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Comparative Effects of Indomethacin and Nabumetone on Urine and Electrolyte Output in Conscious Rats

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Abstract. The effects of indomethacin and nabumetone on urine and electrolyte excretion in conscious rats were examined. Male Sprague-Dawley rats were housed individually for a five-week duration, consisting of acclimatization, control, experimental, and recovery phases. During the experimental phase, rats were given either indomethacin (1.5 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$ in 0.5 ml saline, n = 10), nabumetone (15 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$ 0.5 ml saline, n = 10), or 0.5 ml saline alone (n = 10) for a period of two weeks. Water and food intake, body weight, urine output, and electrolyte excretions were estimated. Data were analyzed using two-way ANOVA. Urine output in the indomethacin- and nabumetone-treated groups was not different from the controls, but was significantly different between the drug-treated groups (P<0.01). Sodium, potassium, calcium, and magnesium excretions were not different between nabumetone-treated and control rats. However, sodium and potassium excretion was significantly lower in rats receiving indomethacin when compared to the control rats. Calcium and magnesium outputs, although did not differ from the controls, nevertheless decreased significantly with indomethacin (P<0.01). It appears that indomethacin and nabumetone when given at maximum human therapeutic doses may affect urine and electrolyte output in conscious rats.

Keywords: indomethacin, nabumetone, conscious rat, renal function

Introduction

The kidney is an important site for arachidonic acid metabolism, displaying abundant cyclooxygenase activity. Two isoforms of this enzyme have been identified; cyclooxygenase-1 (COX-1), the constitutive isoform, produces prostaglandins that are believed to help maintain renal function, whereas COX-2, the inducible isoform, is primarily thought to be involved in the production of prostaglandins during inflammatory processes (1 – 5). However, recent reports point to the presence of COX-2 in the macula densa and surrounding cortical cells of the thick ascending limb of the loop of Henle of normal rat (6, 7) suggesting that it may have a normal physiological role. In fact, COX-2 mRNA and protein expression in the mammalian kidney are among the highest observed in any tissues (8). On the other hand, COX-1 has been shown to be involved in inflammatory reactions (9, 10). These observations suggest that COX-1 and COX-2 may not have such clearly demarcated roles as proposed by the "COX-hypothesis".

Deleterious effects of non-steroidal anti-inflammatory drugs (NSAIDs) on renal function, particularly in pre-existing renal disease or during haemodynamically stressful situations have been attributed to the inhibition of COX in the kidney by these drugs (11, 12). However, there are numerous reports indicating differences in effects of NSAIDs on renal function (13, 14). Previous human studies have indicated that nabumetone may be less nephrotoxic than conventional NSAIDs, as it does not seem to decrease renal prostaglandin synthesis (14 – 16). To our knowledge there is no study in the literature to date comparing the renal effects of indomethacin and nabumetone in conscious rats, and it is unclear if its adverse effects are the same in humans and animals. We therefore investigated the effect of indomethacin and nabumetone on urine and electrolyte output in conscious rats to see if the effects of indomethacin and nabumetone

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were similar under normal, non-stressful situations. Indomethacin is a non-selective COX inhibitor, whereas nabumetone, or more importantly its metabolite 6-methoxy-2-naphthylacetic acid (6-MNA), is considered a relatively selective COX-2 inhibitor.

Materials and Methods

Male Sprague-Dawley rats weighing 200 – 220 g were housed individually in metabolic cages (32 × 21.5 × 20.5 cm, length, width, and height, respectively) for a total duration of 5 weeks. The study protocol consisted of four phases: acclimatization phase (1 week), control phase (1 week), experimental phase (2 weeks), and recovery phase (1 week). All animals were treated identically during the acclimatization, control, and recovery phases. No observations were made during the acclimatization phase where the animals were allowed to acquaint themselves with the metabolic cages. During the experimental phase, however, the animals were given orally either 1.5 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$ of indomethacin (n = 10), or 15 $mg \cdot kg^{-1}$ body $weight \cdot day^{-1}$ of nabumetone (n = 10), dissolved in 0.5 ml of saline or 0.5 ml saline alone for a period of two weeks. The dose that was administered is equivalent to the maximum therapeutic dose used in humans. Food and water were provided ad-libitum and 24-h food intake, water intake, body weight, urine output, urinary excretion of sodium, potassium, magnesium, and calcium were estimated in all animals during all three phases. Sodium and potassium were analyzed using flame photometry (Corning 404; Corning, MN, USA), and calcium and magnesium were estimated using ion selective electrode (model 912; Hitachi, Tokyo).

