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LONG-TERM EFFECTS OF SUCCESSIVE DOSES OF VINCRISTINE ON RAT DENTAL PULP AND DENTINE

ANKICA JAKOVLJEVIĆ

Department of Conservative Dentistry, University School of Dental Medicine, 11000 Belgrade, Yugoslavia

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The aim of the study was to analyze changes occurring in the pulp tissues and dentine of molar teeth of experimental animals after successive administration of the cytostatic Vincristine.

Wistar rats of both sexes were used in this experiment. The experimental animals were subcutaneously injected with five consecutive doses of Vincristine at 0.1 mg/kg b.w. The first dose was given on the 7th or 8th postnatal day, and the next four doses at weekly intervals thereafter. The control group consisted of untreated animals. Both groups were sacrificed by decapitation at the age of 6 or 9 months. The obtained material was prepared for routine histological examination.

The results obtained changes in the pulp and dentine of both molars in treated animals but not in the control ones.

There were histologically evident vascular changes and perivascular edema accompanied the dominant pulp atrophy, with reduction of the odontoblast layer and hyalinisation of connective fibers. Dentine showed an inconsistent formation and calcification with significant non-homogenous incremental lines and loss of structure.

All changes in dental pulp and dentine were more intensive as time proceeded.

Key words: chemotherapy, Vincristine, dental pulp, dentine

INTRODUCTION

At present there are a great number of chematherapy agents entering many cell metabolic processes. Their effect is based on disturbing cell metabolism and protein synthesis. The cytostatic drug Vincristine is one of that group of drugs. It is an integral part of large therapeutic protocols for treating malignant diseases in both children and adults. It is often combined with other drugs, achieving good results (Stefanović, 1989; Calabresi, and Chabner, 1990).

It has been proved that the clinical application of cytostatics increases survival rates, and even leads to complete recovery in a large number of patients

(Calabresi, and Chabner, 1990). Alow level of selectivity and toxicity for normal cells as well as neoplastic cells, causes certain changes and side effects in the host body. Thus, some changes in oral tissue have been noticed (Cancer Journal for Clinicians, 1987). Early side effects are more often present in the oral mucosa while changes on the teeth and jaws appear much later (Barjaktarević, 1988; Inić, 1990).

In patients suffering, from acute lymphoblast leukemia and treated with shemotherapy and radiotherapy, Dahllof et al. 1994, examined the teeth. After 9,5 years of observing the basic disease, they found dental changes by radiographic and hystological analysis. On radiographs, they described short and speared roots; enamel hypoplasia, microdontia and aplasia. Histological analysis of the extracted teeth revealed dominant changes in dentine which had significant incremental lines equivalent to the time of intensive cytostatic treatment and radiotherapy. The authors concluded that chemotherapy causes qualitative changes in dentine and enamel; while radiotherapy causes both qualitative and quantitative ones.

Our previous research showed that successive administration of the cytostatic Vincristine during the period of most intensive growth of rats killed at are age of 3 months, caused changes in both dental pulp and dentine of both molars. These dental pulp changes were: reduction of cell elements; hyperemia and stasis in blood vessels. The histological dentine picture showed irregularly formed dentine with discontinued incremental lines to be dominant (Jakovljević, and Sedlecki, 1993).

Considering the previous results, the aim of this research was to observe the lang term changes in molar pulp and dentine in rats treated with successive doses of Vincristine during their intensive growth period.

MATERIAL AND METHODS

A total of 48 wistar rats of both sexes were included in this experiment.

The control group consisted of 10 female and 12 male rats, while the treated group included 14 female and 12 male rats. The treated group was subcutaneously injected with five doses of Vincristine at 0,1 mg/kg b. w. The first dose was injected on the 7th - 8th postnatal day, and others were given subsequently at weekly intervals. Both control and treated rats were divided into two sub groups and decapitated at the end of the experiment when they were 6 or 9 months old.

After decapitation, the molar parts of the upper jaw, as well as the left half lower jaw were isolated. The material was fixed in Bouin solution and decalfied in 20% neutral EDTA solution. Dehydration was done in a series of alcohols, and the specimens embedded in paraffin. The series of mesiodistal 5-8 μm thick sections were stained by hematoxylin-eosin and Gabe azan and analyzed under a microscope (Olympus-Vanoks-T-AH-2, 1993.).

