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SHORT-TERM STUDY OF THE EFFECT OF PHENACETIN, PHENAZONE AND AMIDOPYRINE ON THE RAT KIDNEY

BY

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Phenacetin has been implicated in kidney damage in man (Spuhler & Zollinger, 1953); however, the number of cases of kidney damage from phenacetin consumption reported from Switzerland, Germany and Scandinavia is greater than that reported from Australia and the United States, though the average consumption of phenacetin per head is similar in all these countries (Ross, 1962). The reported incidence of "analgesic nephritis" is also low in Great Britain, where the consumption of phenacetin is not less than in the other countries (Prescott, 1965).

In preparations examined by Horisberger, Grandjean & Lanz (1958) and Grimlund (1965) the drugs most frequently administered with phenacetin were phenazone and amidopyrine or a close analogue. We felt that any of these drugs could be responsible for the renal effects and we have therefore carried out a preliminary investigation in rats comparing the toxicity of phenacetin, phenazone and amidopyrine, with particular reference to effects on the kidney.

METHODS

Phenacetin, phenazone and amidopyrine were administered orally on 7 days a week as a suspension in 1.25% w/v methyl cellulose in water to young, mature Sprague-Dawley strain male rats (Charles Rivers, U.S.A.), initial weight range, 200–220 g, according to the dosage schedule in Table 1.

Urine cytology and routine urine analysis

Not less than four animals from each group were given an oral water load equivalent to 3% of their body weight. Immediately following this any urine in the bladder was expressed by suprapubic pressure. The cells and particulate material in a measured aliquot of the urine collected during the following 2-hr period were then concentrated fivefold by centrifuging for 5 min at 2,000 r.p.m., with removal of the appropriate quantity of the supernatant fluid. A portion of the cell concentrate was then stained with a peroxidase stain (Prescott & Brodie, 1964), before quantitative assessment in a Fuchs–Rosenthal counting chamber. At this stage the numbers of squamous cells, peroxidase-positive cells, erythrocytes, and cells of probable renal origin, were recorded. After centrifuging, some of the deposit was transferred to albuminized slides, fixed in alcohol, and stained with EA 50 polychrome Papanicolaou stain (Ortho Pharmaceuticals Limited). The provisional morphological classification made in the counting chamber wet preparation was verified using these films.

The remainder of the urine was examined for protein and sugar using Haemocombistix (Ames Division of Miles Laboratories Limited). The amount of protein required to produce an arbitrary series of colour changes on the chemically treated strip was assesesed previously by assaying a series of standards on the Autoanalyser, using both the biuret and a turbidometric method. Thus, a ± reaction is approximately equivalent to a urinary protein of less than 20 mg/100 ml., a + of 20–40 mg/100 ml., a ++ of 40–60 mg/100 ml., and a +++ of not less than 60 mg/100 ml.

Finally, urinary glutamic oxalacetic transaminase was determined on the supernatant fraction by the Sigma Reagent Company's modification of the Reitman/Frankel procedure.

Urine 6-hr concentration test

The 6-hr concentration test used was that of Balaz, Hatch, Zawidska & Grice (1963). Drinking-water was removed from the cage, any urine was expelled from the bladders, and exactly 6 hr later urine was again expelled if possible. The animals were then placed in a metabolism cage equipped with a urine-faeces separator, and the specific gravity of the urine voided in the next hour was determined by micropyknometry. If no urine was obtained during this period the experiment was repeated later.

Serum chemistry

Blood samples were removed by cardiac puncture with the animals under light ether anaesthesia, and serum urea nitrogen and serum creatinine were determined with the Autoanalyser.

Terminal studies

At the end of the dosing period the animals were killed by bleeding under ether anaesthesia. The kidneys were weighed to the nearest milligram, fixed in 10% buffered formal saline and later prepared for histopathological evaluation.

RESULTS

Sixteen animals received phenacetin, sixteen received phenazone and twelve amidopyrine on 7 days a week according to the dose schedule laid out in Table 1. Sixteen control animals received vehicle alone.

TABLE 1

DAILY DOSE SCHEDULE OF ANALGESICS ADMINISTERED TO RATS

Day	Test I Phenacetin and phenazone (mg/kg)	Test II Amidopyrine (mg/kg)
1–6	750	500
7–14	850	750
15–20	1000	850
21–28	1100	950
29–36	1200	1100
37–51	1300	1200 (terminating on day 38)

Urine analyses were carried out frequently, on not less than four animals of each group throughout the test. On day 51 of Test I and day 35 of Test II all surviving animals had their serum urea nitrogen and creatinine determined.