For the analysis of data, two-way ANOVA for repeated measures and Tukey post-hoc test were used to ascertain differences between the three phases within a group and between phases of the different groups. All data are presented as the mean ± S.E.M.

This study was approved by the Animal Ethics Committee of University Sains Malaysia.

Results

Mean food intake, water intake, and body weight of the three groups during the three phases (Table 1)

Food and water intake was measured every two days and body weight was measured every four days. As there were no significant differences between the values in each rat in each phase, the values for each phase for each rat were averaged, and the average was then used to calculate the group mean. No significant differences were evident in food and water intake when the three phases in each group were compared or when the corresponding phases of the three groups were compared (Table 1). Body weight increased significantly in all groups over the period of the study, but no significant differences were evident in the rate of increase in body weight between the animals in the three groups.

Mean urine output in the three groups of conscious rats during the three phases (Fig. 1)

No significant difference was evident in the mean urine output between the three groups during the control phase, although at day 4, urine output for the indomethacin group was lower when compared to animals in the control group ($P<0.05$) (Fig. 1). In the experimental phase, although no significant differences were evident in urine output between the control group and the nabumetone group and between the control group and indomethacin group, but it was significantly between the nabumetone and the indomethacin groups ($P<0.01$). Urine output appears to have decreased slightly in the indomethacin group and increased slightly in rats receiving nabumetone. No significant difference was evident in urine output between the three groups during the recovery phase.

Table 1. Mean food intake, water intake, and body weight of the three groups during the three phases

		Control phase	Experimental phase	Recovery phase
Food intake ($g \cdot day^{-1}$)	Control	24.25 ± 0.47	23.23 ± 0.31	23.78 ± 0.23
	Indomethacin-treated	23.76 ± 0.30	22.85 ± 0.37	23.91 ± 0.34
	Nabumetone-treated	22.34 ± 0.23	22.90 ± 0.42	22.39 ± 0.26
Water intake ($ml \cdot day^{-1}$)	Control	30.02 ± 0.75	30.12 ± 0.59	30.63 ± 0.59
	Indomethacin-treated	27.90 ± 0.36	28.98 ± 0.24	28.49 ± 0.28
	Nabumetone treated	27.46 ± 0.89	28.68 ± 0.70	29.13 ± 0.10
Body weight (g)	Control	222.20 ± 6.68	281.58 ± 5.90	310.01 ± 15.74
	Indomethacin-treated	216.20 ± 5.48	271.18 ± 6.04	299.09 ± 8.47
	Nabumetone-treated	222.89 ± 7.52	270.79 ± 7.95	300.45 ± 9.38

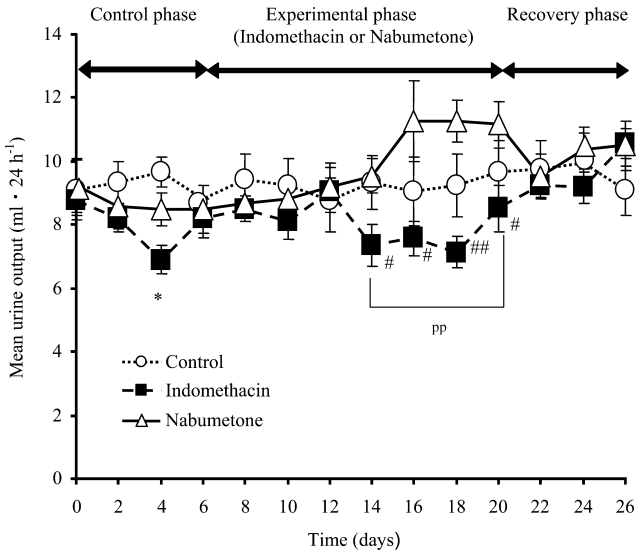

Fig. 1. Mean urine output in the three groups of conscious rats during the three phases. $^{*}P<0.05$, compared to the control group. $^{\#}P<0.05$, $^{\#\#}P<0.01$, compared to the nabumetone group. $^{pp}P<0.01$, between the indicated groups.

