Tetracycline Toxicity in Haemoglobinuria

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One of the important advantages of tetracycline as a therapeutic agent is its relative non-toxicity. The organs in which tetracycline accumulates in large amounts are the liver and kidney (Böttinger, 1955 ; Carroll and Tapp, 1965), and if there were any toxic effects they would be expected to be manifested in these organs. Though it has been reported that high doses of tetracycline given by intravenous injection may result in liver damage in humans (Lepper *et al.*, 1951), and that tetracycline increases nitrogen retention in patients with renal failure, there have been no reports of histological changes in the kidney due to tetracycline. Recently, during experiments to study the effects of various toxic agents and ischaemia on rats with haemoglobinuria due to the intravenous administration of a solution of haemoglobin (Lowe, 1965), it was found that tetracycline causes necrosis of proximal tubules in the kidneys of these animals. This work has now been extended and forms the basis of the present report.

Materials and Methods

The experiments were carried out on a total of 50 female rats of the Wistar strain weighing between 200 and 250 g. They were fed on a standard diet and given water *ad libitum*.

Tetracycline Administration.—Tetracycline was administered in doses of 15, 30, or 60 mg./100 g. body weight by either intravenous or intraperitoneal injection.

Haemoglobin Administration.—A solution of human haemoglobin was made up in the following manner. Red corpuscles were separated from the plasma by centrifugation and washed three times in isotonic saline. They were then suspended in isotonic saline and disrupted in an ultrasonic disintegrator. The resulting solution was centrifuged at 2,000 r.p.m. at 4° C. for 10 minutes to remove any fine particulate matter. The final concentration of the haemoglobin solution was 30 g./100 ml. The solution was administered to the rats by intravenous injection in a dose of 1 ml./100 g. body weight.

Experimental Procedures.—The animals were divided into three main groups: the first received Tetracycline alone ; and the second haemoglobin alone ; and the third received haemoglobin first and then Tetracycline five minutes later. Some of the animals died in a few minutes and were discarded. The remainder were killed at 24 hours by an overdose of ether. The kidneys were fixed in neutral formal-saline. Paraffin sections at 5 μ were stained with haematoxylin and eosin, and, in addition, selected sections were stained by Dunn's method for haemoglobin.

Results

Animals Receiving Haemoglobin Alone.—Haemoglobinuria could be detected within two to three minutes of the injection of haemoglobin. It was pronounced for 6 to 12 hours but gradually diminished over the next six hours and the urine was free of haemoglobin at 24 hours. The kidneys appeared normal macroscopically. In histological sections there were occasional haemoglobin casts in the distal convoluted tubules and collecting tubules, but no necrosis was seen in the epithelium of these tubules. No other change was observed.

Animals Receiving Tetracycline Alone.—Tetracycline is toxic by intravenous injection, particularly if it is given rapidly. All the animals given 60 mg. intravenously died within a few minutes, but most of those given 15 or 30 mg. intravenously survived. On the other hand, there was no mortality after the intraperitoneal injection of 15, 30, or 60 mg. There were no histological changes in the kidneys in any of the animals.

Animals Receiving Haemoglobin Followed by Tetracycline.—The mortality after the Tetracycline was as described above. The animals surviving were those given 15 or 30 mg. intravenously or 15, 30, or 60 mg. intraperitoneally.

In the animals receiving either 30 or 60 mg. the haemoglobinuria persisted longer than in those which had received haemoglobin alone ; haemoglobin could still be detected in the urine at 24 hours.

When a post-mortem examination was performed on these animals the kidneys were seen to be enlarged and darker than normal. The histological changes in the kidneys were similar in the rats receiving either intraperitoneal or intravenous Tetracycline, although they were slightly more marked in the animals which had received the 60-mg. dose of Tetracycline intraperitoneally. There was necrosis of groups of proximal convoluted tubules. The cytoplasm of the epithelium lining these tubules was shrunken and eosinophilic and the nuclei were pyknotic or had completely disappeared. In some tubules the cells appeared to be breaking away from the basement membranes and forming cellular casts. In other necrotic tubules there were brightly eosinophilic casts which gave a positive stain for haemoglobin. Haemoglobin casts were also seen in the distal convoluted tubules, many of which were grossly dilated, but the cells lining these tubules were not necrotic. The glomeruli were normal. In the medulla there were eosinophilic casts in the collecting tubules. Some of these were cellular and others gave a positive stain for haemoglobin.

In animals given the 15-mg. dose of Tetracycline the haemoglobinuria was not prolonged and no changes could be detected in the kidneys.

Comment

Though neither haemoglobin nor Tetracycline alone produces renal damage, the combination of the two causes patchy necrosis of proximal convoluted tubules. Most commercial tetracycline preparations, including Tetracycline, are composed of 25% by weight tetracycline hydrochloride and 75% by weight ascorbic acid. Further experiments were carried out to investigate which of these substances was significant.

Animals Receiving Haemoglobin Followed by Pure Tetracycline Hydrochloride.—Animals were given haemoglobin as before and then, five minutes later, an intravenous injection of pure tetracycline hydrochloride in a dose of 7.5 mg./100 g. body weight (equivalent to 30 mg. of Tetracycline). The animals were killed 24 hours later. The histological changes in the kidneys were indistinguishable from those in the animals given the combination of haemoglobin and an equivalent dose of Tetracycline.