The doses of the drugs administered were near the acute lethal level, so that during the test three of the phenazone, six of the phenacetin, and six of the aminopyrine-dosed animals died. Terminal studies were carried out on the remainder of the animals.

Cellular exfoliation

Figure 1 shows that the rate of cellular exfoliation was considerably raised in the phenazone-dosed animals compared with the controls and to animals given the other two drugs. This elevation in numbers of renal tubular cells was evident even on the second day of dosing and persisted throughout the test period. On six of the nine occasions when the urine was examined, the elevation in urinary cell count was significant [$P<0.05$] in the phenazone-treated animals compared with those receiving methyl cellulose alone. At no time was an elevation of renal cells demonstrable in the urine of phenacetin-treated animals. Amidopyrine gave a significant rise on only one occasion.

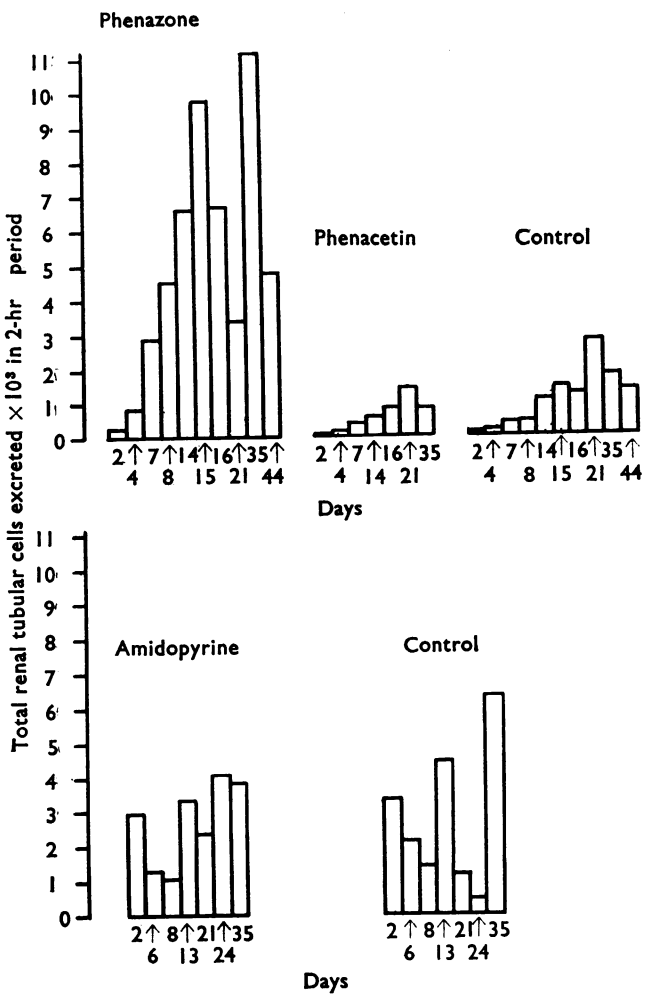

Fig. 1. Total renal tubular cells $\times 10^3$ excreted in a 2-hr period in the urine of not less than four rats treated orally with phenazone, phenacetin and amidopyrine according to the schedule in Table 1. The urine was obtained following an oral water load equivalent to 3% of their body weight.

Routine urine analysis

There was no obvious elevation of the urinary glutamic oxalacetic transaminase at any of the sampling dates, nor could the enzyme levels be correlated with cell count in the phenacetin and amidopyrine groups, but there was a marginal elevation in the phenazone group on several occasions. From the arbitrary score it was evident that there was also a significant increase in the incidence of albuminuria in this group, as is shown in Table 2. There was no evidence of any increased incidence of glycosuria.

TABLE 2

INCIDENCE OF ALBUMINURIA IN RATS DOSED WITH PHENACETIN, PHENAZONE AND AMIDOPYRINE

A score of ± is equivalent to less than 20 mg/100 ml., + 20–40 mg/100 ml., ++ 40–60 mg/100 ml. and +++ greater than 60 mg/100 ml. albumin

	Test I		
	Phenacetin	Phenazone	Controls
Total incidence of albuminuria	6 out of 32 animals 6+	24 out of 47 animals 12+ 11++ 1+++	8 out of 47 animals 8+

	Test II	
	Amidopyrine	Controls
Total incidence of albuminuria	4 out of 36 animals 1+ 2++ 1+++	4 out of 40 animals 3+ 1++

Six-hour urine concentration test

The results of the concentration test given in Table 3 show that with all three compounds there was a statistically significant depression of urinary specific gravity, the greatest effect occurring with phenazone and amidopyrine.

