β-lumicolchicine as a tool to elucidate microtubular function in dentinogenesis

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ß-lumicolchicine, an isomer of colchicine which does not interrupt microtubular function, was given to 8 rats in a dose of 1.5 mg/kg. Four rats served as controls. Histomorphological investigations of odontoblasts and dentin in the maxillary incisors after two weeks revealed no disturbances. This finding, compared with the previously reported effects of colchicine, as well as colcemid, vincristine and vinblastine, indicates dentin production to be dependant on microtubules.

Key-words: Colchicine derivatives; incisor; irregular dentin; rat; vinca alkaloids

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The cytostatic drugs vincristine, vinblastine and colchicine are widely used antitumor agents. Vincristine and vinblastine are alkaloids extracted from the Vinca rosea plant (15). Colchicine is an alkaloid of the Colchicum autumnale plant (38), and so is colcemid (N-metyl, N-deacetyl colchicine), a previously used antitumor drug (30). They all produce a characteristic inhibition of cell mitosis in the metaphase (8, 29). This stathmokinetic effect is probably caused by interference with microtubular structures (41), since the receptors for colchicine, colcemid and the vinca alkaloids are shown to be the microtubular protein tubulin (1, 7, 31). Colchicine, colcemid and vinblastine bind to soluble tubulin preventing its assembly to microtubuli (14, 40, 42), whereas vincristine binds to microtubular subunits tubulin, causing decreased binding affinity of the subunits followed by disruption of microtubules and formation of intracytoplasmatic crystalloids (40).

The lumicolchicines are isomers of colchicine formed by ultraviolet irradiation of colchicine (12, 13). The three isomers (α- ß- and γ-) are chemically closely related. ß- and γ-lumicolchicines are stereoisomers (9), and α-lumicolchicine is a dimer of ß-lumicolchicine (5). Lumicolchicines do not bind to tubulin, nor do they inhibit mitosis (4, 39, 43). However, they inhibit the nucleoside transport in cells (2, 23), bind to non-tubulin structures in nuclear and plasma membranes (32) and alter the cell membrane organization of

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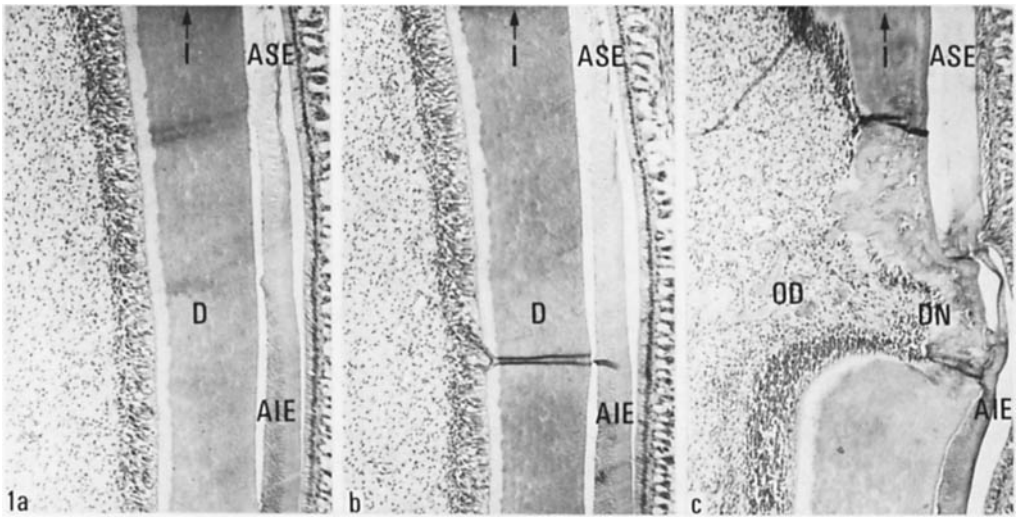

