Renewal and migration of rat incisor mesenchymal cells after doxorubicin administration

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Ten female rats were intraveneously injected with doxorubicin (20 mg/kg) and divided into two equal groups receiving a dose of tritiated thymidine either simultaneously with doxorubicin or 1 h before being killed after 5 days. Six control animals were correspondingly injected with ^{3}H-thymidine. The left and right maxillary incisors were prepared for histologic and microautoradiographic investigation, respectively. The distribution of labeled cells in animals injected 1 h before death showed the regions of proliferation, whereas migration from these regions was evaluated by longer observation time. A zone of reduced dentin deposition lined by irregular odontoblasts being young odontoblasts and late preodontoblasts was observed at the time of doxorubicin injection. Pulpally to the dentinal lesion, islands of irregular predentin were deposited by non-progenitive pulp cells and depolarized odontoblasts. The late preodontoblasts were renewed from progenitive pulp cells, leading to a disturbed mantle predentin deposition. □ *Antineoplastic agents; autoradiography; dentinogenesis; histology; odontoblasts*

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Doxorubicin hydrochloride (Adriamycin®) is an antibiotic of the anthracyclin group used in cancer chemotherapy (1). The cytotoxic effects are due to its intercalation into nuclear DNA and blockade of the transcription (2, 3), leading to inhibition of DNA and RNA synthesis. The influence of doxorubicin on odontoblasts and pulp cells of the continuously growing rat incisor has previously been studied (4). One day after a single injection of doxorubicin, necrosis of all the preodontoblasts and of cells in the progenitive (basal) pulp was observed. On the 5th day of observation, the basal pulp and the incisal part of the preodontoblasts were regenerated, but an irregular predentin tissue with cellular inclusions was found in the pulp, corresponding to the area of the necrotic changes seen 1 day after injection (4).

An indication of cell migratory and proliferative properties can be obtained from autoradiographic studies after administration of tritiated thymidine (5–7). The distribution of ^{3}H-thymidine-labeled nuclei 1 h after injection reveals proliferative cells, since ^{3}H-thymidine is incorporated only in cells synthesizing DNA before mitosis (8, 9). By studying the distribution of labeled nuclei at different time intervals after ^{3}H-thymidine injection, the migration of cells can be followed (10, 11).

The aim of the present study was to elucidate autoradiographically the migration and differentiation of rat incisor mesenchymal cells after doxorubicin administration.

Materials and methods

Sixteen female rats (Wistar, Möllegaards Breeding Centre Ltd, Skensved, Denmark) with a mean weight of 192 g were divided into one experimental and one control group of 10 and 6 animals, respectively. The experimental animals received under light ether anesthesia a single intravenous injection in the tail vein of doxorubicin hydrochloride (Adriamycin®, Farmitalia, Montedison Farmaceutica, Italy) dissolved in sterile water. The dose given was 20 mg/kg body weight and the injected volume 0.25 ml/200 g body weight. Rats of the control group were similarly dosed with 0.25 ml physiological saline

per 200 g body weight. Simultaneously, five of the experimental and three of the control animals were intraperitoneally injected with 250 μCi of methyl-^{3}H-thymidine (specific activity, 25 Ci/mmol; Amersham International Limited, Buckinghamshire, England). The volume administered was 0.5 ml. The other experimental and control animals were correspondingly given 250 μCi of methyl-^{3}H-thymidine 1 h before being killed. All animals were decapitated under ether anesthesia 5 days after the doxorubicin injection. The heads were divided by a median line incision, the maxillae freed from soft tissue, and the lateral nasal wall removed. The maxillae were fixed in 2% Sörensen-buffered glutaraldehyde for 3 days, followed by demineralization in an aqueous solution of equal amounts of 44% formic acid and 20% sodium citrate. The maxillary incisors were divided transversally into pieces of 3 mm apicoincisal length, postfixed in 1% Millonig-buffered osmium for 2 h, and embedded in Vestopal W (Chemische Werke Hüls AG, Mari, FRG) (12).

