Effects of methylmercury chloride on rat incisor odontoblasts and dentinogenesis

Jon Einar Dahl
Department of Pathology, Dental Faculty, University of Oslo, Oslo, Norway

Dahl JE. Effects of methylmercury chloride on rat incisor odontoblasts and dentinogenesis. Acta Odontol Scand 1984;42:251–255. Oslo. ISSN 0001–6357.

Methylmercury chloride, a well-known nerve cell toxicant, was administered to 40 rats in 2 groups, in doses of 10 mg/kg and 20 mg/kg. Histomorphologic investigation of maxillary incisors after 2 and 4 days revealed granular and hydropic changes in parts of the odontoblastema. Deposition of interglobular dentin was seen after 7 and 11 days. The most vulnerable cells seemed to be the mantle dentin-producing odontoblasts, and the alterations found are probably due to interference with the rough endoplasmic reticulum. □ *Endoplasmic reticulum; interglobular dentin; mantle dentin; morphology*

Jon Einar Dahl, Department of Pathology, Dental Faculty, University of Oslo, Box 1052, Blindern, N-0316 Oslo 3, Norway

The interference of some cytotoxic agents with the morphology and function of rat incisor odontoblasts has previously been described (1–10). The effects seen comprise alteration of cell shape, arrested mitoses, necrosis, and modified cellular function manifested as diminished or irregular dentin deposition. All the agents hitherto used, however, have a special affinity for the dividing cell.

Organomercurial compounds such as alkyl- and methyl-mercury have been widely used in agriculture as fungicidal seed dressing, by the paper industry to prevent slime formation, and as catalysts in chemical processes (11–13). The toxicity of organomercurials has been well documented, both by accidental poisoning in man and in experimental animal models. In man, sensory and motoric dysfunction including paresthesia, constriction of the visual field, impairment of fine coordination, and ataxia have been described (11–14). Similar neurological disturbances are found after administration of organomercurial compounds in the rat, rabbit, mink, and cat (15–18). Histomorphologically, the earliest signs of experimental intoxication are dispersion of rough endoplasmic reticulum in neurons (19), followed by diminished protein synthesis in the same cells (20). Later, degenerative and necrotic changes are seen, especially in primary sensory ganglion cells and in Purkinje and granule cells of the cerebellum (15, 18).

The purpose of the present study was to identify possible effects of methylmercury chloride on rat incisor odontoblast and dentin deposition, in order to elucidate further the applicability of the rat incisor as a toxicologic test organ.

Materials and methods

Fifty-two female Wistar rats with a mean weight of 194 g were divided into two experimental groups consisting of 20 animals each; 12 animals served as controls. The experimental animals received a single injection of methylmercury chloride dissolved in 5 mM Na_2CO_2, pH adjusted to 7.4, in the tail vein under ether anesthesia. The doses given were 10 mg/kg and 20 mg/kg in groups I and II, respectively. The injected volume was 1.0 ml/200 g. The control animals received the equivalent volume of pure diluting solution.

Five animals from each experimental group and three animals from the control

group were decapitated under ether anesthesia after 2, 4, 7, and 11 days. The heads were divided by a median incision, and the maxillae were freed from soft tissue and immediately fixed in 4% aqueous formaldehyde. After fixation, the specimens were demineralized in an aqueous solution prepared from equal amounts of 44% formic acid and 20% sodium citrate and embedded in paraffin. Sixty to ninety 5-μm-thick longitudinal sections were cut from each maxillary right incisor and stained with hematoxylin–eosin. The sections were investigated by light microscopy.

Results

Two days after administration of methylmercury chloride changes were observed in the mantle dentin-producing odontoblasts (Fig. 1). In group I (10 mg/kg) the cells were slightly elongated and granular, with signs of intracellular edema. The highest dose (20 mg/kg) produced marked swelling and hydropic changes of the same cells (Fig. 2). After 4 days the odontoblastema of animals in group I appeared as in controls. In group II an area of pale, granular and swollen odontoblasts was seen incisally to the mantle dentin-producing cells (Figs. 1 and 3). In neither group were dentinal alterations found.

