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EFFECTS OF OXYTETRACYCLINE ON THE MECHANICAL PROPERTIES OF BONE AND SKIN IN YOUNG RATS

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The influence of the tetracycline antibiotics on growing bones has been disputed. In the present study 58 young male rats were given intraperitoneal injections twice a day for 2 weeks; half of the rats received oxytetracycline and the other half placebo. The concentration of oxytetracycline in plasma was comparable with therapeutic levels in man. Compared with the control animals the oxytetracycline rats had, at the end of the medication period, a significantly lower weight (7 per cent), shorter bones (1–2 per cent), lower bending strength of both the tibia diaphysis (9 per cent) and the distal femur metaphysis (22 per cent) and even lower tensile strength of intact (17 per cent) and sutured (27 per cent) skin. The effect of oxytetracycline seems to be reversible as no differences between the two groups could be detected 1–3 weeks after the end of medication.

Key words: bones; mechanical properties; oxytetracycline; plasma concentration; rats; skin; tensile strength; tetracycline

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It is well established that tetracycline antibiotics are deposited in developing teeth, leading to discolouration and enamel hypoplasia. These antibiotics are also known to deposit in growing bones, but their effect on bones is disputed. Reduced bone growth (Cohlan et al. 1963, Yen & Shaw 1972) and increased bone fragility (Gudmundson 1971) have been observed, but there have also been reports of no impairment of growing bones by the tetracyclines (Chu et al. 1963, Gruden 1973). To our knowledge, the effect of these antibiotics on the mechanical properties of skin has so far not been investigated.

The purpose of the present study in rats was to assess the effect of oxytetracycline on:

1. Weight gain and longitudinal bone growth.
2. Mechanical properties of a) Bones, b) Wounded and intact skin.

MATERIALS AND METHODS

Fifty-eight outbred male Wistar/Af/Han/Mol SPF rats were used. At the start of the experiments the rats were 20 to 21 days old, weighing from 32 to 37 g. Five animals were kept in each cage and given water and Norwegian standard diet for rats *ad libitum*. The rats were divided in two weight-matched groups of 29 animals, one oxytetracycline treated and the other control.

The oxytetracycline treated rats received 2.8 mg oxytetracycline (Terramycin® Intravenous, Pfizer) in 0.5 ml water as intraperitoneal injections every twelfth hour for 14 days. For practical reasons the amount of medicine given to each animal was constant throughout the medication period. In the middle of the period 49 mg/kg was given twice a day. The control animals received corresponding injections of the vehicle.

The oxytetracycline concentrations were determined by the paperdisc method of AB-biodisk (Stockholm, Sweden) utilizing *Bacillus cereus* (ATCC 11778) as test organism and

antibiotic medium No. 1, BPSA (Difco) (Jalling et al. 1972).

On the first day of medication the rats were operated in aseptic conditions under ether anaesthesia. A 3.5 cm long incision was made through the skin down to the fascial layer on the back, 1 cm to the left and parallel with the caudal part of the spine. The wounds were closed by three interrupted sutures (4–0 Vicryl® Ethicon) which were removed after 1 week. Wound dressing was not used and no wound became infected.

Two, three and five weeks after the start of the experiment nine or ten rats from each group were killed with ether. Immediately after death the femora and tibiae were dissected free. They were kept in isotonic solution and tested within 2 hours. The bending strength of the distal right femur metaphysis was measured 3 mm proximal to the epiphyseal plate as described in another paper (Engesaeter et al. 1978). The strength in the middle of the right tibia was tested by deflecting the proximal end of the tibia laterally relative to the distal end, principally in the same way as described for the femur diaphysis. The left femur and tibia will not be discussed in this paper as they were used for biochemical investigations, which will be reported in a separate paper.

Skin was removed from the back of the rat, from the left side a piece (about 3×5 cm^2) containing the healing incision and from the right side a corresponding piece with intact skin (Figure 1). The skin was frozen in Petri dishes at minus 20°C in a natural stretched condition for 3–14 days before tensile testing. By means of a special template (Figure 1) and scalpel the skin pieces were cut, in the frozen state, into appropriate specimens. The template has four main parallel clefts (30 mm long and 10 mm apart), between each there are two shorter parallel clefts (7 mm long and 3 mm apart). The wounded skin was cut into three 10 mm wide strips with the wound positioned in the centre (Figure 1). The three intact skin specimens were made with a 3 mm wide central narrowing to prevent rupture at the clamps and curling when tested (Figure 1).

