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Dental Abnormalities in Rats after a Single Large Dose of Cyclophosphamide[1]

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SUMMARY

Delayed drug-related mortality in rats treated with a single high dose (75 mg/kg) of cyclophosphamide complicated experiments using this drug treatment. We observed that this delayed mortality was due to dental abnormalities including broken teeth, absent teeth, extra long teeth, and/or supernumerary teeth. These dental abnormalities developed about 140 days after treatment and, if left untreated, interfered with eating. Eventually, the untreated rats starved. Clipping their long teeth and feeding the rats powdered chow eliminated the deaths. Researchers should be aware that high doses of cyclophosphamide may result in dental abnormalities several months after the treatment.

INTRODUCTION

Cyclophosphamide, *N*,*N*-bis-(β-chloroethyl)-*N*-*o*-propylenephosphoric acid ester diamide (Cytoxan, Mead Johnson Laboratories, Evansville, Ind.), has been marketed since 1959 as a cancer chemotherapeutic agent and, more recently, as an immunosuppressant. Its action *in vivo* is believed to be that of an alkylating agent that cross-links the guanine bases in double-stranded DNA, inhibiting cell division or causing mutation (2). In Wistar-derived rats, high single i.p. doses of cyclophosphamide (150 mg/kg body weight) are extremely toxic and produce high mortality within 10 to 14 days (5). Smaller single i.p. doses of approximately 75 mg/kg have been reported to produce immediate side effects in rats, such as ruffled hair (7), leukopenia (10), or interrupted odontogenesis (5). In addition, we (4) and others (8, 11) have reported a slow progressive wasting in rats, with mortality peaking approximately 3 to 4 months after a single injection. We now believe that this previously unexplained, high delayed mortality after cyclophosphamide treatment is the result of simple starvation caused by bizarre tooth abnormalities and the consequent inability to eat.

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MATERIALS AND METHODS

Animals

Albino rats descended from Wistar stock were used. Brother-sister matings of 74 generations insured that they were isogeneic. Skin transplant experiments also demonstrated that the animals were isogeneic. In some experiments only females were used; in others both sexes were used.

Food

Normal animals were provided with Purina mouse chow food pellets. In Experiments 2 and 3 animals that were losing weight were given a supplement of powdered Purina mouse chow.

Experiments

Experiment 1. The object of Experiment 1 was to investigate the possibility of spinal cord regeneration occurring with the use of immunosuppressive drugs (4).

After spinal cord transection, female rats were divided into control and treatment groups. Twenty-four control animals were given no special treatment, whereas 120 test animals were placed on various regimens of immunosuppressants. The animals were observed at weekly intervals for evidence of functional recovery and for evaluation of their general health. Electrophysiological testing for motor tract regeneration was not conducted until the animals had survived for 6 months after spinal cord transection and treatment. A group of 25 animals given single i.p. injections of cyclophosphamide, 75 mg/kg, showed the best evidence for long descending motor tract axon regeneration.

Experiment 2. The object of Experiment 2 was to investigate further in a 2nd group of rats the encouraging results of single i.p. injections of cyclophosphamide, 75 mg/kg, on spinal cord regeneration as found in Experiment 1. Only female rats were used.

The animals of Experiment 2 were divided equally into 2 principal groups: (1) 96 animals with spinal cords completely transected, and (2) 90 animals with only the dorsal half of the cords surgically sectioned. These principal groups were each further divided into subgroups: (*a*)

one-third of each group of animals was given injections of 0.9% NaCl solution within 1 hr after transection; (*b*) one-third of the animals was given injections of cyclophosphamide, 75 mg/kg, within 1 hr after transection; and (*c*) one-third of the animals was given injections of cyclophosphamide, 75 mg/kg, 24 hr after transection.

Their general condition was evaluated weekly. After 6 months these animals were to be tested for long motor tract regeneration. As soon as we became aware of a correlation between weight loss and the dental abnormalities, we instituted a plan of cutting back extra long teeth or extra teeth and placing animals with abnormal teeth and weight loss on powdered chow instead of the usual pellets.

Experiment 3. In both Experiments 1 and 2, all treated animals also had had spinal cord surgery and resultant paraplegia. The object of Experiment 3 was to rule out paraplegia as a contributing factor in the development of the dental abnormalities found in those rats. This test group consisted of 5 male (mean weight, 124 g) and 5 female (mean weight, 106 g) 6-week-old albino rats that received no treatment except a single i.p. injection of cyclophosphamide, 75 mg/kg, dissolved in sterile water. The rest of our large colony of more than 100 rats served as a control group, inasmuch as no untreated animals had shown similar dental abnormalities.

