Med. J. Cairo Univ., Vol. 62, No. 2, June : 419-432, 1994

Effect of Tiaprofenic Acid and Piroxicam on Serum and 24 Hour Urinary Excretion of Calcium in Normal and Adjuvant-Induced Arthritic Rats

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Abstract

Prostaglandin synthesis inhibitors of the non-steroidal anti-inflammatory group have been shown to decrease calcium excretion in experimental animal, in human volunteers and in calcium stone formers. Moreover, other studies showed that NSAIDs inhibited hypercalcaemia and osteolysis in rats. So, this study was performed in conscious male albino normal rats to determine the changes in Ca^{+2} zexcretion and its concentration in the serum following oral administration of prostaglandin synthesis inhibition by tiaprofenic acid (10 mg/kg) and piroxicam (1 mg/kg) for 21 days. It was found that tiaprofenic acid reduced the mean ± SE 24 hour urinary Ca^{+2} excretion significantly after 10 and 21 days. This reduction did not reverse after one month from stoppage of its administration. Serum Ca^{+2} was lowered significantly after 21 days from its administration and persisted after one month from withdrawal of the drug. Piroxicam reduced significantly Ca^{+2} excretion and serum concentration of Ca^{+2} after 10 days and more reduction appeared after 21 days from administration. This reduction was not reversed after one month from stoppage of its administration. On the other hand, in arthritic rats, tiaprofenic acid reduced significantly urinary Ca^{+2} excretion and serum Ca^{+2} after 10 days from its administration and more reduction was observed after 21 days. As regards arthritic rats receiving piroxicam, it was found that 24 hour urine volume, urinary excretion of Ca^{+2} and serum Ca^{+2} concentration were decreased significantly after 10 day from its administration. This reduction became more apparent after 21 days. One month after withdrawal of tiaprofenic acid and piroxicam, some improvement in these parameters was observed but still a significant reduction was noted compared to the control group (arthritic rats).

Introduction

NON-STEROIDAL anti-inflammatory drugs (NSAIDs) are hailed as a novel therapeutic possibility for calcium (Ca^{+2}) urolithiasis [1]. There is a choice of a very wide range of NSAIDs at the present time. It was found that indomethacin reduced significantly urinary Ca^{+2} excretion in normal volunteers and in hypercalciuric stone formers [2 & 3]. Recently, diclofenac-Na has been used to decrease the rate of calculus recurrence [4]. Furthermore, Sharma et al [5] found that diclofenac-Na significantly decreased the urinary Ca^{+2} concentration and 24 hour Ca^{+2} excretion in spinal cord injury patients who are suffering from urolithiasis - a documented complication in traumatic paraplegics due to hypercalciuria.

Therefore, the present study was undertaken to investigate the effect of two representatives of NSAIDs namely tiaprofenic acid and piroxicam which are potent reversible inhibitors of cyclooxygenase [6 & 7] on renal handling and homeostasis of Ca^{+2} in normal rats.

Because many workers reported that rheumatoid arthritis is a disease associated with deranged calcium metabolism and many patients have disordered Ca^{+2} homeostasis [8] this study was extended to include the effect of the previous two tested NSAIDs on serum and urinary Ca^{+2} adjuvant induced arthritic (AIA) rats which is an experimental model of rheumatoid arthritis.

Material and Methods

I- Anti-inflammatory Drugs:

1. Piroxicam powder (Pfizer Incorp., USA). [4-hydroxy-2-Methyl-N- (2- pyridyl)-2H-1,2-benzothiazine-3-carboxamide-1, 1, dioxide].
2. Tiaprofenic acid powder (Les Laboratories, Roussel, France), [5-benzoyl-α-methyl-2-tiophene acetic acid].

II-Freund's Complete Adjuvant (F. C. A.) (Behringwerke Ag, Marburg, germany)

III-Reagents used for calcium determination were obtained from Bio-Analytics reagents and products, USA.