Daily sodium, potassium, calcium, and magnesium excretion in the three groups of rats during the three phases (Table 2)

All measurements were made every two days and the values for each rat in each phase were averaged and the average was then used to calculate the group means. Administration of nabumetone did not result in any significant differences in the urinary excretion of sodium, potassium, calcium, or magnesium, either within the group or when compared to the corresponding phases of the control group. In the indomethacin group, however, mean urinary sodium and potassium outputs during the experimental phase were significantly lower when compared to the corresponding period in the control group (Table 2). Sodium and potassium outputs during the experimental phase in rats given indomethacin were also significantly lower when compared to their outputs during the recovery phase ($P<0.01$). Although sodium and potassium outputs during the experimental phase were slightly lower in rats receiving indomethacin when compared to the corresponding period in rats given nabumetone, the differences, however, were not significant. Calcium and magnesium excretion generally declined from the control phase to the recovery phase in all the groups and was not significantly different between the three groups. However, the decrease in the excretion of calcium and magnesium was somewhat greater in rats receiving indomethacin. Calcium excretion during the experimental phase in rats receiving indomethacin was significantly lower than during the control phase ($P<0.01$). However, it increased slightly during the recovery phase. Magnesium excretion decreased significantly in rats receiving indomethacin ($P<0.01$) and remained low even during the recovery phase ($P<0.01$).

Discussion

This study investigated the effect of oral administration of indomethacin and nabumetone on urine and electrolyte excretion in conscious rats. Most previous studies investigating the effects of NSAIDs on renal function have been performed on anesthetized animals

Table 2. Daily sodium, potassium, calcium, and magnesium excretion in the three groups of rats during the three phases

		Control phase	Experimental phase	Recovery phase
Sodium output (μmol · day^{-1})	Control	1046.37 ± 35.04	1020.06 ± 35.18	1110.74 ± 29.82
	Indomethacin-treated	983.78 ± 15.01	935.21 ± 14.56$^{\varphi\varphi,pp}$	1067.16 ± 51.12
	Nabumetone-treated	974.77 ± 48.20	1002.78 ± 50.60	1049.17 ± 43.53
Potassium output (μmol · day^{-1})	Control	467.98 ± 12.60	439.15 ± 9.78	402.01 ± 16.20
	Indomethacin-treated	422.97 ± 12.08	386.21 ± 22.68$^{\varphi\varphi,pp}$	447.80 ± 14.82
	Nabumetone-treated	411.14 ± 11.52	435.16 ± 23.45	467.98 ± 29.52
Calcium output (μmol · day^{-1})	Control	50.62 ± 3.67	41.80 ± 4.04	45.16 ± 4.61
	Indomethacin-treated	47.96 ± 1.57	35.23 ± 3.83$^{\varphi\varphi,\#\#}$	42.30 ± 1.28
	Nabumetone-treated	49.08 ± 3.17	43.37 ± 5.89	43.59 ± 1.10
Magnesium output (μmol · day^{-1})	Control	9.90 ± 0.70	8.18 ± 1.49	8.02 ± 0.92
	Indomethacin-treated	13.58 ± 0.40	6.19 ± 1.76$^{\#\#}$	6.34 ± 1.99$^{\#\#\#}$
	Nabumetone-treated	11.70 ± 1.13	9.92 ± 1.77	9.66 ± 2.09

$^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$, compared to the respective control phase. $^{\varphi\varphi}P<0.01$, compared to the respective recovery phase. $^{pp}P<0.01$, compared to the control group.

where hemodynamic stress could compromise some of the findings. The doses of indomethacin and nabumetone used were equivalent to the maximum human therapeutic dose. We had previously also used a similar dose protocol for naproxen (17).

No significant differences were evident in food and water intake or body weight changes between the three groups over the period of study (Table 1). There is little information in the literature on the effects of indomethacin or nabumetone on food and water intake and body weight changes in the rat. Administration of naproxen to conscious rat was also found not to affect food and water intake or changes in body weight (17). It could therefore be concluded that like other NSAIDs, indomethacin and nabumetone, when given at these doses for a period of two weeks, do not affect food and water intake or body weight changes in the rat.