To measure the tensile strength each end of the skin specimen was fixed by a clamp with a 10 mm distance between the two clamps (Figure 1). The specimens with the clamps were then mounted in the same tensile machine as used for the bone testing. The elongation rate in the tensile measurements was constant, 0.125 mm/mm/s.

In addition to these 58 rats described, six animals were killed at the start of the experiment to obtain origin values for bone lengths and tensile strength of intact skin. The mechanical properties of the bones, however, could not be assessed as the bones were too short to fit in the test apparatus.

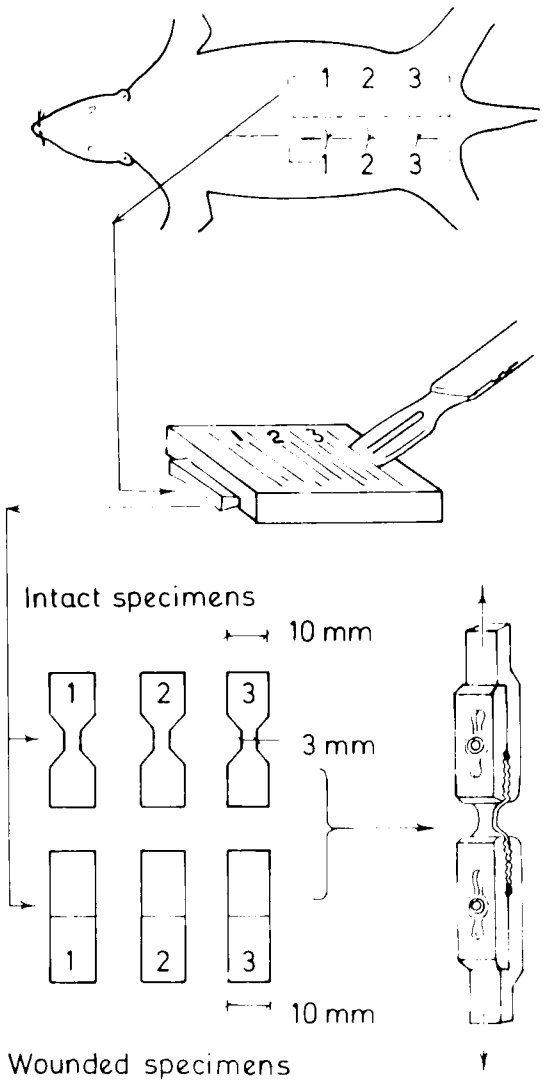

Figure 1. Tensile strength testing of the skin. Skin from the back of the rat is used, wounded from the left and intact skin from the right side. With a special template the skin is cut into standardized test specimens, which are fixed by clamps and pulled to rupture in the tensile machine.

The median with 25- and 75-fractiles is used as an expression for the average and the dispersion of the measured values. Statistical significance was evaluated by the Wilcoxon test for two samples (one-tailed test) and differences were considered significant if $P \leqslant 0.05$ (Diem & Lentner 1975).

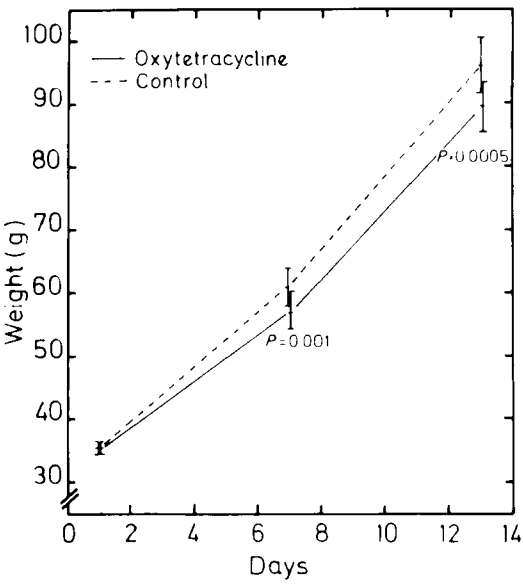

Figure 2. The weight gain in the oxytetracycline and in the control rats in the medication period. (Median with 25- and 75-fractiles.)