Animals Used:

This study was conducted on 36 male albino rats weighing 120-150 g. Animals were allowed food and water ad libitum.

Study Design:

The animals were divided into the following 4 groups:

Group (1): 6 normal rats served as a control (N).

Group (2): 6 Normal rats received tiaprofenic acid (10 mg/kg) orally daily for 21 days (T).

Group (3): 6 normral rats received piroxicam (1 mg/kg) orally daily for 21 days (P).

Group (4): 18 rats injected with 0.1 ml of F. C. A. in the left hind paw and were further subdivided into:

Group (4a): 6 rats injected with the F. C. A. and served as a model of arthritis (A).

Group (4b): 6 arthritic rats received tiaprofenic acid (1 mg/kg) orally daily for 21 days (A + T).

Group (4c): 6 arthritic rats received piroxicam (1 mg/kg) orally daily for 21 days (A + P).

The Procedure

In groups (2) and (3), on day one, the tested anti-inflammatory drugs were administered orally daily as freshly prepared aqueous suspensions for 21 days. The dose of tiaprofenic acid (10 mg/kg) and piroxicam (1 mg/kg) were chosen on the basis that they produced 62.4% and 65% mean inhibition of rat paw swelling, respectively in rats with adjuvant induced arthritis as measured by air displacement method.

In groups (4) b and c, on day one the anti-inflammatory drugs were administered orally in the previous specified doses. One hour later, F. C. A. was injected in the left hind paw. Thereafter, the anti-inflammatory drugs were administered orally daily from day 2 to day 21.

On day 10 and 21, rats in all groups were housed in metabolic cages (one animal/cage), the 24 hour urine was collected. Blood samples were obtained by means of capillary glass tubing from the retro-orbital plexus by procedure described by Schremere [9] on day 11 and 22.

In groups (2, 3, 4) b and c, the tested 2 NSAIDs were withdrawn suddenly on day 22 and the rats remained alive ad libitum for one month without any medication. 24 hour urine and serum samples were collected by the same previous procedures in all groups of rats after one month.

The 24 hour urine sample was collected to measure its volume and was analyzed to estimate the 24 hour urinary excretion of Ca^{+2}.

The serum samples were analyzed for the measurement of serum Ca^{+2} concentration using Kit from Bio-Analytics (USA). The procedure was based on the work of Gitelman [10] and involved the reaction of cresolphthalein complexone with Ca^{+2} to form a colored complex which is measured photometrically at 570 nm.

Statistical analysis of the Data:

Results are given as the mean (± SE) and analyzed by Student's *"t"* test and one-way ANOVA test on I. B. M. computer mode A. T. with Microstat Lotus 123 and Hg package system. Probability values (p) of less than 0.05 were considered significant.

Results

The mean (± SE) urine volume in normal rats receiving piroxicam (group 3) was significantly reduced after 10 and 21 days from its administration by 5% and 17% respectively. No significant reduction was

observed in rats receiving tiaprofenic acid (group 2). One month from stoppage of medication, some improvement occurred, but still there was significant reduction in the urine volume (table 1).

Table (2) illustrates the results of the mean (± SE) 24 hour urinary Ca^{+2} excretion (μmol/24h) in groups ("1", "2", & "3"). It was significantly decreased in groups (2) and (3) after 10 days by 17% and 39% and by 43% and 46% after 21 days of administration of both tested NSAIDs, respectively. One month after stoppage of medication, the mean (± SE) 24 hour urinary Ca^{+2} excretion was still significantly reduced compared to the control group.

Table (3) represents the effects of the 2 tested drugs on serum concentration (mol/L). It was observed that the administration of tiaprofenic acid in rats in group (2) produced a significant reduction in the mean (± SE) serum Ca^{+2} after 21 days by 4% but not after 10 days. In group (3), piroxicam reduced significantly the mean (± SE) serum Ca^{+2} concentration after 10 days by 10% and produced more decrease after 21 days by 15% which was not reversed after one month from stoppage of piroxicam.

