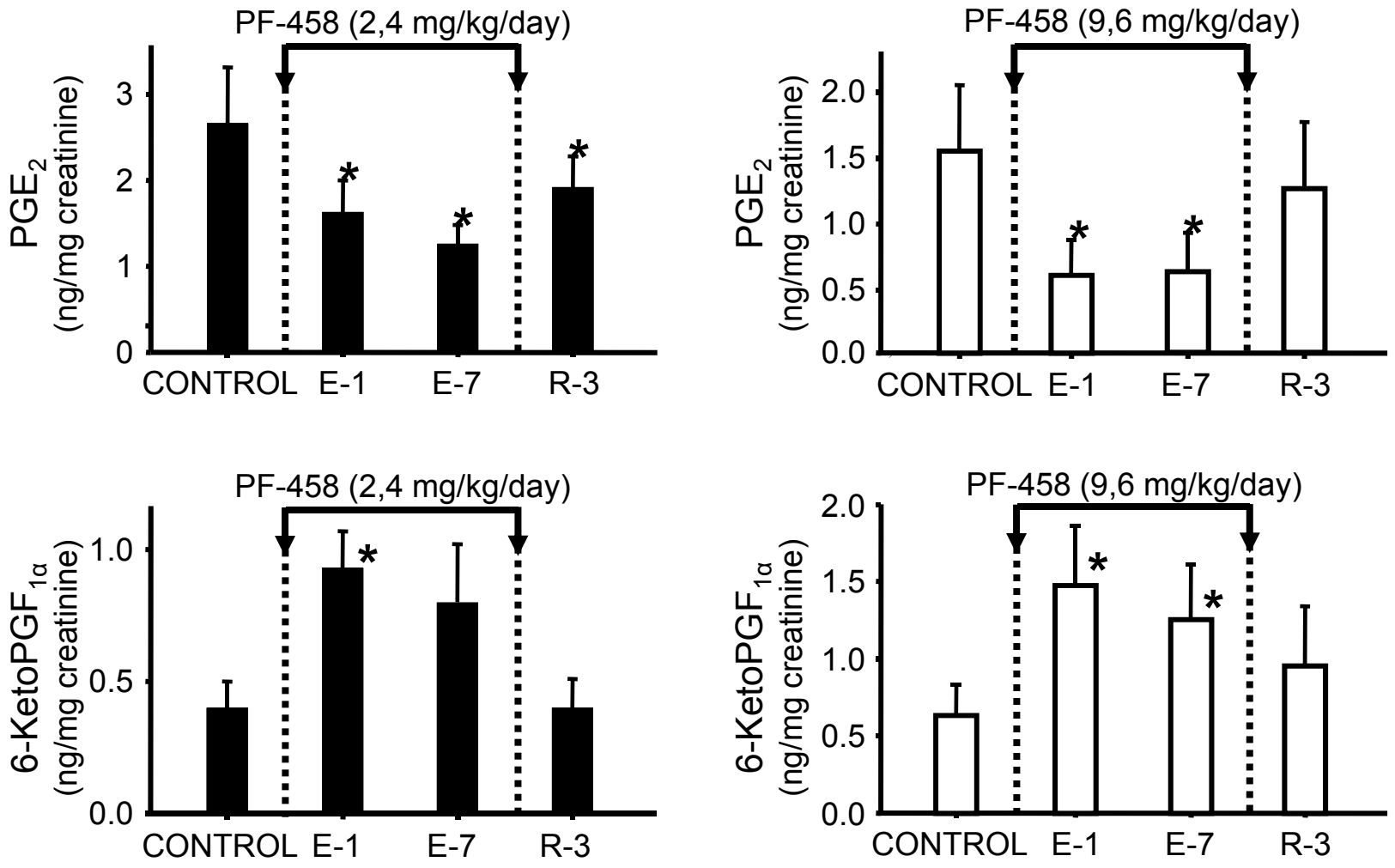
PF-458 (2,4 mg/kg/day)
PF-458 (9,6 mg/kg/day)
PGE2 (ng/mg creatinine)
6-KetoPGF1α (ng/mg creatinine)
CONTROL
E-1
E-7
R-3
0
1
2
3
0.0
0.5
1.0
1.5
2.0
*