After injection, the rats were examined for evidence of tooth malformation every 2 to 5 days for a period of 7 months. Rats showing both dental abnormalities and weight loss were given powdered food to supplement their normal diet of hard food pellets.

RESULTS

Experiment 1. Treatment with immunosuppressive drugs gave encouraging evidence of regeneration of axons across the site of a previous spinal cord transection in rats (4). The best results were obtained in the group treated with a single 75-mg/kg dose of cyclophosphamide at the time of transection. This treatment group, however, had a very high delayed mortality that we could not explain. Thirteen of 25 animals died of drug-related causes. The 1st deaths occurred at 70 and 100 days after treatment. An additional 11 treated animals died between 120 and 180 days.

Experiment 2. Because we were alert to the possibility of delayed drug toxicity, we observed these animals with special concern for evidence of weight loss. It became obvious that treated animals developed abnormalities of the teeth and were unable to maintain body weight. Once a vigorous treatment program was instituted for the dental abnormalities, no further deaths occurred in the treatment group. Eventually, 88 of 116 rats either died or showed tooth abnormalities that we felt required treatment. The 70 control animals showed no deaths or dental abnormalities.

Experiment 3. All 10 treated rats showed gross tooth abnormalities within 6 months after injection (Chart 1). No control rats were found to have similar abnormalities.

The tooth abnormalities fell easily into 3 categories (Figs. 1, 2, and 3): (*a*) temporarily short or completely missing maxillary incisors (all 10 rats); (*b*) extremely long maxillary incisors (4 rats); and (*c*) well-developed supernumerary mandibular incisors (5 rats). In 2 rats, each of the 3 categories was represented at some time during the 7-month period after injection. Broken or missing teeth always preceded long teeth but did not necessarily precede supernumerary teeth.

The ubiquitous missing or temporarily shortened maxillary incisors usually were the 1st indication of dental anomaly, although in 1 case supernumerary teeth appeared first. Missing incisors were preceded by a gradual loosening and disorientation of the tooth and then by disarticulation. Three months postinjection, 1 rat had temporarily shortened maxillary incisors with abnormally white and chalky distal surfaces, but these reverted to apparently normal teeth after 1 month.

The 4 rats in which maxillary incisors were found to be completely missing at 3 months after injection had grown extremely long incisors by the 4th month. These long incisors were often thickened, poorly oriented, and partially fused together at midline. In some cases they curved backward into the mouth and eventually even back up into the palate (Fig. 4). Five rats that lost incisors 3 months after treatment had shown no sign of growing replacements by the 7th month.

Supernumerary mandibular incisors developed from tiny sharp sprouts protruding from gingiva lateral to the normal 2 mandibular incisors of the rats (Fig. 5). These sprouts developed into apparently normal-looking mandibular incisors. However, their abnormal lateral orientation caused them to irritate and eventually penetrate through the lower lip (Fig. 3). Supernumerary mandibular incisors occurred in 4 of the 5 female rats, but in only 1 of the 5 males.

All 3 types of tooth abnormality impaired ability to eat. The long maxillary incisors prevented proper chewing and occasionally reached and irritated the palate as they curved

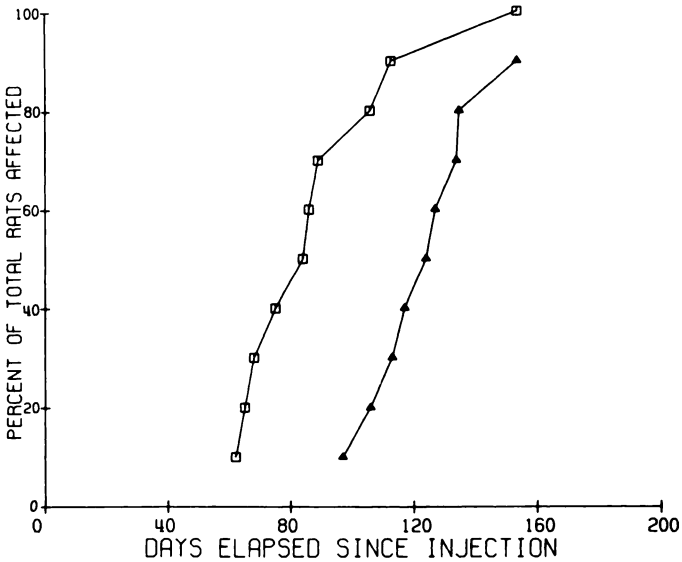

Chart 1. Dental abnormalities in Experiment 3. □, onset of first-noted dental abnormalities; Δ, the time when the dental abnormalities became severe enough to interfere with feeding and thus required powdered food. All animals developed dental abnormalities, and 9 of 10 animals required powdered food.

backward and prevented swallowing because of their large size. The supernumerary mandibular incisors irritated the buccal surface of the lips. The missing or shortened teeth also interfered with chewing. The impaired ability to eat was reflected in the weight loss noted at the time of the appearance of each tooth abnormality. In the case of rats with initially broken teeth, weight was lost until the teeth regrew and attained normal length but was lost again as the teeth grew too long for proper chewing.