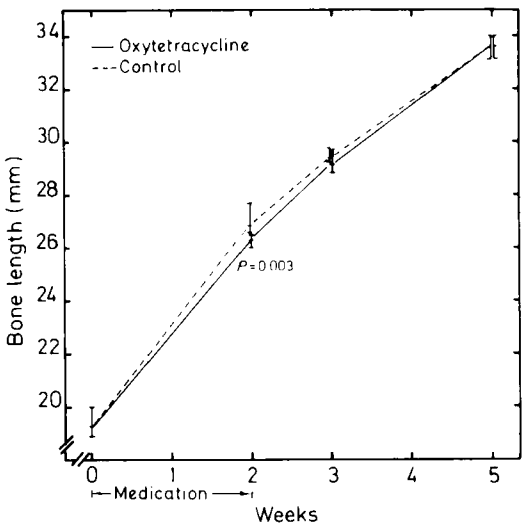

Figure 3. The longitudinal growth of the right tibia in the oxytetracycline and in the control rats. (Median with 25- and 75-fractiles.)

RESULTS

On the fifth and twelfth day of medication the plasma concentration of oxytetracycline was measured 2, 6 and 12 hours after the intraperitoneal injections. The results are shown in Table 1.

The weight of the rats increased six times in this 5-week experiment, from 35 to 215 g. During the first 2 weeks, i.e., in the medication period, the growth was, however, significantly lower for the oxytetracycline than for the control rats (Figure 2). In the last 3 weeks of the experiment there was no difference in weight between the two groups, as the oxytetracycline rats caught up with the control rats a few days after the end of the medication period.

Principally the same experience was gained when studying the length of the bones. At the end of the medication period both tibia and femur were significantly shorter in the oxytetracycline than in the control group. No difference was found after 3 and 5 weeks (Figure 3).

A corresponding difference was found in the bending strength of the bones in the two groups. The distal femur metaphysis of the

Table 1. The plasma concentration of oxytetracycline on the fifth day (5 rats) and on the twelfth day (4 rats) of the medication. (Median with 25- and 75-fractiles.)

Day of medication	Dose mg/kg/12h	Plasma concentration (μg/ml)		
		2h after injection	6h after injection	12h after injection
5th	51	8.0 (8.0–9.3)	2.4 (1.9–2.6)	< 1.0
12th	31	6.0 (5.3–6.8)	0.8 (0–1.6)	< 1.0

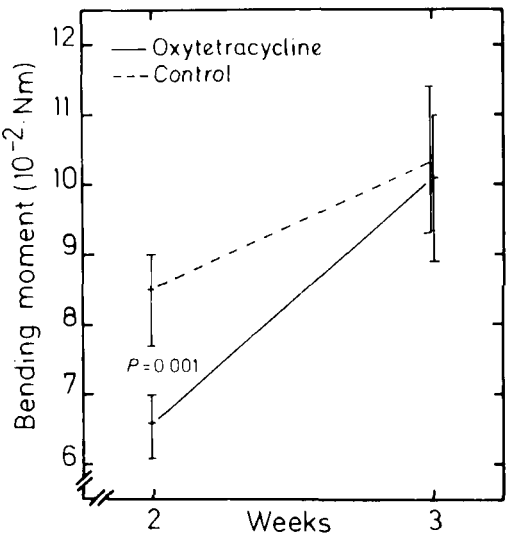

Figure 4. The bending moment necessary to produce fracture in the distal right femur metaphysis (3 mm proximal to the epiphyseal plate) in the oxytetracycline and in the control rats. (Median with 25- and 75-fractiles.)

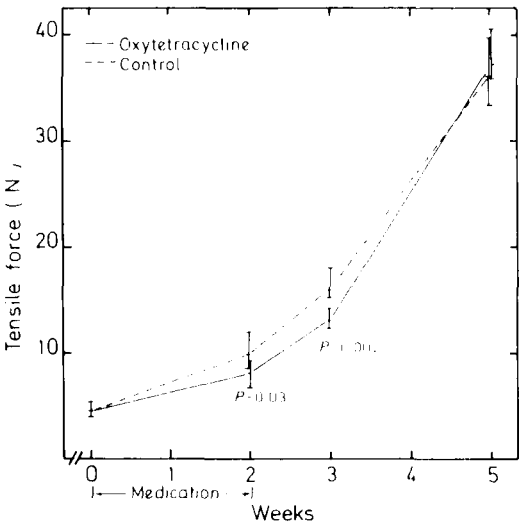

Figure 5. The tensile force necessary to pull apart the intact skin specimens from the oxytetracycline and from the control rats. (Median with 25- and 75-fractiles.)

oxytetracycline rats was 22 per cent weaker than the metaphysis of the control rats at the end of medication, while no difference was observed later (Figure 4). After 2 weeks the bending strength in the middle of the tibia was also significantly less (9 per cent) in the oxytetracycline than in the control group, and once again there was no difference after 3 and 5 weeks.