Figure 4

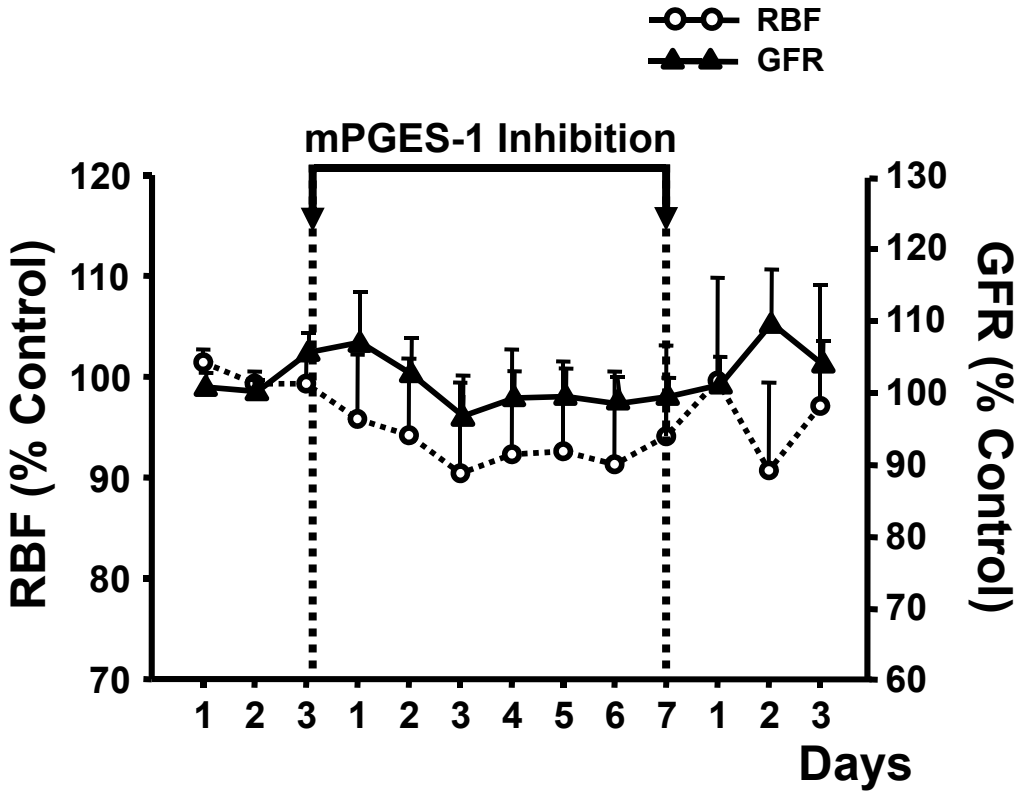
RBF
GFR
mPGES-1 Inhibition
RBF (% Control)
GFR (% Control)
120
110
100
90
80
70
130
120
110
100
90
80
70
60
1 2 3 1 2 3 4 5 6 7 1 2 3
Days

Figure 5

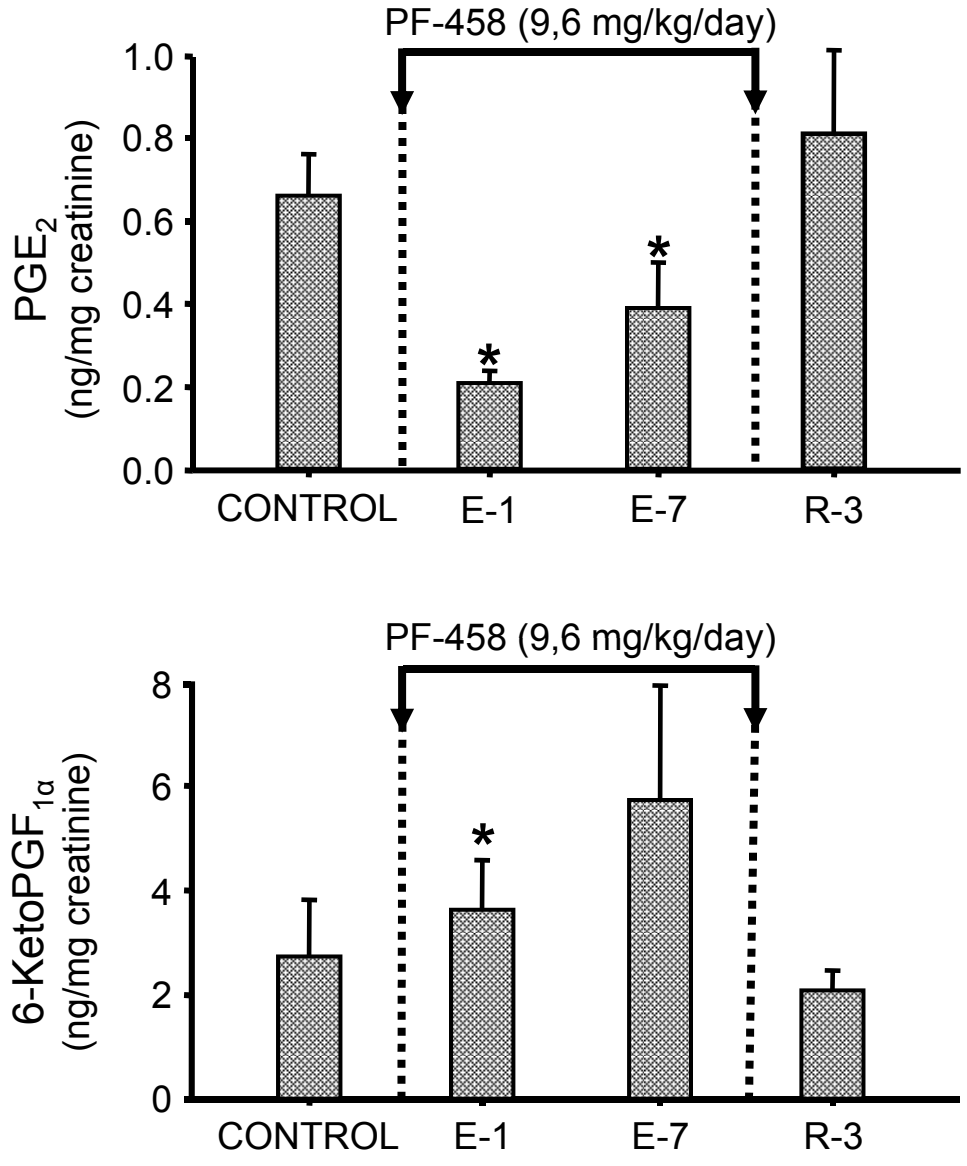
PF-458 (9,6 mg/kg/day)
1.0
0.8
0.6
0.4
0.2
0.0
PGE2
(ng/mg creatinine)
*
*
CONTROL
E-1
E-7
R-3
PF-458 (9,6 mg/kg/day)
8
6
4
2
0
6-KetoPGF1α
(ng/mg creatinine)
*
CONTROL
E-1
E-7
R-3

Figure 6