No significant changes were observed between normal rats group (1) and AIA rats group (4a) in all parameters measured (table 4).

Table (1): Effect of Tiaprofenic Acid and Piroxicam on the mean ± SE 24 hour Urine Volume [ml / 24h / 100 g B.W.] in Normal Rats.

Drugs	10 days after administration of the tested drug	21 days after administration of the tested drug	One month after withdrawal of the tested drug
Control	15.42 ± 0.154	15.417 ± 0.201	15.42 ± 0.154
Group 2 Tiaprofenic acid (10 mg / kg orally)	15.17 ± 0.211	14.92 ± 0.3	14.916 ± 0.239
Group 3 Piroxicam (10 mg / kg orally)	14.5 ± 0.183*	12.67 ± 0.33*	13.42 ± 0.3**

* Significant by One way ANOVA test ($p < 0.05$)

** Significant by Student "t" test ($p < 0.001$)

Table (2): Effect of Tiaprofenic Acid and Piroxicam on the Mean ± SE 24 Hour in Normal Rats.

Drugs	10 days after administration of the tested drug	21 days after administration of the tested drug	One month after withdrawal of the tested drug
Control	61.875 ±3.162	61.875 ± 3.162	61.88 ± 0.162
Group 2 Tiaprofenic acid (10 mg / kg orally)	51.08 ± 5.544*	35.125 ± 3.657*	36.598 ± 2.545**
Group 3 Piroxicam (10 mg / kg orally)	37.38 ± 4.241*	33.00 ± 0.652*	34.84 ± 1.551**

* Significant by One way ANOVA test ($p < 0.05$)
** Significant by Student "*t*" test ($p < 0.001$)

Table 5 shows that the mean (± SE) 24 hour urine volume (ml/24h/100 g B. W.) was significantly reduced in group (4b) (A + T) after 21 days by 9%. It was found that this effect was reversed after one month from withdrawal of tiaprofenic acid. Whereas in group (4c) arthritic rats received piroxicam), it was observed that the significant reduction in urine volume occurred after 10 and 21 days by 11% and 25% respectively and were not reversed after one month from withdrawal of piroxicam.

In table (6) a significant reduction was found in the mean (± SE) 24 h urinary Ca^{+2} excretion was found in group (4b) by 54% after 10 days and by 56% after 21 days. In group (4c) it was reduced by 68% and 71% after 10 and 21 days from medication respectively. Some improvement was observed after one month from stoppage of tiaprofenic acid and piroxicam administration although a high significant decrease was still observed in both groups (4b) and (4c) compared to the control AIA rats, group (4a).

As regards the effect of tiaprofenic acid and piroxicam on the mean (± SE) serum Ca^{+2}, it was noticed that these tested drugs produced a significant decrease in the mean + SE serum Ca^{+2} concentration after 10 days by 23% in group (4b) and by

Table (3): Effect of Tiaprofenic Acid and Piroxicam on the Mean ± SE Serum Calcium Excretion [mmol / L] in Normal Rats.

Drugs	10 days after administration of the tested drug	21 days after administration of the tested drug	One month after withdrawal of the tested drug
Control	2.617 ± 0.065	2.6167 ± 0.065	2.617 ± 0.065
Group 2 Tiaprofenic acid (10 mg / kg orally)	2.583 ± 0.070	2.483 ± 0.04	2.46 ± 0.048
Group 3 Piroxicam (10 mg / kg orally)	2.333 ± 0.061*	2.217 ± 0.083*	2.237 ± 0.086*

* Significant by One way ANOVA test ($p < 0.05$)
** Significant by Student "t" test ($p < 0.001$)

Table (4):Effect of Adjuvant on the Mean ± SE 24h Urinary Volume [ml / 24h / 100 g B.W.], the Mean ± SE 24h Urinary Calcium Excretion [µmol / 24h] and the Mean ± SE Serum Calium [mmol / L] in Adjuvant indduced Arthritic Rats (A.I.A.).