TABLE 3

6-HR URINE CONCENTRATION TESTS IN RATS

The specific gravity estimated by micro pycknometer at week 8 for the phenacetin- and phenazone-dosed rats and at week 5 for the amidopyrine dosed animals

	Test I		
	Phenacetin	Phenazone	Controls
Mean ± S.E.	1·0426 ±0·0038	1·0304 ±0·0039	1·0597 ±0·0052
t	2·65	4·53	
P	0·019	<0·001	

	Test II	
	Amidopyrine	Controls
Mean ± S.E.	1·0353 ±0·0038	1·0546 ±0·0041
t	3·36	
P	0·008	

Body weight and clinical condition

The terminal mean weights of the surviving animals are shown in Table 4. At the end of the 51 days in Test I with phenacetin and phenazone, and 38 days in Test II with amidopyrine, body weights of all the remaining test animals were depressed compared with the controls. The depression in body weight, which was significant in all instances, was more pronounced with amidopyrine than with the other two compounds.

TABLE 4

MEAN TERMINAL BODY WEIGHTS IN GRAMS OF RATS DOSED WITH PHENACETIN AND PHENAZONE (51 DAYS) AND AMIDOPYRINE (38 DAYS)

Numbers of animals are given in parentheses

	Test I		
	Phenacetin	Phenazone	Controls
Mean	376 (10)	361 (13)	428 (16)
t	2·70	4·23	
P	<0·05	<0·001	

	Test II	
	Amidopyrine	Controls
Mean	321 (6)	437 (12)
t	6·15	
P	<0·001	

Apart from the effect of body weight none of the animals showed any external signs, but serum urea nitrogen and serum creatinine levels were elevated in two out of six of the amidopyrine and one out of thirteen of the phenazone-dosed animals at the end of the test.

Organ weights

The mean gross kidney weight (Table 5) rose considerably in the amidopyrine-dosed animals. Table 5 shows the mean value compared with that for the other two analgesics and the controls.

TABLE 5

MEAN GROSS KIDNEY WEIGHTS IN GRAMS FROM SURVIVING RATS AT THE END OF THE TEST PERIODS. BOTH KIDNEYS WERE WEIGHED

Numbers of kidneys examined are given in parentheses

	Test I		
	Phenacetin	Phenazone	Controls
Mean	1·352 (20)	1·414 (26)	1·366 (32)
t	0·32	1·36	
P	>0·5	>0·2	

	Test II	
	Amidopyrine	Controls
Mean	1·896 (12)	1·350 (24)
t	4·01	
P	<0·001	

Histology

The most striking histological finding in paraffin sections of kidneys was in animals given amidopyrine. There was a sharply localized necrosis of the papillary tip in five out of six of these animals. This lesion was associated with a varying degree of tubular atrophy and dilatation, which in some cases occurred with an infiltration of cells of the chronic inflammatory type (Fig. 2). There was some congestion of the capillaries throughout the medulla, and occasional casts in the collecting tubules were also present in some animals.

In contrast, however, no papillary necrosis was seen in any of the phenacetin- or phenazone-treated animals. In one phenazone-treated animal there was much congestion throughout the entire tissue, with the occasional focus of cellular infiltration—again of the chronic inflammatory type—in the interstitial spaces.

Otherwise the kidneys from all other animals were normal.

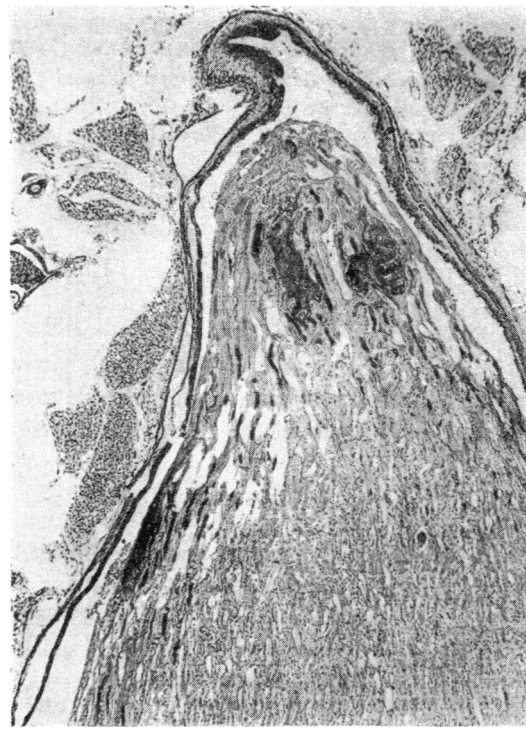

Fig. 2. Photomicrograph (×125) of a haematoxylin and eosin stained paraffin section of the renal papilla of a rat treated orally with amidopyrine according to the dose schedule in Table 1. This shows a sharply localized necrosis in the papillary tip.

