

The use of renal enzymes as early indication of renal toxicity after multiple-dose administration of aspirin

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Summary

Ten volunteers participated in a study comparing the effects on renal enzymes of multiple oral doses of aspirin relative to no treatment. The total urinary output was collected daily for 21 days from all subjects. The first 7 days were treatment-free. During the second 7-day period the subjects received aspirin 1 500 mg 3 times daily. This was followed by another treatment-free period of 7 days.

The activity of the enzymes alanine aminopeptidase and *N*-acetyl- β -glucosaminidase were determined in each daily urine specimen. Statistical analysis revealed that aspirin significantly increased the output of both enzymes.

S Afr Med J 1983; 63: 687-688.

Alanine aminopeptidase (AAP) is the principal enzyme of the brush-border membrane of the proximal tubule in the kidney.¹ *N*-acetyl- β -glucosaminidase (NAG) is a lysosomal enzyme found in high concentrations in the epithelial cells of renal tubules.² AAP and NAG are excreted in the urine of normal people, but much larger quantities are excreted by patients with renal disease and those taking nephrotoxic drugs.^{1,2}

The aim of our study was to compare the effects of multiple oral doses of aspirin, relative to no treatment, on renal excretion of the abovementioned enzymes.

Subjects and methods

Ten healthy male volunteers participated. Prior to the trial each was subjected to a comprehensive physical examination, ECG, chemical blood tests, urinalysis and haematological tests.

Informed consent was obtained from each subject, the South African Medicines Control Council and the Ethics Committee of the University of the Orange Free State.

Trial design. This was a multiple-dose open study in which each subject participated in three phases: I (days 1 - 7) — no treatment; II (days 8 - 14) — aspirin 1 500 mg 3 times daily; III (days 15 - 21) — no treatment.

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During all three phases total urine output was collected daily.

Medication. Acetylsalicylic acid (Disprin) 1 500 mg was administered 3 times a day with meals on days 8 - 14.

Parameters measured. Total urine output was collected daily for 21 days, and the enzymes AAP and NAG were assayed in each specimen. The method of Mondorf *et al.*¹ was used for AAP estimation. For NAG measurement the method of Maruhn³ was employed.

Results

Results for AAP are shown in Fig. 1 and Table I and those for NAG in Fig. 2 and Table II.

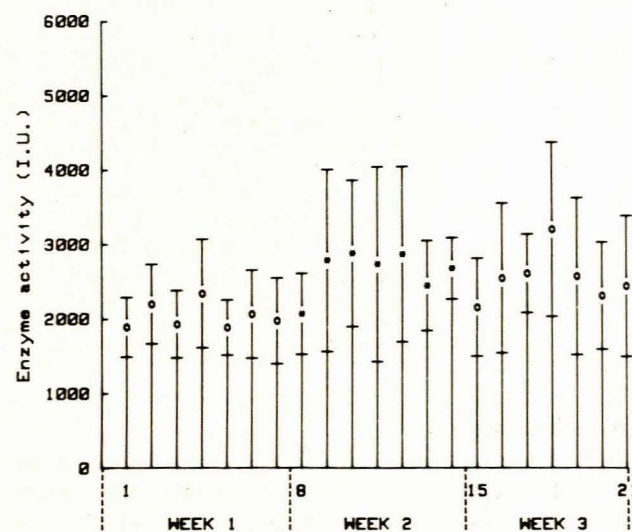


Fig. 1. Mean (\pm SD) daily AAP activity (in IU) (o — no treatment; * — aspirin).