	24h Urinary Volume		24h Urinary Ca^{+2} Excretion		Serum Ca^{+2}	
	After 10 days	After 21 days	After 10 days	After 21 days	After 10 days	After 21 days
Control group (1)	14.4167 ± 0.154	15.4167 ± 0.101	61.8750 ± 3.162	61.875 ± 3.162	2.6167 ± 0.065	2.617 ± 0.065
Adjuvant group (4a)	15.25 ± 0.281	15.5833 ± 0.396	59.6667 ± 4.043	59.625 ± 4.043	2.60 ± 0.073	2.55 ± 0.092

Non-Significant by Student "t" test ($p > 0.05$).

Table (5): Effect of Tiaprofenic Acid and Piroxicam on the mean ± SE 24 hour Urine volume [ml / L 24h / 100 g B.W.] in Adjuvant-induced Arthritic Rats (A.I.A.).

Drugs	10 days after administration of the tested drug	21 days after administration of the tested drug	One month after withdrawal of the tested drug
Adjuvant "A" group (4a)	15.25 ± 0.281	15.58 ± 0.396	15.4167 ± 0.3
A + Tiaprofenic acid(10 mg / kg orally) group (4b)	14.75 ± 0.214	14.08 ± 0.154*	14.83 ± 0.167
A + Piroxicam (1 mg / kg orally) group (4c)	13.5 ± 0.447*	11.67 ± 0.247*	12.75 ± 0.214**

* Significant by One way ANOVA test ($p < 0.05$)
** Significant by Student "t" test ($p < 0.001$)

Table (6): Effect of Tiaprofenic Acid and Piroxicam on the mean ± SE 24 hour Urinary Calcium Excretion [µmol / L 24h urine vol .] in Adjuvant-induced Arthritic Rats (A.I.A.).

Drugs	10 days after administration of the tested drug	21 days after administration of the tested drug	One month after withdrawal of the tested drug
Adjuvant "A" group (4a)	59.67 ± 3.587	59.625 ± 4.043	59.67 ± 3.587
A + Tiaprofenic acid(10 mg / kg orally) group (4b)	27.38 ± 0.688	20.54 ± 0.918*	27.633 ± 0.997**
A + Piroxicam (1 mg / kg orally) group (4b)	18.50 ± 0.3*	17.166 ±1.433*	25.69 ± 0.27**

* Significant by One way ANOVA test ($p < 0.05$)
** Significant by Student "t" test ($p < 0.001$)

Table (7): Effect of Tiaprofenic Acid and Piroxicam on the mean ± SE Serum Calcium [mmol / L] in Adjuvant-induced Arthritic Rats (A.I.A.).

Drugs	10 days after administration of the tested drug	21 days after administration of the tested drug	One month after withdrawal of the tested drug
Adjuvant "A" group (4a)	2.60 ± 0.073	2.55 ± 0.092	2.6 ± 0.073
A + Tiaprofenic acid(10 mg / kg orally) group (4b)	2.0 ± 0.113*	1.98 ± 0.098*	2.11 ± 0.077**
A + Piroxicam (1 mg / kg orally) group (4c)	1.57 ± 0.084*	1.63 ± 0.056*	1.89 ± 0.084**
	* Significant by One way ANOVA test ($P < 0.05$)		* Significant by Student "*t*" test ($P < 0.001$)

22% in group (4c) and the decrease became more apparent after 21 days (39% and 36% respectively) (table 7). This table also shows that this significant reduction was present after one month from withdrawal of medication in groups (4b and c).

Discussion

In the present study, it was found that administration of both tiaprofenic acid and piroxicam produced a significant reduction in the urinary excretion of Ca^{+2} after 10 and 21 days from their administration. This reduction was not reversed after one month from withdrawal of the 2 tested drugs.