Animals Receiving Haemoglobin Followed by Ascorbic Acid.—Animals were given haemoglobin as before, and then, five minutes later, an intravenous injection of ascorbic acid in a dose of 22.5 mg./100 g. body weight (equivalent to 30 mg. of

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Effect of Tetracycline on the Excretion of Sodium, Potassium, and Chloride in Rats Loaded Orally with Physiological Saline

Group	No. of Animals	Body Weight (g.)	4-hr. Urine Output Calculated for 100 g. Body Weight (ml.)	4-hr. Output (μ Eq/100 g. Body Weight)			Na/K ratio
				Na	K	Cl	
Control	9	202.2 $\pm$ 21.2*	4.1 $\pm$ 0.4	695 $\pm$ 500	162.3 $\pm$ 18.6	948 $\pm$ 168	4.18 $\pm$ 1.5
Tetracycline	9	193.8 $\pm$ 20.8	6.2 $\pm$ 1.9	968 $\pm$ 308	236.1 $\pm$ 14.6	1,344 $\pm$ 168	4.12 $\pm$ 1.3

* Standard deviation.

Tetracycline). The animals were killed 24 hours later. The findings in these kidneys were similar to those in animals given haemoglobin alone, but the haemoglobin casts in the distal convoluted tubules and collecting tubules were more numerous. It may therefore be concluded that the factor responsible for the renal damage is the tetracycline and not the ascorbic acid.

Discussion

There are two possible mechanisms whereby the necrosis might be produced. In the first instance tetracycline hydrochloride lowers the pH of the urine to about 3, as compared with a pH of 8 in our control rats. Secondly, the haemoglobin may directly sensitize the tubules to the effects of tetracycline.

Role of Acidity in Producing the Lesions

Some workers have reported that renal damage is produced in normal rabbits if haemoglobin is administered to animals with acid urine (Baker and Dodds, 1925). In contrast to this Yuile *et al.* (1945) failed to produce damage in normal rabbits by giving haemoglobin to animals with acid urine. They, however, found that in rabbits with damaged kidneys the haemoglobin casts persisted longer and were more numerous in the acid state. It is possible, therefore, that in our rats the acidity of the tetracycline hydrochloride solution might be an important factor in the production of the renal damage. This is unlikely, since the ascorbic acid solution induced a similar lowering of the pH of the urine and yet did not cause necrosis when administered to the rats with haemoglobinuria. Two additional experiments were carried out to investigate this point further.

In one group the animals were given haemoglobin, and then, five minutes later, an intravenous injection of N/1,000 hydrochloric acid. The volume administered and the acidity of the solution were the same as in the 7.5-mg. dose of tetracycline hydrochloride. The pH of the urine in these animals was lowered by this injection to a level similar to that induced by an intravenous injection of 30 mg. of Tetracycline or 7.5 mg. of tetracycline hydrochloride. No lesions were found in the kidneys.

The second group of rats were given haemoglobin, and then, five minutes later, an intravenous injection of 7.5 mg. of pure tetracycline hydrochloride in 0.1-molar glycine buffer at pH 7–8. Lesions were found in the kidneys of these animals identical with those produced by a similar dose of tetracycline hydrochloride in acid solution.

It would appear from these experiments that the acidosis produced by acid solutions of tetracycline does not play any significant part in the production of the necrosis.

Sensitization of Tubules by Haemoglobin

It has been shown that haemoglobin increases the sensitivity of the tubules to short periods of ischaemia (Carroll *et al.*, 1965). We have, however, no evidence that tetracycline reduces the renal blood flow. The Table shows the urinary output and electrolyte excretion in response to oral administration of 5% of the body weight of physiological saline in normal animals

and in animals which had received an intravenous injection of 10 mg. of tetracycline hydrochloride shortly after the saline. It can be seen that the tetracycline did not impair the diuresis or the excretion of electrolytes. It is unlikely therefore that there is any significant reduction in blood flow or glomerular filtration rate during the four hours of the observation. On the other hand, this experiment does not eliminate the possibility that after tetracycline administration there might be a short-lasting ischaemia which would be sufficient to produce renal damage in the presence of haemoglobin. Carroll *et al.* (1965) have shown that a period of renal ischaemia lasting 10 minutes is enough to produce necrosis of tubules when haemoglobin is present in the kidney.

It has been shown elsewhere (Lowe, 1965) that when nephrotoxins are administered to rats which have haemoglobinuria the necrosis in the kidney is more extensive than that produced by the same dose of the nephrotoxin alone. In addition, nephrotoxins in amounts which do not produce necrosis in the kidney when given alone may do so when they are given with haemoglobin. It seems probable by analogy that tetracycline is a substance whose toxicity for the kidney is revealed only when it is associated with haemoglobinuria.

Antibiotics may be required by patients with haemoglobinuria due to incompatible blood transfusions, haemolytic anaemia, drugs, or infections. The present findings suggest that tetracycline should be used with care in these patients. Although the doses of tetracycline used in the present experiments are higher than those usually recommended for therapeutic purposes, the lowest dose which produces necrosis of renal tubules in rats is in the same range on a weight-for-weight basis as the daily intravenous dose used in the cases described by Schultz *et al.* (1963). These workers reported fatal hepatic necrosis after such daily doses for six days but did not record renal damage in their patients.

Summary

Tetracycline given intravenously to rats in a dose of 7.5 mg./100 g. body weight does not result in damage to the kidney. However, when haemoglobinuria is induced first and then the same dose of tetracycline is given, this causes patchy necrosis of the proximal convoluted tubules. It appears that the haemoglobin sensitizes the tubules to either an ischaemic or a cytotoxic effect of tetracycline. The acidosis which results from the administration of tetracycline does not play any part in producing the necrosis.

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