From each piece of the right maxillary incisors, 10–15, 1- to 2-μm-thick non-serial longitudinal sections were made approximately 50 μm apart. The sections were mounted on glass slides and dipped in liquid photographic emulsion (Kodak NTB-2, Nuclear Track Emulsion, Eastman Kodak Co., USA). After drying and exposure for 6 weeks in dry atmosphere at 4 °C, the sections were developed (Kodak D-19, X-ray development, Eastman Kodak Co.), fixed (Kodak Unifix, Eastman Kodak Co.), and washed. Approximately 60, 1- to 2-μm-thick serial longitudinal sections were made of the left maxillary specimens.

All sections were stained with methylene blue–azure II–basic fuchsin (13) and subjected to light microscopic investigation. Labeled cells were defined as cells having more than five grains over the nucleus detected autoradiographically (14).

Results

In control animals, the mesenchymal cells of the incisors consisted of progenitor (basal) and non-progenitor pulp cells, early and late preodontoblasts, young mantle dentin-producing odontoblasts, and mature, circumpulpal dentin-producing odontoblasts (Figs. 1 and 2a) (4, 11). In rats given ^{3}H-thymidine 1 h before death labeled nuclei were found only among early and late preodontoblasts and progenitor pulp cells (Figs. 1b and c and 2a). Equivalent labeling and also labeled young and mature odontoblasts were observed in rats killed 5 days after injection of ^{3}H-thymidine. In those animals, labeled pulp cells were seen adjacent to the labeled odontoblasts (Figs. 1d and e).

In doxorubicin-administered animals, reduction of the dentin thickness was seen in a zone extending approximately 0.5 mm incisally from the borderline between the young and mature odontoblasts. The width of dentin throughout the zone was estimated to be 15–50 μm (Figs. 2b and 3a). In this zone, the odontoblasts appeared shortened, irregular, and disorientated. No labeling of odontoblasts was seen in animals given ^{3}H-thymidine 1 h before death, but in rats simultaneously given doxorubicin and ^{3}H-thymidine, odontoblasts situated in the apical third of the lesion were labeled. A cellular and irregular predentin was deposited pulpally to the altered odontoblasts (Figs. 2b and 3a and b). Only a few of the predentin-embedded cells were labeled and only in animals observed 5 days after ^{3}H injection. The young odontoblast layer appeared duplicated, with irregular cells and interspersing islands of predentin. Nuclear labeling was found in young odontoblasts of animals to which ^{3}H-thymidine and doxorubicin were given at the same time (Fig. 3c). The late preodontoblast layer in both experimental groups contained labeled, cuboidal cells, whereas the early preodontoblast zone appeared necrotic (Fig. 2b). The basal pulp tissue was more edematous after doxorubicin administration than in the controls and contained labeled cells, although the density of the labeling was highest in rats dosed with ^{3}H-thymidine 1 h before death.