After 7 and 11 days both dentin and odontoblasts in group I animals appeared as in the controls. However, a zone of irregular, globularly mineralized dentin was seen pulpally to the normal dentin in the group II animals. An incremental line separated the irregularly and normally mineralized dentin (Fig. 4). The localization of the irregular dentin at 7 days was incisal to the areas of changes observed after 4 days and was even more incisal 11 days after injection (Fig. 1). The odontoblasts appeared as in the controls (Fig. 4).

No animals exhibited clinical symptoms of methylmercury intoxication.

Discussion

With the doses applied, methylmercury chloride produced altered morphology of the mantle dentin-producing odontoblasts. This was the only dearrangement caused by the lowest dose (10 mg/kg) and was seen after 2 days. In group II similar changes were observed after 2 days, whereas after 4 days the changes were located in more incisally positioned odontoblasts. In the nerve cells of rats dosed with methylmercury chloride (7.5 mg/kg/day), ribosomes were lost from the rough endoplasmic reticulum, accompanied by the accumulation of dispersed granular material. These early

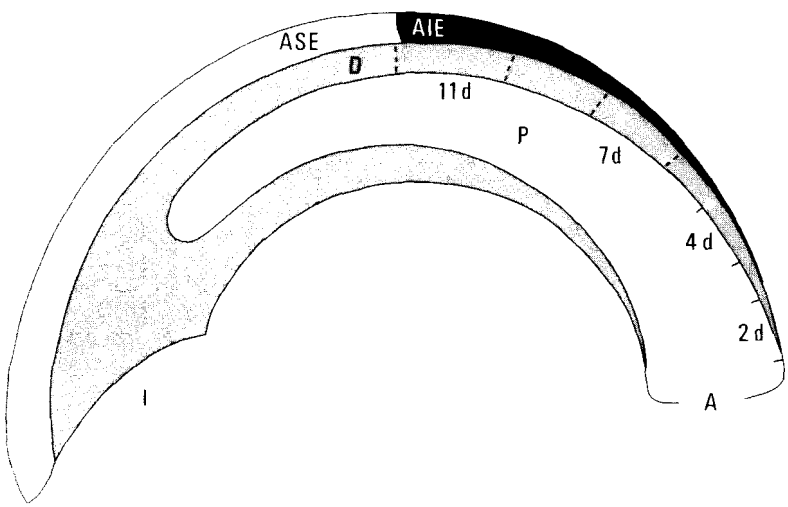

Fig. 1. Schematic presentation of the maxillary rat incisor and the localization of the lesions in the odontoblasts after 2 days (2d) and 4 days (4d) and the in dentin (D) after 7 days (7d) and 11 days (11d). AIE = acid-insoluble enamel; ASE = acid-soluble enamel; D = dentin; P = pulp; I = incisally; A = apically.