Fig. 1. Longitudinal sections from the region where the enamel turns acid soluble in maxillary rat incisor 2 weeks after injection of 1.5 mg/kg ß-lumicolchicine (1a) showing no changes compared with isotonic saline solution (1b) (controls), and 1.5 mg/kg colchicine (1c) producing a dentinal niche (DN) with osteodentin (OD). I, incisally, D, dentin, AIE, acid insoluble enamel, ASE, acid soluble enamel. (Fig. 1c is taken from the material used in reference 26 with permission from the authors.) X 50.

tissue-cultured cells (10), as does colchicine. For identification of these and other possible effects of colchicine not related to interference with microtubular structures, the lumicolchicines seem to be a useful tool.

The interference of vinca and colchicum autumnale alkaloids with dentinogenesis in the rat incisor have been elucidated both in short term (22, 27, 30, 33, 36, 37) and long term (22, 26, 27, 30, 34, 35) studies. The effects seen after 5 hours, 1 and 2 days are mostly altered cell shape, arrested mitoses, necrosis and irregular dentin production. After a week or more, niche-like defects developed, and irregular dentin and osteodentin were produced. Some of these effects are probably caused by interaction of the drugs with microtubules (26), but other mechanisms of action could also be possible.

The aims of the present study were to record long term effects of one of the colchicine isomers (ß-lumicolchicine) on dentinogenesis, and to compare these effects, which would not be related to microtubular dysfunction, with those previously reported for colchicine, colcemid, vincristine and vinblastine in order to elucidate the role of microtubules in dentin production.

MATERIALS AND METHODS

Preparation and characterization of ß-lumicolchicine

ß-lumicolchicine is not commercially available and had to be prepared.

To a photochemical reactor (Hanovia) supplied with a 100 watts medium pressure arc tube, cooled with tap water at 14°C, 1.2 l 96 % ethanol and 5 g colchicine were added. The mixture was flushed with nitrogen for 30 minutes under stirring before the ultraviolet lamp was illuminated. After 20 h, only traces of colchicine could be demonstrated by thin layer chromatography. The reaction mixture was concentrated and chromatographed on a silica gel

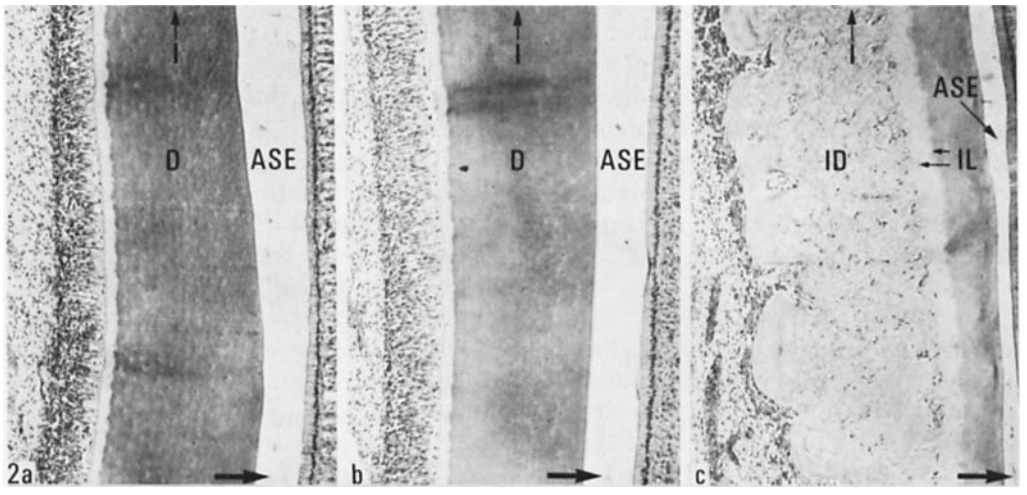

Fig. 2. Longitudinal sections from a region more incisal than in Fig. 1 two weeks after injection of 1.5 mg/kg ß-lumicolchicine (2a), isotonic saline solution (2b) and colchicine (2c) revealing no dentinal disturbances in 2a and 2b but an irregular dentin (ID) pulpally to two incremental lines (IL) in 2c. I, incisally, D, dentin, ASE, acid soluble enamel, arrow pointing at remnants of acid insoluble enamel. (Fig. 2 c is taken from the material used in reference 26 with permission from the authors.) X 50.

