Preliminary Communications

Papillary Necrosis in Rats Caused by Aspirin and Aspirin-containing Mixtures

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Summary: Nearly half the rats gavage-fed with aspirin and aspirin-containing mixtures developed papillary necrosis in 20 weeks. This incidence is similar to that found in rats on A.P.C. mixtures with high and low concentrations of *p*-chloroacetanilide, an impurity of phenacetin. Aspirin alone produced necrosis in 7 out of 19 rats (36.8%) whereas phenacetin in the same dose had failed to cause any renal damage over six to nine months. If these results also apply to man they suggest that aspirin and not phenacetin may be the major factor in analgesic nephropathy in patients taking A.P.C. mixtures. An augmented clearance of aspirin appeared to afford some protection to the medulla, and it is suggested that this observation may have important clinical and epidemiological applications.

INTRODUCTION

Spühler and Zollinger (1953) first drew attention to the association between phenacetin and interstitial nephritis. There is now considerable evidence that prolonged intake of analgesics is associated with a form of renal disease characterized by papillary necrosis (Hultengren, 1958; Lindeneg *et al.*, 1959; Burry *et al.*, 1966; Dawborn *et al.*, 1966; Gault *et al.*, 1968). Phenacetin, though never prescribed alone, has been singled out and blamed for this lesion because it is the common factor in analgesic mixtures (Prescott, 1966; Kincaid-Smith, 1967; Shelley, 1967). The other analgesics often prescribed with phenacetin are aspirin, caffeine, amidopyrine or its analogues, and phenazone. All of them have been shown to be nephrotoxic in animals and humans (Wendt, 1938; Axelsson, 1958; Boyd, 1959; Fazekas *et al.*, 1960; Clausen, 1962, 1964; Harvald, 1963; Abrahams *et al.*, 1964; Fellers *et al.*, 1965; Prescott, 1965, 1969; Brown and Hardy, 1968).

Saker and Kincaid-Smith (1969) showed that rats gavage-fed with an aspirin, phenacetin, and caffeine (A.P.C.) mixture in a dose taken by some patients with analgesic nephropathy will develop renal papillary necrosis in six to nine months. Phenacetin in twice the dose contained in the A.P.C. mixture failed to cause any renal damage, suggesting that some factor other than phenacetin in A.P.C. was responsible for papillary necrosis. To study the cause and pathogenesis of analgesic nephropathy we have extended the work of Saker and Kincaid-Smith in an attempt to define the ingredient of A.P.C. mixtures which causes papillary necrosis.

MATERIAL AND METHODS

Trial 1.—Sixty Sprague-Dawley and fifty Wistar female nulliparous rats, equally distributed between groups of rats, were fed analgesics for 20 weeks in two separate studies. One of these trials was conducted during the winter and the other during summer. The analgesic mixtures were given by gavage twice daily, five days a week. The number of animals in the two studies and the analgesic mixtures used are shown in Table I. The maximum dose of the drug administered was achieved over two weeks by progressively increasing the dose every two to three days. All animals were subjected to 16

hours' overnight dehydration daily. After eight weeks of analgesic feeding one rat from each analgesic group was killed each week over the next 12 weeks. A proportion of animals from group 1, Table I, and the control group were subjected to oligaemic shock by removal of 2 to 3 ml. of blood by cardiac puncture under light anaesthesia. These animals were kept on dehydration for 48 hours before killing. At the end of the trial the animals were killed and the kidneys removed for histological examination.

TABLE I.—Analgesic Mixtures Used in Trial 1

Analgesic	Dose (mg./kg./day)	No of Rats
Group 1: A.P.C. (high impurity)	900 (A = 380)	40
Group 2: A.P.C. (low impurity)	900 (A = 380)	10
Group 3: A + P	750 (A = 380)	10
Group 4: A + C	700 (A = 500)	10
Group 5: A	500	20
Group 6: Control	—	20

A = Aspirin. P = Phenacetin. C = Caffeine.