Rats that were going to develop dental abnormalities could be identified by a change in their behavior. These rats spread food pellets across their cage tops, probably searching for softer or smaller food pellets. Thus a quick glance at the cage tops revealed which rats were developing tooth difficulties.

DISCUSSION

During our work on spinal cord regeneration (Experiments 1 and 2) (4), we found a delayed toxicity of cyclophosphamide, as others (8, 11) had reported. Rats began to waste away at about 90 days after i.p. cyclophosphamide, 75 mg/kg, and mortality peaked at around 130 days. At 140 days into Experiment 2 we noted that 1 moribund rat had extremely long maxillary incisors and extra mandibular incisors. A thorough dental examination of our experimental colony revealed that about 76% (88/116) of our rats receiving cyclophosphamide injections had some form of gross tooth abnormality; none (0 of 70) of the control rats receiving injections of 0.9% NaCl solution had dental abnormalities. A program of clipping long and extra teeth, providing a powdered food pellet supplement for rats with abnormal teeth, and monitoring rats for body weight loss prevented the deaths of any more rats.

We gave the 10 rats described in Experiment 3 injections of cyclophosphamide to determine if the paraplegia of the rats in previous experiments was a factor in their dental malformations. It was not.

A search of the literature revealed some excellent research involving the short-term (2-week) histomorphic effects of single doses of cyclophosphamide on dentinogenesis of the rat incisor. Koppang's work (5) showed " . . . (1) interrupted odontogenesis, (2) circular and mesiodistal dental constrictions, (3) niche-like dentinal defects, (4) differentiations of post-experimental tooth, and (5) cyst formation and circulatory disturbances" in rats so treated. Her autoradiographic work on incisors (6) demonstrated ". . . the predontoblasts to be the odontoblast developmental stage most sensitive to cyclophosphamide succeeded by precursors of predontoblasts. The injury was reflected as reduced production of circumpulpal dentin . . . " for 24 to 48 hr. Another researcher (9) investigated the effects of cyclophosphamide injections on newborn and weaned rats but not on adult rats. He reported findings of malformed incisors of varying shapes and suggested that the delayed death in such animals was caused by their inability to eat properly. We found no previous reports of supernumerary teeth in rats.

Danforth (3) has described the occurrence of duplicate lower incisors in the mouse. However, this trait is somewhat different than the trait that we observed. In his experiment the anomaly appeared 2 generations after animals had been treated with nitrogen mustard *in utero* and was heritable. In our experiment duplicate incisors appeared directly in the treated generation; it is not possible that the anomaly arose from damage to the germinal tissue. Also, duplicate incisors in his experiment appeared behind the normal ones; in our experiment they appeared laterally and slightly anteriorly. Finally, Danforth postulates 3 separate mutations would be required to explain the pattern of inheritance of this trait. He suggests that 2 of these mutations were preexisting in the strain of mice used and that the 3rd mutation was induced by treatment with nitrogen mustard. If the anomaly in our rats is controlled by the same 3 genes, it seems extremely unlikely that 2 of the mutations would also be preexisting and that the 3rd would be induced in 70 to 100% of the animals by a single injection of cyclophosphamide.

Why cyclophosphamide should cause these abnormalities is not clear. Cyclophosphamide has the action of blocking DNA replication. This blockage may be complete, resulting in cell death, or incomplete, possibly resulting in mutation. The short-term effect of cyclophosphamide on rat incisors seems to be the interruption of tooth growth. Long-term effects, on the other hand, do not seem to be caused by depletion but by excessive growth or a change in tissue quality, both of which may be a result of mutation. DNA replication may be interrupted at a point along a gene involved with feedback inhibition of protein production. Thus, proteins may be produced excessively and growth may be abnormally rapid. In a large population of active cells it would seem likely that at least some cells will be in the final stages of DNA replication when cyclophosphamide is administered. These cells may be injured in such a way as to cause mutation and eventually give rise to defective tissue.

While the dental abnormalities in rats after cyclophosphamide may have no clinical significance, they are of importance in research using rats and may signify the need to be especially alert for dental changes in children treated with this drug. Rat teeth grow continually and therefore are more similar to the teeth of children than to those of adults. We plan further work on the mechanism of this abnormal tooth growth.

ACKNOWLEDGMENTS

Analysis of data in these experiments and the preparation of Chart 1 were performed by the computer service of the University of Michigan (1).