Discussion

Cells in the synthesis phase at the time of

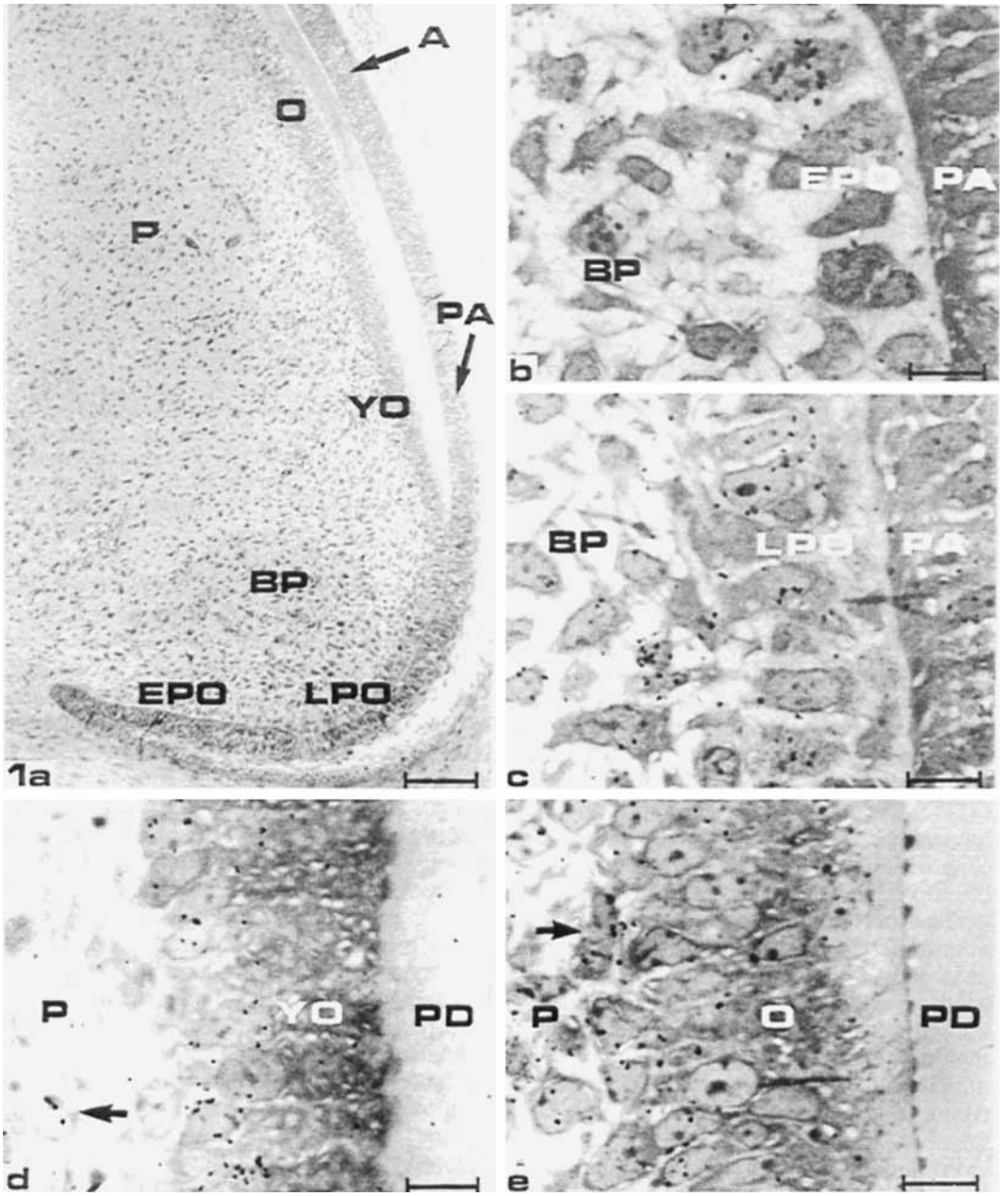

Fig. 1. Microphotographs of longitudinal Vestopal W-embedded sections of the apical part of maxillary incisor from control animals injected with ^{3}H-thymidine. (a) Low-magnification overview of basal pulp cells (BP) and pulp tissue (P), of early preodontoblasts (EPO), late preodontoblasts (LPO), and young odontoblasts (YO), all adjacent to preameloblasts (PA), and of mature odontoblasts (O) at the enamel-secreting ameloblast (A) level. Bar = 100 μm. (b and c) Higher magnification of basal pulp cells (BP), randomly arranged cuboidal early preodontoblasts (EPO), and more elongated, neatly arranged late preodontoblasts (LPO) of animal given ^{3}H-thymidine 1 h before being killed. PA = preameloblasts. Bar = 10 μm. (d–e) Closely packed, columnar young odontoblasts (YO) and pseudostratified mature odontoblasts (O) containing labeled nuclei, from animal 5 days after ^{3}H-thymidine administration. Arrow points at labeled cell in the non-proliferative pulp (P). PD = predentin. Bar = 10 μm.