Trial 2.—In this trial 80 female nulliparous Sprague-Dawley rats were used. The analgesic mixtures were administered for 20 weeks as in the first trial, the analgesics used being shown in Table II. Fifty rats were sustained on a constant water diuresis with 5% glucose as drinking-water (Andriole and Epstein, 1965). Thirty rats were subjected to dehydration as in the first trial and simultaneously given acetazolamide and sodium bicarbonate with the analgesics. Half the rats from this group were also subjected to oligaemic shock as in the previous trial. The animals were killed weekly over a period of 8 to 20 weeks.

TABLE II.—Analgesic Mixtures Used in Rats in Trial 2

Analgesic	Dose (mg./kg./day)	No. of Rats
<i>Rats on a Constant Water Diuresis with 5% Glucose</i>		
Group 1: A.P.C.	900 (A = 380)	10
Group 2: A + NAPA + C	900 (A = 380)	10
Group 3: A + C	700 (A = 500)	10
Group 4: A	500	10
Group 5: Control	—	10
<i>Rats on 16 Hours' Dehydration a Day</i>		
Group 1: A.P.C. + acetazolamide (20 mg./kg./day)	900 (A = 380)	10
Group 2: A + acetazolamide (20 mg./kg./day)	400	10
Group 3: A + sod. bicarb. (50 mg./kg./day)	500	10

A = Aspirin. P = Phenacetin. C = Caffeine. NAPA = Paracetamol.

Serial renal function tests including an assessment of medullary blood flow were performed in some rats. The results of these studies and details of the pathological findings will be published separately.

PATHOLOGICAL DEFINITIONS

Histological grading was done on a blind basis.

The kidneys were included as papillary necrosis in Tables III and IV when they showed frank necrosis, either diffuse or patchy, in the papillae. Diffuse papillary necrosis (see Fig.) showed total necrosis of the papillae with a clear line of demarcation between necrotic and viable renal tissue. The patchy medullary lesion showed either necrosis of loops of Henle and vasa recta with some surviving collecting ducts or areas of necrosis of all elements with intervening areas of surviving tissue. Minor degrees of change including necrobiosis are excluded for the purposes of this report.

TABLE III.—Incidence of Renal Papillary Necrosis with Aspirin-containing Mixtures over 8 to 20 Weeks—Rats on 16 Hours' Dehydration a Day

Analgesic	No. of Rats	Papillary Necrosis
Control	19	0
A.P.C. (high impurity)	31	13 (41.9%)
A.P.C. (low impurity)	10	6 (60%)
A+P	10	4 (40%)
A+C	10	1 (10%)
A (500 mg./kg./day)	19	7 (36.8%)
*Phenacetin (500 mg./kg./day)	27	0 (over 6-9 months)

*From Saker and Kincaid-Smith (1969).

TABLE IV.—Incidence of Renal Papillary Necrosis with Aspirin-containing Mixtures over 8 to 20 Weeks

Analgesic	No. of Rats	Papillary Necrosis
<i>Rats on a Constant Water Diuresis with 5% Glucose</i>		
Control	10	0
A.P.C.	9	0
A+NAPA+C	9	2 (22.2%)
A+C	9	1 (11.1%)
A	9	3 (33.3%)
<i>Rats on 16 Hours' Dehydration a Day</i>		
A.P.C. + acetazolamide	8	1 (12.5%)
A + acetazolamide	10	0
A + sodium bicarbonate	10	2 (20%)

Necrotic rat papilla showing outlines of necrotic tubules. The darker-staining areas are calcified material. The changes are similar to those seen in analgesic nephropathy in man. ($\times 210$.)

RESULTS

The kidneys from 19 rats were excluded from evaluation because of early deaths from aspiration pneumonia and gastrointestinal ulceration and bleeding or advanced autolytic changes.