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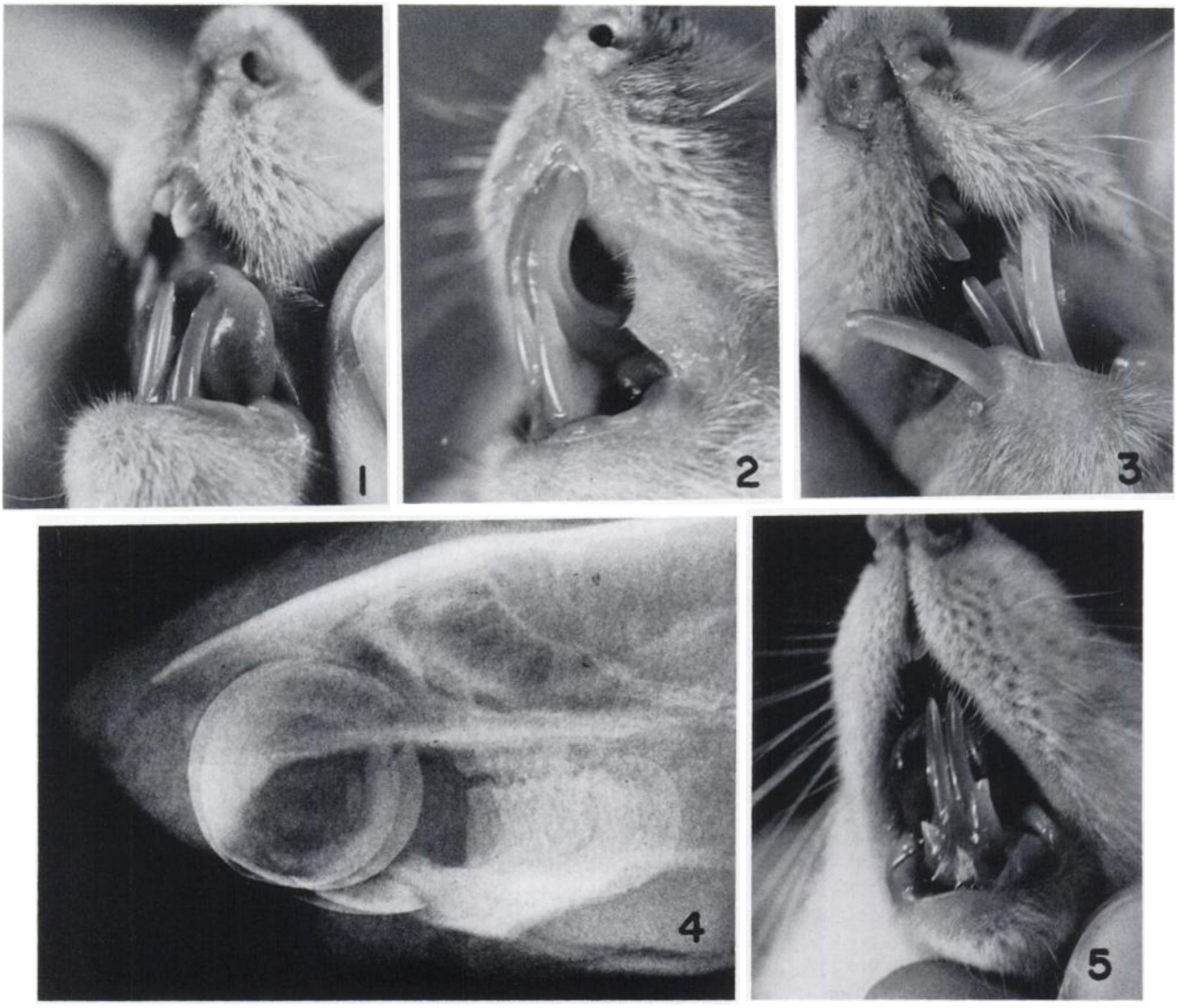

Fig. 1. Tooth abnormalities caused by single high-dose cyclophosphamide treatment. Note the short (broken off) maxillary incisors.

Fig. 2. In this animal, overgrowth of the maxillary incisors after cyclophosphamide treatment was actively interfering with its ability to eat and to swallow.

Fig. 3. After 140 days of treatment, supernumerary mandibular incisors have grown in this animal to unusual lengths, 1 of them growing directly through the lower lip.

Fig. 4. Lateral skull X-ray of a rat with long maxillary incisors. Overgrowth of these teeth after cyclophosphamide treatment eventually prevented normal eating.

Fig. 5. Early sprouts of supernumerary teeth. This rat has small sprouts of supernumerary mandibular incisors beginning to grow anteriorly and laterally from the mandible adjacent to his regular mandibular incisors.