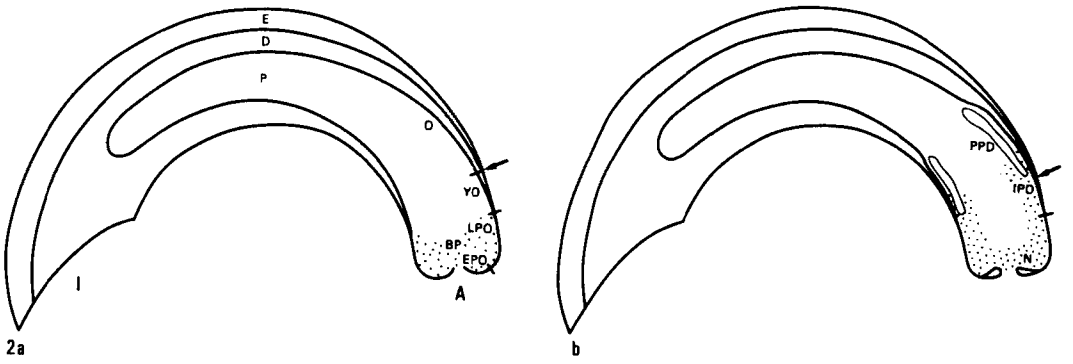

Fig. 2. Schematic presentation of longitudinal sections of maxillary incisors of control animals (a) and 5 days after a single injection of doxorubicin (b). (a) Enamel (E) and dentin (D) surround the pulp (P), odontoblasts (O), and young odontoblasts (YO). Localization of proliferating basal pulp cells (BP), early preodontoblasts (EPO), and late preodontoblasts (LPO) is shown. Proliferating cells are indicated by dots. I = incisally. A = apically. (b) Localization of reduced dentin deposition and irregular predentin in the pulp (PPD), interspersing islands of predentin among young odontoblasts (IPD), necrotic early preodontoblasts (N), and labeled cells 5 d after injection of ^{3}H-thymidine (dots).

^{3}H-thymidine administration will incorporate the radioactively labeled nucleotide in their DNA (8, 9), and, since most cells in the S-phase proceed to mitosis, the distribution of autoradiographically detected labeled cells at 1 h after injection of ^{3}H-thymidine displays the pool of proliferative cells (5). In agreement with previously published results (7, 11), the basal pulp cells and the preodontoblasts of control animals were labeled (Figs. 1b and c), thus representing proliferative cells. Five days after injection, the labeled preodontoblasts had matured into odontoblasts and, together with the adjacent basal pulp cells, had migrated incisally with tooth growth (Figs. 1d and e) (11). Further, the cells of basal pulp and preodontoblast regions remained labeled as the ^{3}H-thymidine built into cellular DNA was distributed to the daughter cells during mitosis (15).

Five days after a single injection of doxorubicin, a zone of reduced dentin thickness, pulpally lined by disorientated, irregular odontoblasts, was observed (Figs. 2b and 3a). These cells were unlabeled in animals dosed with ^{3}H-thymidine 1 h before decapitation, showing a lack of proliferative capacity, similar to normal odontoblasts (7, 11). In rats simultaneously dosed with the nucleotide and doxorubicin, labeled cells appeared only in the apical third of the lesion (Figs. 2b and 3a and b). Synthesizing DNA at the time of doxorubicin injection, these cells were late preodontoblasts proceeding into young odontoblasts before doxorubicin discharged its cytotoxic effect, and they were therefore less severely injured. The zone of remaining non-labeled cells corresponded in length approximately to the young odontoblast region of control animals. This indicates that when doxorubicin was administered the unlabeled cells of the lesion were young odontoblasts moving incisally with tooth growth and that doxorubicin also affected these non-proliferating cells.

Deposition of irregular predentin with cellular inclusions in the pulp (Fig. 3b) is believed to be a reparative phenomenon induced by pulp cell destruction (16), but it is also caused by loss of polarity of secreting cells (17, 18). Some depolarization of odontoblasts and necrosis of pulp cells are registered 1 day after doxorubicin treatment (4). All predentin-embedded cells appeared unlabeled, and, also at the time of doxorubicin injection, most of them were non-cycling cells, most probably pulp cells. These cells and the depolarized odontoblasts were most likely responsible for deposition of the irregular predentin.