In agreement with the finding of the present work, may NSAIDs have been reported to decrease urinary Ca^{+2} excretion [11, 12, 13 & 14]. Both indomethacin (10 mg/kg) and flurbiprofen (10 mg/kg) significantly reduced urinary Ca^{+2} excretion after i. v. infusion in male Sprague-Dawely rats [15]. Moreover, it was found that flurbiprofen produced a statistically significant reduction in urinary Ca^{+2} excretion in recurrent idiopathic stone formers [16]. Buck et al. [2] reported that Ca^{+2} was significantly decreased following PG synthetase inhibition with

indomethacin in conscious normal Sprague-Dawley rats and anesthetized monkeys. The same investigators observed that exogenous PGEz infusion resulted in a marked calciuretic response without producing changes in glomerular filtration rate (G. F. R.). These studies suggest that PGs are important intrarenal hormones which determine the renal handling of Ca^{+2} by regulating renal haemodynamic and renal tubular function.

There is also good circumstantial evidence to suggest that PG may influence Ca^{+2} reabsorption by the kidney as PG have been shown to alter Na^{-}, K^{+} and Ca^{+2} transport across frog skin which can be regarded as a model epithelium for renal tubules [17]. More significantly, indomethacin has been effective in normalizing the severe hypercalciuria associated with Barter's syndrome and congenital tubulopathies [18].

Cox et al. [19] found that indomethacin concentration less than 2.5 mg/ml did not influence renal function while higher concentration caused a decrease in urinary flow and Ca^{+2} excretion in perfused rat kidney. They also reported that the presence of 133 ng/ml PG in perfusate fully opposed these effects on kidney function.

Hemal et al. [1] studied the effect of diclofenac-Na^{+} 50 mg t. i. d. for 4 weeks on 31 recurrent oxalate nephrolithiasis patients who were normocalciuric. They observed that the 24 hour urinary excretion of Ca^{+2} and uric acid remained unchanged at 2 weeks and 4 weeks of therapy. Whereas after 2 and 4 weeks, there was a significant decrease in the 24 hour urinary excretion of glycosaminoglycans (GAGs) and urinary concentration of GAGs which are potent inhibitors of Ca^{+2} oxalate crystallization. So, there is a risk of calculus formation.

In contrast, Sharma et al. [5] investigated the effect of diclofenac-Na+ (50 mg t. d. s.) for 2 weeks and 4 weeks in 12 traumatic paraplegics who had sustained their injury to 6 months and they were hypercalciuric. There was no significant changes in 24 hour urinary volume, uric acid GAGs excretion. However, urinary Ca^{+2} concentration and 24 hour urinary Ca^{+2} excretion decreased significantly following 2 weeks and 4 weeks treatment with diclofenac-Na^{+}.

Lifschitz [20], suggested that PG appear to promote water excretion by increasing renal blood flow to medulla, tends to decrease papillary hypertonicity, limiting maximal concentrating ability. The decrease in urine volume by tiaprofenic acid and piroxicam was probably mediated through inhibition of renal PG biosynthesis resulting in enhancement of ADH on collecting renal tubules leading to a decrease of water excretion [21].

This study showed that the administration of tiaprofenic acid (10 mg/kg) for 10 days in normal rats produced a nonsignificant decrease in serum Ca^{+2} but after 21

daye the mean (±SE) serum Ca^{+2} concentration significantly decreased. Meanwhile, the piroxicam after 10 and 21 days from its administration reduced significantly the mean serum Ca^{+2}. The hypocalcaemic effect persisted after one month from withdrawal of the medication. In agreement with this results, Wiseman [6] reported that piroxicam may produce hypocalcaemia, leucocytosis and renal papillary necrosis, a syndrome associated with nephritides in dog.

The effect of NSAIDs on Ca^{+2} metabolism has been studied in animals, and indomethacin in particular, exhibits hypocalcaemia, hyperphosphataemia and parathyroid hormone inhibitory action in rats [22].