TABLE I. CUMULATIVE WEEKLY AAP ACTIVITY (IU)

Subject No.	Wk 1	Wk 2	Wk 3
1	11 802,14	11 657,96	10 934,43
2	15 573,07	28 064,78	16 606,76
3	10 946,66	15 749,20	15 018,79
4	14 502,38	16 105,48	23 205,68
5	10 622,98	14 522,65	11 646,05
6	14 018,55	16 797,69	14 254,55
7	9 342,28	10 721,99	13 017,90
8	13 078,71	18 421,16	16 937,90
9	9 927,30	11 770,15	16 292,47
10	13 691,13	16 941,04	17 474,18
Mean	12 350,52	16 075,21	15 538,87
SD	2 116,14	4 941,27	3 509,06

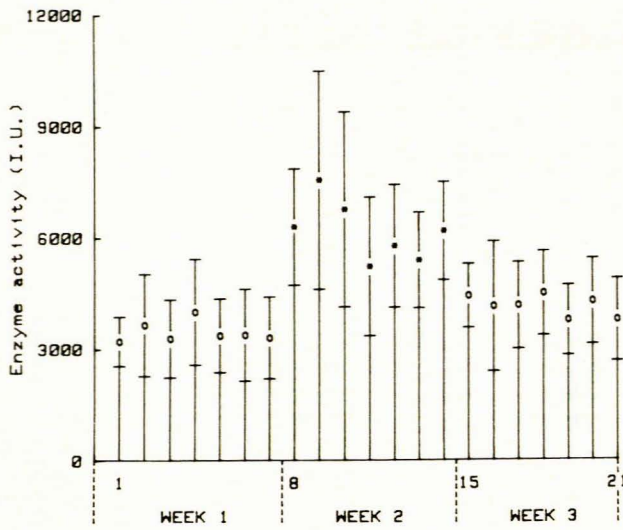


Fig. 2. Mean (\pm SD) daily NAG activity (in IU) (o — no treatment; * — aspirin).

Subject No.	Wk 1	Wk 2	Wk 3
1	19 079,84	23 383,69	20 194,31
2	29 608,08	55 726,80	35 328,49
3	17 957,58	29 246,55	22 196,18
4	25 934,36	37 697,71	31 498,11
5	23 076,29	38 666,67	21 737,15
6	24 102,93	43 578,78	24 377,60
7	14 279,07	26 134,57	18 844,40
8	20 912,48	43 667,18	26 398,01
9	13 210,87	26 207,52	23 449,95
10	21 051,42	43 612,48	23 781,79
Mean	20 921,29	36 792,20	24 780,60
SD	5 067,54	10 355,02	5 100,59

Statistical analysis

The cumulative weekly activity of the two enzymes in urine was calculated for each subject for each of the 3 weeks by adding up the individual daily enzyme activities for the 7 days of each week (Tables I and II). Using Student's *t* test for paired values,

the results for each week were compared in order to establish whether aspirin had a significant effect on the output of the two renal enzymes.

Statistical analysis revealed the following:

AAP. The total urinary output of AAP was significantly higher in week 2 than in week 1 ($P < 0,05$), but not in week 2 compared with week 3 ($P > 0,05$). The total urinary output of the enzyme in week 3 was significantly higher than that in week 1 ($P < 0,05$).

NAG. The total urinary output of NAG in week 2 was significantly higher than in week 1 ($P < 0,05$), and in week 2 it was significantly higher than in week 3 ($P < 0,05$). There were no significant differences between weeks 1 and 3 ($P > 0,05$).

Discussion

When considering the results for AAP it should be borne in mind that isoxepac, an anti-inflammatory drug, has been shown to display consistent concentration-dependent suppression of AAP activity *in vitro*, whereas no inhibition has been observed with salicylic acid.⁴ As a precaution, all urine samples were nevertheless dialysed against water as suggested by the authors, thereby completely removing salicylic acid and metabolites from the urine, as could be shown by a sensitive thin-layer chromatographic method.

The levels of AAP point to persisting damage to the brush-border membrane of the proximal tubule 1 week after drug withdrawal. The levels of NAG were not elevated 1 week after drug withdrawal. This may indicate a differential degree of tissue damage or tissue recovery. AAP and NAG activities in urine appear to hold promise as early indicators of potential nephrotoxicity produced by chemical substances.

Since the results of these determinations may be influenced by the presence of a drug in the urine, the intrinsic attenuating influence of such a drug or its metabolite(s) on AAP and NAG activities should be investigated before a formal trial is embarked on.

REFERENCES

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