In the experimental animals, the young

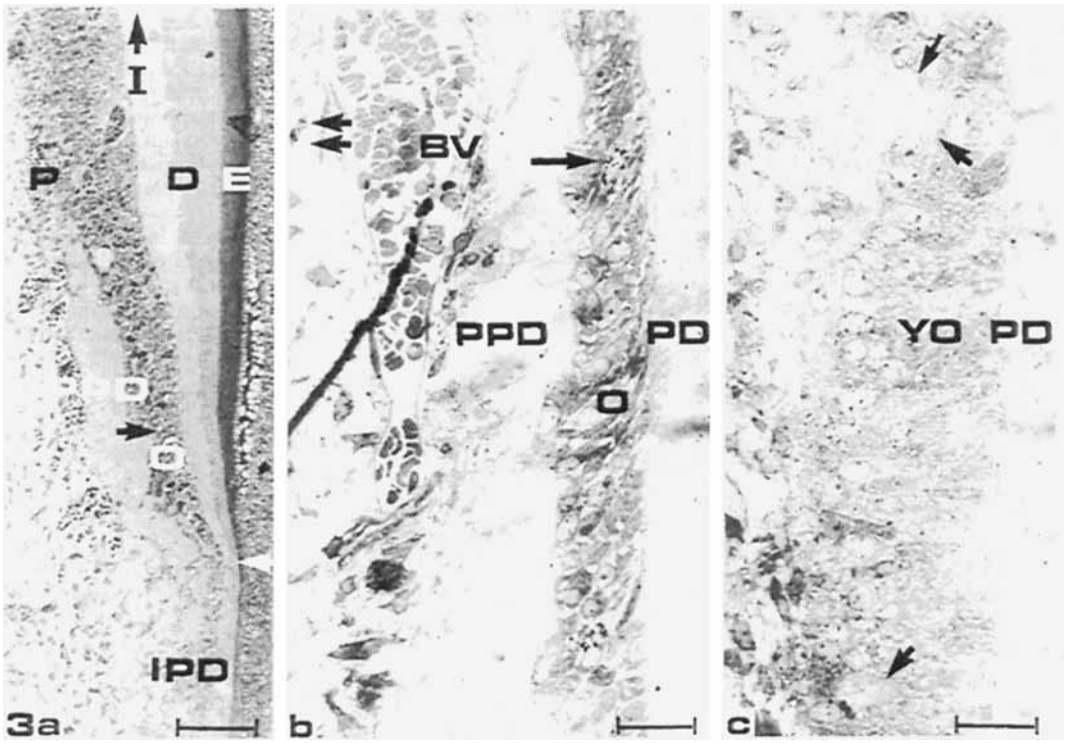

Fig. 3. Microphotographs of longitudinal Vestopal W-embedded sections of maxillary incisors 5 days after simultaneous injection of doxorubicin (20 mg/kg) and ^{3}H-thymidine. (a) Marked reduction of dentin thickness, deposition of irregular predentin (PPD) in the pulp, and young odontoblasts with interspersing islands of irregular predentin (IPD). D = dentin and predentin, E = enamel, I = incisally, O = odontoblasts, P = pulp. Black arrow points at the most incisal labeled odontoblast. White arrow is situated at the preameloblast/ameloblast borderline. Bar = 100 μm. (b) Higher magnification of predentin in the pulp (PPD) and of irregular, displaced odontoblasts (O). Arrow points at the most incisal labeled odontoblast nucleus. Double arrow marks the most incisal labeled pulp cell. PD = predentin, BV = blood vessel. Bar = 25 μm. (c) Irregularly arranged young odontoblasts (YO) with labeled nuclei and interspersing islands of predentin (arrows). PD = predentin. Bar = 25 μm.

odontoblasts appeared irregular with abnormal predentin deposition and were labeled in animals given ^{3}H-thymidine 5 days before death (Figs. 2b and 3c). These cells originated from cells of the proliferative pool destroyed by doxorubicin, apparently basal pulp cells, since the complete preodontoblast zone was necrotic 1 day after the injection (4). Since the early preodontoblasts remained necrotic throughout the experimental period, the renewal of late preodontoblast zone by proliferative cells after 5 days must have occurred from cells of the progenitor pulp tissue, as formerly indicated (4).

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