Of the urinary parameters recorded, although no significant differences were evident in urine output between the control rats and rats receiving indomethacin or nabumetone, urine output was significantly higher during the experimental phase in rats receiving nabumetone when compared to the corresponding phase in rats given indomethacin ($P<0.01$, Fig. 1). This was primarily due to a slight but non-significant decrease in urine output in rats receiving indomethacin and a slight but non-significant increase in urine output in rats given nabumetone. The reason for the small changes may be due to the dose used, which might be somewhat low for this species, although our earlier study with naproxen, using a similar human dose, revealed significant increases in urine flow (17). Although studies with higher doses could help clarify this, indomethacin, nevertheless, has been shown to decrease urine output in conscious rats (13, 18). This effect of indomethacin on urine flow has been attributed to its ability to inhibit prostaglandin synthesis, in particular prostaglandin E_2 (PGE_2), which inhibits vasopressin-stimulated water reabsorption in the collecting duct via EP3 receptor activation (19, 20). Consequently there is increased tubular reabsorption of water resulting in decreased urine output. The decreased urine could also be due to the effects of indomethacin on renal blood flow and glomerular filtration rate. In addition to decreasing urine flow, indomethacin administration also significantly decreased sodium, potassium, calcium, and magnesium excretions, where sodium and potassium excretions were significantly lower than those in the control group (Table 2). PGE_2, a potent regulator of renal haemodynamics and salt and water transport, inhibits NaCl reabsorption in the thick ascending limb (21) and in the corticol collecting duct (22, 23). The inhibition of its synthesis by indomethacin might have been responsible for the decreased urinary excretion of sodium. Both oral (18) and intravenous (13) administration of indomethacin to conscious rats has been shown to decrease urinary calcium excretion. Similar results have also been reported with meclofenamate, indomethacin, and piroxicam in anesthetized rats (24). These effects of indomethacin on urinary excretion of calcium and magnesium in conscious rats have been found not to depend on changes in renal plasma flow or glomerular filtration rate, but dependent on endogenous inhibition of prostaglandin synthesis. Nabumetone administration, however, did not result in significant changes in the excretion of these electrolytes, although mean calcium and magnesium excretions were slightly reduced during the experimental and recovery phases.

The reason for the difference in the effects of indomethacin and nabumetone on urine flow and the excretion of some electrolytes is unclear. One possible explanation for the difference might be the difference in the abilities of the two drugs in inhibiting the two COX isoenzymes. COX-1 generated prostaglandins cause diuresis (25) and COX-2 generated prostaglandins may have antidiuretic effects, as COX-2 expression in the renal medulla is markedly stimulated by water deprivation (26, 27). Moreover, COX-1 is the constitutive form, whereas COX-2 is believed to be the inducible form, although recently the latter has been found to be constitutive in renal vessels (28). In view of their opposing effects, it may be proposed that a balance between COX-1 and COX-2 activities may determine fluid and electrolyte excretion by the kidney. Indomethacin, being a non-selective COX inhibitor, inhibited both COX-1 and whatever little COX-2 isoenzyme that may be present normally, thereby decreasing urine output in conscious rats. Although nabumetone is not entirely selective for COX-2, its metabolite 6-MNA is a more potent inhibitor of COX-2 than COX-1. Therefore, it is possible that a greater inhibition of COX-2 isoenzyme than COX-1 during nabumetone administration may be responsible for the slight increase in urine output in conscious rats. Alternatively, it is also possible that the difference in urine output following the administration of indomethacin and nabumetone may reflect a mechanism of nabumetone that is not related to inhibition of prostaglandin biosynthesis and that this mechanism overcomes or exceeds any effects due to inhibition of prostaglandin biosynthesis by nabumetone. This possibility is suggested by evidence of a differing effect of aspirin and indomethacin on renal handling of magnesium and phosphate in the rat (13). We do not know if higher or lower doses would have given similar or different results. Nevertheless, our findings seem to suggest that indomethacin and nabumetone when used at

doses equivalent to the maximum human therapeutic dose differ in their effect on urine and electrolyte output in conscious rats. Further studies with higher doses of these drugs in different circumstances and on the expression of the isoenzyme COX-1 and COX-2 along the nephron in normal and stressful circumstances may elucidate the underlying mechanisms.

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