DISCUSSION

Most analgesics are commonly taken in the form of mixtures. The composition of these mixtures varies from country to country, but in broad terms in Europe the principal components are phenacetin and phenazone, or a near analogue such as amidopyrine, whereas in the United Kingdom and North America the analgesic constituents are usually aspirin and phenacetin. Phenazone and amidopyrine are not used to any extent in the Anglo-Saxon countries because of their recognized toxicity, particularly that of amidopyrine with its propensity to cause agranulocytosis.

In assessing the possible toxic effect of these mixtures it is virtually impossible to determine from human studies which compound is the one responsible and in the circumstances only animal studies carried out on the individual components can provide a satisfactory guide. Most studies in animals, however, have been inconclusive. A two-year study by Woodard, Post, Cockrell & Cronin (1965) in dogs with large doses of phenacetin failed to indicate any renal abnormality. In rats and rabbits, before a nephrotoxic action could be demonstrated it was necessary to infect the kidney with either *E. coli* (Miescher, Schnyder & Krech, 1958) or *Staphylococci* (Studer, Zbinden & Fust, 1958). On the other hand Abrahams, Rubenstein & Levin (1963) claim to have

induced papillary damage in rats to which tablets of phenacetin and also aspirin, phenacetin and caffeine were administered for 15 months. They, however, used Wistar rats, which have a high incidence of natural renal malformation, which could complicate the picture (Sellars, Rosenfeld & Friedman, 1960).

Animal experimentation, therefore, has so far provided little information about the effect of phenacetin on the kidneys and the circumstantial evidence points to the possibility of another agent being the cause.

Fazekas, Fazekas & Bertok (1960) studied experimentally in rabbits and cats the effect of high doses of amidopyrine, and have clearly demonstrated an effect on kidney function. Initially they found an oliguria, followed by a persistent polyuria, the animals ultimately dying in uraemia. Histological studies by the same authors showed that amidopyrine caused extensive necrotic changes in the kidney, particularly to the glomeruli and the vasa recti. The histological picture in these species, shown by Fazekas, Fazekas & Bertok (1962), does not, however, correspond to the classical picture of human pyelonephritis or interstitial nephritis as described by Thiel, Spuhler & Uehlinger (1964), but their results show unequivocally that amidopyrine causes serious damage to the kidney. On the other hand, the results of our experiments in rats have not only confirmed the nephrotoxic action of amidopyrine, but have also demonstrated that amidopyrine produces papillary necrosis similar to the chronic papillary necrosis in man (Knudsen, personal communication).

There are differences in the levels of exfoliation of renal tubular cells in the urine between the two groups of control animals, but phenazone caused a statistically significant elevation of cellular exfoliation in the urine over the control values, whereas amidopyrine, which caused extensive necrosis of the renal papillae, did not. Prescott (1956) demonstrated in man that mild analgesic drugs will cause an increase in the number of cells in the urine, aspirin having by far the greatest effect, whereas the effect of phenacetin was very much less, being only slightly greater than the control values. Taken in conjunction with Prescott's observations, our results indicate that the elevation of cells excreted in the urine need not necessarily suggest that the drug will cause actual renal damage. Nevertheless, phenazone and phenacetin have an effect on kidney function in rats as shown by a reduction in urinary concentrating ability, phenazone having a greater action in this respect than phenacetin. Kincaid-Smith (1967) has studied the pathogenesis of the renal lesion associated with the abuse of analgesics and has formed the view that renal papillary necrosis is the primary lesion. In view of the fact that amidopyrine and related drugs are used extensively in Europe, and from our own animal studies showing that amidopyrine will cause renal papillary necrosis, it would be of interest to carry out a retrospective epidemiological survey of the use of analgesic preparations to determine whether the incidence of nephritis associated with analgesic abuse is related to phenacetin or to the pyrazalone group of drugs.

SUMMARY

1. Phenacetin, phenazone and amidopyrine were given to rats in large doses by mouth over a period of 5 to 8 weeks and the effects of these drugs on kidney function were studied.

2. Phenazone causes a persistent celluria with evidence of slight kidney damage, whereas amidopyrine causes papillary necrosis but little if any celluria ; phenacetin causes neither.

3. The ability of the kidney to excrete a concentrated urine is adversely affected particularly by amidopyrine, to a lesser degree by phenazone, and to a very small extent by phenacetin.

4. It is possible that cases of "analgesic nephritis," reported from Europe, may be caused by the presence of amidopyrine and perhaps phenazone in most of the analgesic preparations used.

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