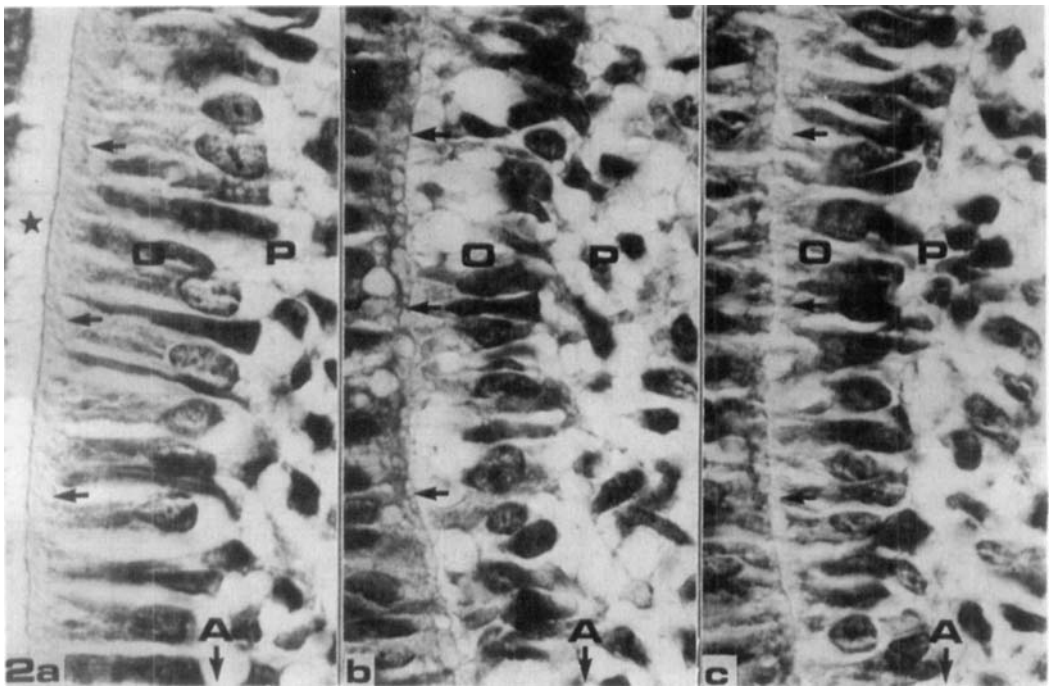

Fig. 2. The mantle dentin-producing odontoblast 2 days after injection of methylmercury chloride in doses of 10 mg/kg (2a), 20 mg/kg (2b), and of diluting solution (controls) (2c). Slightly elongated, granular and swollen odontoblasts (O) in 2a and marked hydrophic changes of odontoblasts (O) in 2b. P = pulp cells; A = apically; arrows pointing at mantle dentin. The star indicates an artefact. (Hematoxylin and eosin; ×800.)

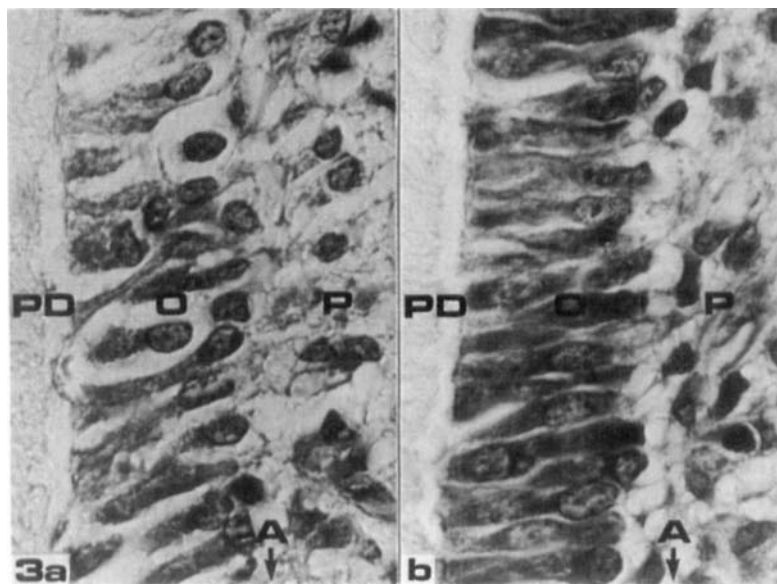

Fig. 3. Marked hydropic changes in pale and granular odontoblasts (O) 4 days after injection of methylmercury chloride in a dose of 20 mg/kg (3a), compared with controls (3b). PD = predentin; P = pulp cells; A = apically. (Hematoxylin and eosin; ×800.)