The combined results of the two studies conducted in Trial 1 are shown in Table III. Over a period of 8 to 20 weeks on aspirin-containing mixtures nearly half the rats developed necrosis. The animals fed on A.P.C. in the summer study in this trial showed a higher incidence of frank and severe papillary necrosis (69.2%) compared with those in the winter study (22.2%). This appeared to be related to the fact that in the summer study the rats were often exposed to prolonged periods of hot and dry weather and the most severe forms of necrosis were seen in rats which died spontaneously on hot days. There were no facilities to maintain the temperature of the animal-room at a constant level. The incidence of necrosis in rats fed on a proprietary A.P.C. mixture containing high impurity content (0.1% *p*-chloroacetanilide) is similar to that in rats on a relatively pure A.P.C. mixture (0.1% *p*-chloroacetanilide). Aspirin alone produced papillary necrosis in 7

out of 19 rats (36.8%). This incidence is not significantly different from that in the A.P.C. groups. In spite of a higher dose of aspirin, a combination of aspirin and caffeine produced necrosis in only 10% of rats compared with 40% in rats fed on aspirin combined with phenacetin.

A constant water diuresis or the administration of acetazolamide or sodium bicarbonate appeared to reduce the effect of analgesic mixtures on the renal medulla (Table IV). The incidence of papillary necrosis was 38.8% in the dehydration group compared with 16.7% in rats on a constant water diuresis and 10.7% in rats having added acetazolamide and sodium bicarbonate.

Oligaemic shock was induced in 58.6% of analgesic-fed rats and in 69% of control rats before killing; 21.9% of the analgesic-fed animals died spontaneously. The incidence of papillary necrosis in analgesic-fed rats on dehydration subjected to oligaemic shock (51.6%) was higher than in animals dying spontaneously (45.5%) or those killed without cardiac puncture (37.5%), but these differences were not statistically significant.

DISCUSSION

In the past, renal papillary necrosis has been produced in animals with both aspirin and phenacetin. The very high doses used in these experiments have been strongly criticized (Gilman, 1964). Abrahams *et al.* (1964) and Saker and Kincaid-Smith (1969) gave doses of A.P.C. to rats similar to those taken by some patients with papillary necrosis and produced a renal lesion identical to that seen in patients with analgesic nephropathy in nearly 50% of animals. In an attempt to shorten the period of feeding before necrosis develops we have in our present study doubled the dose of analgesics and induced necrosis in 8 to 20 weeks. The short-term feeding has enabled us to study a greater number of combinations of analgesics and to identify the probable toxic agent in analgesic nephropathy.

Renal papillary necrosis was observed in the same percentage of rats fed aspirin alone as that found in rats given an A.P.C. mixture over 8 to 20 weeks. Phenacetin alone in the same dose over nine months failed to cause any renal damage (Saker and Kincaid-Smith, 1969). On the basis of these studies it seems likely that aspirin is the main factor causing papillary necrosis in rats fed on A.P.C. mixtures.

For many years salicylates have been known to cause renal damage, and there have been both clinical and experimental reports of a variety of lesions, including haematuria and renal failure (Hanzlik, 1927; Lipman *et al.*, 1949; Locket, 1957; Campbell and MacLaurin, 1958; Granville-Grossman and Sergeant, 1960; Parsons, 1963; Scott *et al.*, 1963; Ben-Ishay, 1964; Prescott, 1965; Dubach and Josch, 1967; Robinson *et al.*, 1967). Renal papillary necrosis has been produced in animal experiments with aspirin alone (Clausen, 1962, 1964; Fellers *et al.*, 1965; Robinson *et al.*, 1967). Though aspirin is commonly taken with other analgesic compounds, cases of analgesic nephropathy and papillary necrosis with aspirin alone have also been reported (Harvald, 1963; Parsons, 1963; Gault *et al.*, 1968; Prescott, 1969).