column with an ether/ethanol mixture. The first gram eluted from the column was chromatographed once more on a new silica gel column. From the eluate the initial 0.5 g were recrystallized from an ether/ethanol mixture to give ß-lumicolchicine crystals melting at 184–186°C (183°C (13)) in a melting point microscope. Mass spectrum (recorded on V.G. Micromass 7070F attached to V.G. 2200 data system) and infrared spectrum (Perkin Elmer 457 IR) were consistent with expected spectra. Ultraviolet (Cary 14 Recording Spectrophotometer) and ^{1}H NMR (Varian 100 MHz NMR) spectra concurred with published spectra for ß-lumicolchicine (6, 13).

Animal experiment

Out of twelve Wistar female rats (average weight 185 g) eight constituted the experimental group and four served as controls. The experimental animals received a single intravenous injection of ß-lumicolchicine (prepared as described above) dissolved in isotonic saline solution in a dose of 1.5 mg/kg body weight. The injected volume was 3 ml/kg body weight. The control animals received an equivalent volume of isotonic saline solution.

All animals were decapitated under ether anesthesia two weeks after injection. The heads were divided by a midline incision, the maxillae were freed from soft tissue and immediately fixed in 4 % aqueous formaldehyde. After demineralization in an aqueous solution prepared from equal amounts of 44 % formic acid and 20 % sodium citrate, the specimens were embedded in paraffin, and approximately 60, 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

No changes in cell structure or dentin deposition were discovered in the experimental or the control groups (Figs. 1 and 2).

DISCUSSION

ß-lumicolchicine in a dose of 1.5 mg/kg body weight had no histomorphologically detectable effect on dentinogenesis in rat incisors after two weeks, while colchicine, colcemid, vincristine and vinblastine produced striking cellular and dentinal alterations (22, 26, 30, 34).

Formation of a dentinal niche, mostly containing osteodentin, (Fig. 1) was observed after administration of all drugs but ß-lumicolchicine. Niches of similar morphology and localization were also seen in animals treated with cyclophosphamide (18) and roentgen rays (16). This appears to represent an unspesific, although characteristic, reaction induced by injury of late preodontoblasts and early mantel dentin producing odontoblasts (17, 19, 20, 33). The osteodentin is a reparative phenomenon initiated by degradation or necrosis of preodontoblasts and odontoblasts, and deposited by mesenchymal cells in the pulp tissue (26, 27, 36).

Microtubules are believed to play an important role in maintenance of cell shape (28). In tissue-cultured cells, general cell shape was correlated to distribution of microtubules, and colchicine and other microtubule disrupting agents caused alterations in cell shape and loss of normal polarity (3). Altered morphology of odontoblasts (short, apolar and pyknotic cells) was seen after administration of all antitumor agents but ß-lumicolchicine. This effect on odontoblast morphology is believed to be specifically due to interference with microtubular structures.

Colchicine, colcemid, vincristine and vinblastine interfered with the proceeding dentinogenesis. Incisally to the niche-like defect, one or two incremental lines were detected in normal dentin, and pulpally to these line(s), an irregular dentinal tissue with only occasional dentinal tubules but numerous inclusions of cells. Incremental lines probably represent an immediate and direct effect on the dentinal apparatus just after injection of the drugs (26), while protracted effects are manifested as irregular dentin. This dentin seems to be produced by abnormal odontoblasts with severely altered secretion mecanism, and not by cells differentiated from the pulp (34, 36).