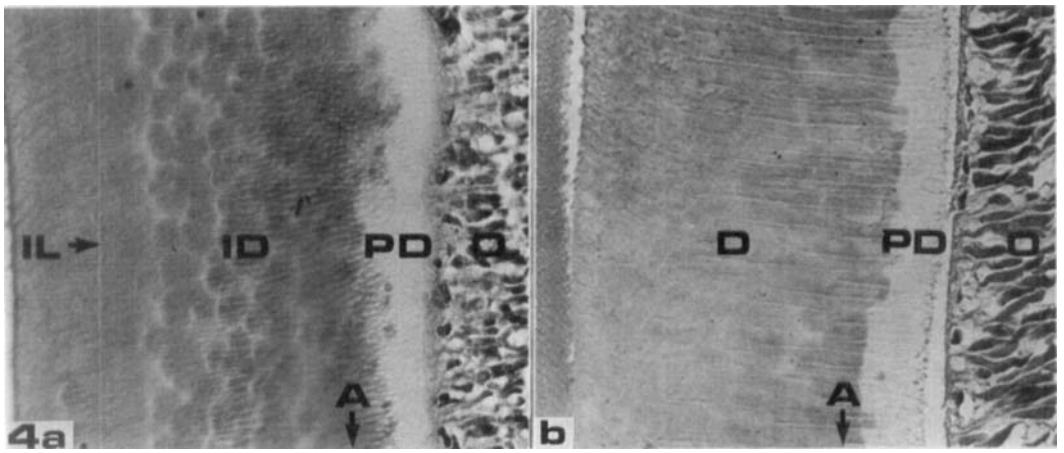

Fig. 4. Irregular mineralized dentin (ID) in longitudinal sections from rats decapitated 7 days after administration of methylmercury chloride (20 mg/kg) (4a) compared with normal dentin (D) in control animals (4b). IL = incremental line; PD = predentin; O = odontoblasts; A = apically. (Hematoxylin and eosin; ×195.)

changes were seen after 2 days of administration and are believed to be reversible (19). The mantle dentin-producing odontoblasts are nondividing and metabolically highly active cells (21, 22), thus resembling nerve cells. The marked transitory granular appearance of these odontoblasts might indicate degranulation of rough endoplasmic reticulum, followed by a resynthesis of these organelles.

Hydropic alterations are also seen in rat incisor odontoblast after administration of vincristine but are regarded as unspecific (9). However, swelling of kidney tubule cells and of neurons is reported as specific after methylmercury intoxication (16, 18) and is probably due to breakdown of cytoplasmic proteins (23).

After 7 and 11 days irregular mineralized dentin was found in group II animals. Assuming an unimpaired growth of the incisors—that is, about 2.1 mm/week (24)—this lesion corresponded to the dearrangement seen in the mantle dentin-producing odontoblasts after 2 days (Fig. 1). The histomorphologically normal deposited predentin that later mineralized globularly (interglobular dentin) reflects alteration in the mineralization process (25). According to the epitactic nucleation theory of mineralization, a specific (macro)molecule should be present in the predentin to initiate calcification (26). The suggested transitory dearrangements of rough endoplasmic reticulum might well cause alterations in the composition of secreted protein matrix, leading to inferior mineralization. The incremental line preceding the interglobular dentin is often seen and is caused by transitory interference with the dentinal apparatus (27).

In conclusion, methylmercury chloride seems to attack young, mantle dentin-producing odontoblasts, as manifested by altered morphology of these cells and later by formation of interglobular dentin. The mechanism of action is probably an interference with rough endoplasmic reticulum, leading to disturbances in the mineralization process. The effect, however, was less pronounced than after the administration of microtubuli-disrupting agents (28).

References

1. Koppang HS. Studies on the radiosensitivity of the rat incisor. Scand J Dent Res 1967;75:413–50.
2. Koppang HS. Histomorphologic investigations on the effect of cyclophosphamide on dentinogenesis of the rat incisor. Scand J Dent Res 1973;81:383–96.
3. Mettner W, Pliess G. Wirkungen der Cytostatica