Patients with rheumatoid arthritis are an important group in whom the clinical association between aspirin ingestion and papillary necrosis may be further studied. Most patients with rheumatoid arthritis consume large quantities of analgesics, especially salicylates. The incidence of papillary necrosis and interstitial nephritis in necropsy and renal biopsy surveys in patients with rheumatoid arthritis is high. The incidence in different reports has varied from 21.3% to 28.1% (Clausen and Pedersen, 1961; Brun *et al.*, 1965; Lawson and Maclean, 1966; Bulger *et al.*, 1968). In a necropsy survey conducted by us at the Royal Melbourne Hospital a 100% incidence of papillary necrosis was found in eight patients coming to necropsy during the one-year period of survey

(Nanra *et al.*, 1970). This very high incidence of papillary necrosis in rheumatoid arthritis in our hospital has been further confirmed in another nine patients coming to necropsy in the following year.

The role of caffeine in analgesic nephropathy with A.P.C. mixtures is not clear. The suggestion in the present study that caffeine may reduce the nephrotoxicity of aspirin may be explained on the basis of its diuretic action (Mudge, 1965). On the other hand there is both clinical and experimental evidence to indicate that caffeine itself is nephrotoxic (Vinci, 1914; Wendt, 1938; Boyd, 1959; Boyd *et al.*, 1965; Prescott, 1966).

p-chloroacetanilide has been recognized as a contaminant in phenacetin over the past 30 years since it has been manufactured from 4-nitrochlorobenzene. It has been suggested that *p*-chloroacetanilide is the nephrotoxic component in phenacetin (Harvald *et al.*, 1960; Schreiner, 1962). In a 22-week study in rats with *p*-chloroacetanilide, however, Schnitzer *et al.* (1965) found no evidence of nephrotoxicity but observed profound effects in red-cell haemolysis and production of sulph-haemoglobinaemia and methaemoglobinaemia. Our results in animals with A.P.C. mixtures containing high and low impurity make it unlikely that *p*-chloroacetanilide is directly related to the production of papillary necrosis.

Dehydration has been incriminated as a predisposing factor in papillary necrosis (Kincaid-Smith, 1968; Saker and Kincaid-Smith, 1969; Nanra *et al.*, 1970), and experimental papillary necrosis with serum and vinylamine can be prevented by maintaining high urine flow rates by a previous administration of mannitol (Ljungquist and Richardson, 1966; Fuwa and Waugh, 1968). These findings suggest that the concentrating mechanism in the renal medulla probably plays an important part in the genesis of papillary necrosis. A constant water diuresis in our study has afforded some protection to the renal medulla. The apparent protective effect of administration of acetazolamide and sodium bicarbonate may be explained on the basis of increased clearance of aspirin by suppression of nonionic diffusion in the presence of an alkaline urine (Woodbury, 1965). The protection afforded to the kidney by increased urine flow rates and augmented clearance of aspirin may have important clinical applications in the prophylaxis and management of analgesic nephropathy.

Medullary ischaemia has been incriminated in the pathogenesis of papillary necrosis (Kincaid-Smith, 1968; Nanra *et al.*, 1970), and the medulla seems to be particularly liable to damage by ischaemia (Sheehan and Davis, 1959). Renal papillary necrosis has been found in children at necropsy following severe shock (Bernstein and Meyer, 1961), and we have often noted the association of an acute episode of papillary necrosis after a bout of haematemesis or severe dehydration in patients who abuse analgesics. An ischaemic infarct of the renal medulla may be explained by the fact that medullary blood flow is not controlled by autoregulation (Thurau, 1964), and severe medullary ischaemia would therefore follow systemic hypotension. The higher incidence of papillary necrosis in our animals with shock appears to be consistent with these observations, but the results in our study have not been conclusive.

RANJIT S. NANRA, M.B., B.S., M.R.A.C.P.,
Assistant Physician, Medical Renal Unit,
Royal Melbourne Hospital.

PRISCILLA KINCAID-SMITH,* M.D., F.R.C.P., F.R.A.C.P.,
Reader in Medicine, University of Melbourne;
Physician-in-Charge, Medical Renal Unit,
Royal Melbourne Hospital.

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