SHORT COMMUNICATION

06.84.23

Prevention of the toxic effect of uranium on bone formation by tetracycline

R.L. CABRINI, M.B. GULIELMOTTI and A.M. UBIOS

Departamento de Radiobiología, Comisión Nacional de Energía Atómica y Cátedra de Anatomía Patológica, Facultad de Odontología, Universidad de Buenos Aires, Argentina.

Received: June, 21, 1984
Accepted: August, 29, 1984
Key words: bone formation - uranium tetracycline - histometry - bone morphometry.

In a previous experiment we demonstrated the inhibitory effect of uranium on bone formation (1). The healing of alveoli after tooth extractions is impaired by uranium intoxication; only a small amount of bone was formed in their fundus. Tetracycline (TC) was used as a marker in intoxicated and non-intoxicated animals with the aim to evaluate the rate of bone formation in alveolar wound healing. Surprisingly TC reverted the toxic effect of uranium on bone formation.

The three left mandibular molars were extracted from 60 male Wistar rats weighing 90 g under sodium pentobarbital anaesthesia, 0.025 g/kg of body weight intraperitoneal (IP). The animals were divided up into 4 groups: Group I (10 animals) received no further treatment; Group II (10 animals) received an IP injection of 2 mg/kg of body weight of uranyl nitrate dissolved in buffer trismaleate pH 7.2 (0.02% w/v) immediately after the extractions; Group III (20 animals) received identical treatment to that of Group II coupled with 3 IP injections of tetracycline chlorhydrate (50 mg/kg of body weight) given on the 1st, 7th and 14th day post-extractions; Group IV (20 animals) received IP injections of tetracycline chlorhydrate (50 mg/kg of body weight) at the same times as for Group III.

All animals were killed 17 days after the extractions by ether inhalation. Their left jaws were fixed in 10% formaline and roentgengraphed on mammagraphic film. Ten jaws from Group III and 10 from Group IV were embedded in methyl-metacrilate. Bucco-lingual sections of the first molar messial socket were obtained in a Jung K microtome and examined in a fluorescence microscope. The rest of the jaws were decalcified in nitric acid, embedded in paraffin, sectioned at the level of the first molar messial socket in a bucco-lingual orientation and stained with hematoxylin and eosin.

Total alveolar volume, and volume density of bone in the apical third of the sockets (2) were established by means of standard stereological methods (3). The determinations were performed on tracings obtained from projections of the sections (4) using an image analysing system (Kontron Messgeräte MOP-AM-03).

Radiographic images of Group I, III and IV were similar; they exhibited the edentate alveolar ridge almost completely occupied by homogeneous radiopaque tissue. Only the animals from Group II showed a radioluscent image of their sockets.

Similar histologic features were observed in Groups I, III and IV: sockets were almost totally filled by reticular bone. Trabecular surfaces exhibited intense bone activity whereas the wound surface was completely covered by new epithelium. The alveoli of Group II were almost totally occupied by granulation tissue. Only in the apical fundus newly formed woven trabeculae were seen. In addition, no differences were observed in TC labeling images between intoxicated and non intoxicated animals.

Histometric values of total alveolar volume and volume density of bone in the apical third of the sockets are presented in Table 1.

These results reveal that TC prevented the inhibitory effect on bone formation in alveolar wound

TABLE 1: HISTOMETRIC VALUES OF ALVEOLAR WOUND HEALING

	Group I	Group II	Group III	Group IV
Total alveolar volume	3,400 μm^2 ± 180	1,500 μm^2 ± 800	4,124 μm^2 ± 424	3,979 μm^2 ± 535
Alveolar volume in the apical third	0.40 ± 0.06	0.26 ± 0.04	0.45 ± 0.02	0.42 ± 0.04

± S.D.
$p < 0.01$ between groups I-III-IV and group II (Duncan's test).

healing caused by an acute intoxication with uranyl nitrate (1). In intoxicated animals a small amount of new bone only appeared in the alveolar fundus. This fact suggests that the inhibitory effect of uranium on bone formation could be due to cell damage at osteoblastic or preosteoblastic level. Cell alterations by uranium intoxication were reported in kidney (5) and in skin (6). In skin, uranium was detected both on cell membrane and in the cytoplasm (6). This fact indicated that further consequences could be prevented if the penetration of the metal into the cells were avoided. Osteoblast participati in uranium transport is probable since it is well known that bone is the critical organ in chronic exposures to uranium (7-8). Uranium was detected autoradiographically as "hot spots" in sites of active bone formation (9).

In the present experiment no differences in bone formation between intoxicated and non-intoxicated animals were detected after TC injections. Taking into account that TC is capable of forming chelats with heavy metals, the formation of a chelat uranium-TC is feasible. If this were so and the chelat is formed in the extracellular compartment, it could be eliminated in the urine (10) thus preventing the passage of uranium through the cell which in turn allows bone formation to progress normally.

Since TC label was observed in the bone of both normal and intoxicated animals the chelat TC-uranium may pass through the cell without damaging it or uranium may compete with calcium to form chelats and the chelat uranium-TC would be eliminated in the urine.

The preventive effect of TC in uranium intoxication is especially important in connection with people directly involved in the handling of uranium compounds. Further studies are necessary to determine the exact pathway of uranium when TC is applied.

Acnowledgements

This work was supported in part by Grant N° 8461 e/83 of the National Research Council Argentina.

The tetracycline chlorhydrate was kindly supplied by Bernabo & Cia.

The authors wish to acknowledge Dr. M.J. Piloni's help in obtaining metacrilate sections and Mi(P. Mandalunis' excellent technical assistance. It is also acknowledge Dr. R.L. Macchi's assistance with the statistical analysis, is also acknow ledged.

References

1. GUGLIELMOTTI M.B., UBIOS A.M. de, REY B.M., CABRINI R.L. Effects of acute intoxication with uranyl nitrate in bone formation. Experientia. (1984) 40: 474-476.
2. GUGLIELMOTTI M.B., CABRINI R.L. Alveolar wound healing and ridge remodelling after tooth extractions in rats. A histologic, radiographic and histometric study. J. Oral Maxillofac. Surg. (In press).
3. WEIBEL E.R., ELIAS H. Introduction to stereology and morphology. In: Quantitative methods in morphology. (1967) pp. 3-19, Springer Verlag, Berlin, Heidelberg.
4. ITOIZ M.E., CARRANZA (Jr.) F.A., CABRINI R.L. Histologic and histometric study of occlusal trauma in rats. J. Periodont. (1963) 35: 305-314.
5. HALEY D.P. Morphologic changes in uranyl nitrate induced renal failure in saline- and water-drinking rats. Lab. Invest. (1982) 46: 196-208.
6. DE REY B.M., LANFRANCHI H.E., CABRINI R.L. Percutaneous absorption of uranium compounds. Env. Res. (1983) 30: 480-491.

7. NEUMAN W.F., NEUMAN M.W., MURYAN B.J. The deposition of uranium in bone. I Animal studies. J. Biol. Chem. (1984) **173**: 705-709.
8. ADAMS N., SPOOR N.L. Kidney and bone retention functions in the human metabolism or uranium. Phys. Med. Biol. (1974) **19**: 460-471.
9. NEUMAN M.W., NEUMAN W.F. The deposition of uranium in bone. II Radioautographic studies. J. Biol. Chem. (1948) **173**: 711-716.
10. Council on Dental Therapeutics of the American Dental Association in: Accepted Dental Terapeutics. Tetracyclines. (1982) pp. 246-247, 39th Ed., Chicago.