auf die Zahnentwicklung der Albinoratte. I. Mitteilung. Frankfurt Z Pathol 1956;67:96–108.
4. Mikkelsen HB. Acute and protracted effects of vinblastine on ameloblasts and dentinogenesis in rat incisors. Scand J Dent Res 1978;86:313–24.
5. Nogueira TO, Stene T, Koppang HS. Colchicine's effects on rat incisor odontoblasts and dentinogenesis. Scand J Dent Res 1981;89:48–58.
6. Pliess G. Wirkungen der Cytostatica auf die Zahnentwicklung der Albinoratte. III. Mitteilung. Frankfurt Z Pathol 1958;69:437–51.
7. Pliess G. Wirkungen der Cytostatica auf die Zahnentwicklung der Albinoratte. IV. Mitteilung. Frankfurt Z Pathol 1959;70:621–32.
8. Pliess G, Baren F. Wirkungen der Cytostatica auf die Zahnentwicklung der Albinoratte. II. Mitteilung. Frankfurt Z Pathol 1957;68:622–32.
9. Stene T. Effect of vincristine on odontoblasts in rat incisor. Scand J Dent Res 1978;86:346–56.
10. Stene T, Koppang HS. The effect of vincristine on dentinogenesis in the rat incisor. Scand J Dent Res 1976;84:342–4.
11. Bakir F, Damluji SF, Amin-Zaki L, et al. Methylmercury poisoning in Iraq. Science 1973;181:230–41.
12. Hunter D, Bomford RR, Rusel DS. Poisoning by methylmercury compounds. J Med 1940;9:193–213.
13. Takeuchi T, Morikawa N, Matsumoto H, Schiraishi Y. A pathological study of Minimata disease in Japan. Acta Neuropathol (Berl) 1962;2:40–67.
14. Weiss B, Doherty RA. Methylmercury poisoning. Teratology 1975;12:311–14.
15. Carmichael N, Cavanagh JB, Rodda RA. Some effects of methylmercury salts on rabbit nervous system. Acta Neuropathol (Berl) 1975;32:115–25.
16. Davies TS, Nielsen SW. Pathology of subacute methylmercurialism in cats. Am J Vet Res 1977; 38:59–67.
17. Somjen GG, Herman SP, Klein R, et al. The uptake of methylmercury (^{203}Hg) in different tissues related to its neurotoxic effects. J Pharmacol Exp Ther 1973;187:602–11.
18. Wobeser G, Nielsen NO, Scheifer B. Mercury and mink. II. Experimental methylmercury intoxication. Can J Comp Med 1976;40:34–45.
19. Jacobs JM, Carmichael N, Cavanagh JB. Ultrastructural changes in the dorsal root and trigeminal ganglia of rats poisoned with methylmercury. Neuropathol Appl Neurobiol 1975;1:1–19.
20. Cavanagh JB, Chen FCK. Amino acid incorporation in protein during the 'silent phase' before organomercury and p-bromo-phenylacetylurea neuropathy in the rat. Acta Neuropathol (Berl) 1971;19:216–24.
21. Stene T, Koppang HS. Autoradiographic investigation of proliferative responses in rat incisor pulp after vincristine administration. Scand J Dent Res 1980;88:96–103.
22. Stene T, Koppang HS. Autoradiographic investigation of dentin production in rat incisors after vincristine administration. Scand J Dent Res 1980;88:104–12.
23. Chang LW, Hartmann HA. Ultrastructural studies of the nervous system after mercury intoxication. Acta Neuropathol (Berl) 1972;20:122–38.
24. Pindborg JJ. Den kroniske fluor- og cadmiumforgiftnings indflydelse på den hvide rottes incisiver, med særligt henblik på emaljeorganet [Thesis]. Copenhagen: Munksgaard, 1950;1–136.
25. Mjör IA. Dentin and pulp. In: Mjör IA, Pindborg JJ, eds. Histology of the human tooth. Copenhagen: Munksgaard, 1973;45–76.
26. Neuman WF, Neuman MW. The chemical dynamics of bone mineral. Chicago: University of Chicago Press, 1958.
27. Nogueira TO, Stene T, Koppang HS. Long term effects of colchicine in rat incisors. Scand J Dent Res 1980;88:15–21.
28. Dahl JE, Stene T, Koppang HS, Koppang R, Stokke T. β-lumicolchicine as a tool to elucidate microtubular function in dentinogenesis. Acta Odontol Scand 1981;39:195–200.

Received for publication 7 June 1983