Oxytetracycline was also found to influence the mechanical properties of skin. The tensile strength of sutured skin specimens was 27 per cent less ($P=0.02$) in the oxytetracycline than in the control animals at the end of medication. Also this parameter was equal in the two groups 1 and 3 weeks later. The intact skin specimens from the oxytetracycline group were about 17 per cent weaker than the specimens from the control group both at 2 and 3 weeks, but no difference was found at 5 weeks (Figure 5).

DISCUSSION

In the present study oxytetracycline medication caused reduced weight gain, reduced longitudinal bone growth, increased bone fragility and reduced skin tensile strength.

The oxytetracycline dosage may perhaps seem to be a point of contention in this investigation. The amount given in mg/kg day is true enough two to five times higher than the recommended maximum human dose (30 mg/kg/day). The plasma concentration is, however, by no means above human therapeutic levels. A single intravenous injection of 7.5 mg/kg oxytetracycline in man gives serum concentrations after 1–2 hours of 6–10 μg/ml, after 5–7 hours 4–5 μg/ml and after 11–12 hours still 2–3 μg/ml (Otten et al. 1975). These concentrations are comparable to those in the present experiment and as 7.5 mg/kg is well within the recommended human dosage, the concentrations in the present study correspond to human therapeutic levels. Comparing the dosage in the present investigation with that in others, however, is difficult as the medicine is administered in different ways and the plasma concentration of tetracycline is reported in few publications.

The influence of oxytetracycline in the present study seems to be reversible as no difference in growth or in strength could be

detected 1 week after the cessation of medication, except for the tensile strength of intact skin from the oxytetracycline rats. This parameter was, however, equal in the two groups 3 weeks after the end of medication. Reversible inhibition of bone growth of tetracycline was also reported by Cohlan et al. (1963) in premature infants and by Yen & Shaw (1973) in young monkeys. Gudmundson (1971), however, observed increased fragility of rat femur and tibia for 90 days after only 1 week of intramuscular injections of oxytetracycline (10 mg/kg/day).

The possibility may be considered that the reduced growth observed in the oxytetracycline rats during the medication was a consequence of impaired intestinal function, caused either by disturbance of intestinal flora or by an inflammatory reaction in the peritoneal cavity after the oxytetracycline injections. In accordance with the latter, at the end of medication, the peritoneum was found to be more reddish in the oxytetracycline than in the control rats, but no signs of inflammation were observed later in either group. However, Cohlan (1963) and Yen & Shaw (1972) also reported reduced growth and they did not use intraperitoneal administration of the medicine.

At the end of medication the femur and tibia in the oxytetracycline rats were shorter – 1 and 2 per cent, respectively – than the bones in the control rats, while the bending strength of the tibia diaphysis was reduced by 9 per cent and that of the distal femur metaphysis by 22 per cent compared with the bones of the control animals. This may indicate that the bone strength is reduced to a greater extent than the longitudinal bone growth. Why then should the reduction in bone strength be more pronounced in the femur metaphysis than in the tibia diaphysis? The explanation may be found in the mode of growth of the long bones. As the longitudinal growth of both tibia and femur takes place mainly in the epiphyseal plates near the knee and as the femur growth during the medication was about 6mm, all the tested metaphyseal bone material (3 mm proximal to the epiphyseal plate) was produced under the influence of oxytetracycline. In the tibia diaphysis, however, only parts of the tested bone material were made under oxytetracycline influence, viz., the shell of periosteum derived bone. If oxytetracycline really causes production of a weaker bone material, it would be expected that the metaphyseal bone would be more affected than the diaphyseal bone, as the former is entirely produced under the influence of oxytetracycline while the latter only partly.

Also the reduction in the tensile strength of both intact and sutured skin (17 and 27 per cent, respectively) from the oxytetracycline rats appears to be greater than the general growth inhibition (weight difference of 9 per cent in the two groups) can explain.

In what way the oxytetracycline impairs the mechanical properties of both bone and skin remains unanswered. As collagen is the dominant organic component both in bone and skin, the influence of oxytetracycline on the biosynthesis and maturation of collagen seems extremely interesting in this context.

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