The same, severe alteration of dentin production is seen after administration of several different antitumor agents with varying chemical structure (Fig. 3) and mecanism of action (40, 42). These agents have one thing in common, i.e. the ability of binding to tubulin (1, 7, 31), though at different sites (43), and disrupting microtubular function (14, 41). The seven membered ring *I* of colchicine and colcemid (Fig. 3) is of critical importance for their ability to interact with tubulin (40). In the lumicolchicines, this ring is converted into one four and one five membered ring (40), the result being that they do not bind to tubulin (43). Consequently, the lumicolchicines do not possess the antimitotic effect of colchicine and colcemid (39). However, the non-microtubular dependent properties of colchicine are also found in the lumicolchicines (23, 32).

Because ß-lumicolchicine does not seem to affect dentin deposition, probably due to its lacking ability to disrupt microtubular function, the dearrangements in circumpulpal dentin produced by the antitumor agents are evidently caused by interference with microtubules. This strongly indicates a microtubular dependent secretion mecanism in odontoblasts, corresponding to other secretory cells (11, 21, 24, 25).

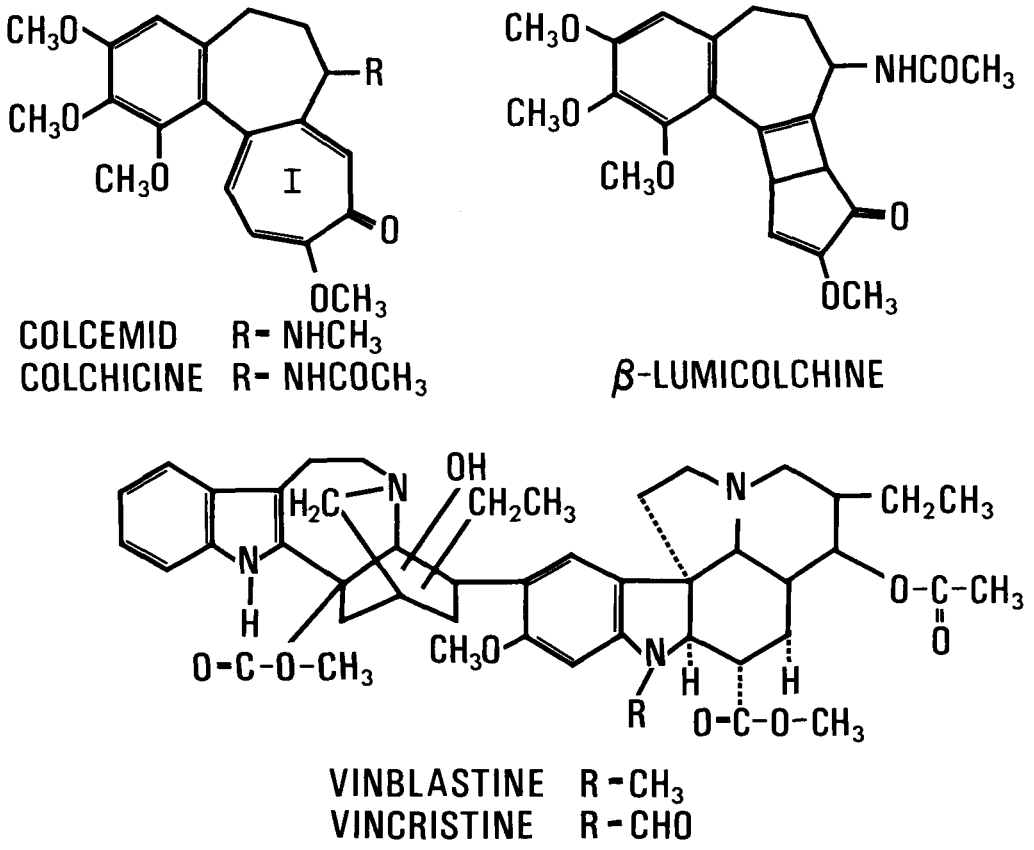

Fig. 3. Chemical formulae of alkaloids from the Colchicum autumnale and Vinca rosea plants.

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