Moreover, PG synthesis inhibitors of the NSAI group have been shown to inhibit hypercalcaemia and osteolysis in rats with Walker tumour [23], hypercalcaemia in patients with various non-haematological tumours [24], osteolysis and osteoclast proliferation in rabbits [25].

Powels and his colleagues [26], reported that aspirin reduced osteolysis and hypercalcaemia induced by human mammary carcinoma and experimental rabbit carcinoma probably by inhibiting the stimulation of osteoclasts by PGE_2. This result was confirmed by Glasko and his colleagues [27] who advised that the NSAIDs should be tested in controlled clinical trial in patients with apparently early mammary carcinoma as a form of adjuvant therapy following mastectomy.

Northover [28], observed that indomethacin and other NSAIDs interfere with membrane transport of Ca^{+2} from the cells to the extracellular fluid and this may be an explanation of the lowering of blood Ca^{+2} level. Fernandes et al. [29] suggested that tiaprofenic acid may act on the cell membrane and interferes with transport mechanism explaining hypocalcaemic effect of tiaprofenic acid. Bijlasma and Rabelink [3], did not found significant changes in serum concentration of Ca^{+2}, phosphorus and 25 hydroxy vit. D3 and 1. 25 hydroxy vit. D3 but a decrease in Ca^{+2} excretion was noted in a study of Ca^{+2} homeostasis in 8 healthy volunteers during 8 days treatment with 150 mg indomethacin.

In arthritic rats, no significant changes were observed in all parameters measured compared to normal. Meanwhile, this work showed a decrease in 24 hour urine volume, Ca^{+2} excretion and serum Ca^{+2} concentration in AIA rats received tiaprofenic acid and piroxicam. Some improvement was noticed after one month of withdrawal of medication but significant reduction still observed in both groups.

Adjuvant-induced arthritis in rats is considered a model of rheumatoid arthritis [31]. Adjuvant-induced arthritis acts as a risk factor for renal side effect of PG synthesis inhibitors as NSAIDs where renal

PG are important in maintaining renal perfusion and function and are elaborated in response to activation of adrenergic and renin-angiotensin system [12]. Thus, any inhibition of renal PG in this location, became more important and more apparent than in healthy animals as the results of this study showed.

It is known that PG of E series play an important role in bone remodelling [32] and the activation of osteoclasts, the major bone resorbing cells [25]. Accordingly, PG synthesis inhibitors of NSAIDs exert their effect in adjuvant arthritis via inhibition of bone reactivity and acts as effective inhibitors of bone resorption and redeposition.

Bijlsma and Rablink [30], reported that rheumatoid arthritis is associated with generalized loss of bone mass. One of the factors that may be involved in the pathogenesis of this bone loss is the chronic use of NSAIDs which are known to increase gastrointestinal permeability of Ca^{+2} and they may also influence glomerular filtration rate and renal excretion of Ca^{+2}. In addition, the same investigators suggested that NSAIDs may inhibit osteoblastic function as well as osteoclastic bone resorption. Waters et al. [33] studied the effect of aspirin on bone mass and bone PG in immobilization of osteoporosis in 12 growing dogs. They found that aspirin treatment was associated with 65% reduction in bone PGE and 13% bone mass sparing effect.

All these studies suggested that metabolism was disturbed in rheumatoid arthritis. Kennedy et al. [34] reported the presence of hypercalcaemia although parathyroid hormone concentrations were raised. Hypocalcaemia may also present in rheumatoid patients with advanced bone disease [35].

According to the results of this study, the ability of NSAIDs in reducing Ca^{+2} excretion can be researched clinically as a trial in reducing the frequency of Ca^{+2} renal stones particularly in hypercalcaemic hypercalciuric patients with recurrent stone formation. Moreover, those drugs can be investigated in condition of some maliganant tumors metastasis in bones due to their ability to decrease hypercalcaemia, osteoporosis and osteoclastic activity.

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