RESULTS

The normal dental pulp and dentine of upper and lower jaw molars in the control group of rats at 6 and 9 months old are presented in Figure 1 end 2.

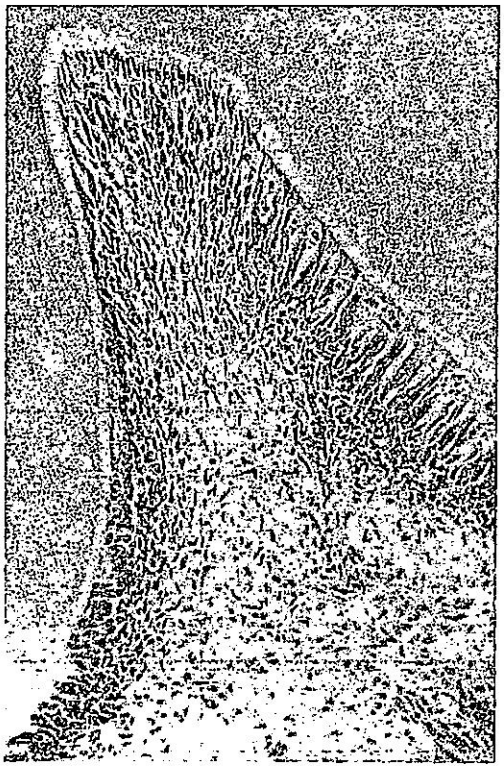

Figure 1. Dental pulp and dentine beneath a cusp in the first molar of a six-month-old control rat. H. E. x 50.

- Treated rats at six months old

- A significant dental pulp atrophy was present in the first and second molar in six-month-old treated rats. Vascular changes could also be noticed. Significant hyperemia in dental pulp blood vessels overfilled with blood cells was present (Figure 3). At some places, blood vessel walls were damaged, accompanied with extravasacy of blood cells (Figure 4). The changed dental pulp had perivascular

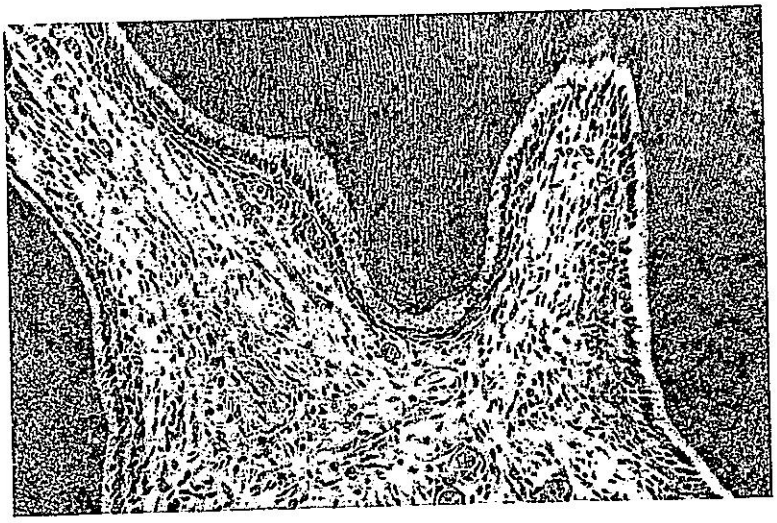

Figure 2. Dental pulp and dentine in the first molar coronary pulp of a nine-month-old control rat. H. E. x 50.

edema together with significant connective fibres and unhomogenous distribution of cellular elements (Figure 4). The odontoblast layer was changed. The number of odontoblasts was reduced (Figure 4), while the odontoblast arrangement was disturbed (Figure 3). Predentine was of uneven width (Figure 3, Figure 5). Dentine had an irregular canalicular arrangement. At some places, dentine tissue mass took the form of newly - formed tetiary dentine having apparent periodical incremental lines (Figure 3, Figure 5).

Treated rats at nine months old

There were histological changes bothin dental pulp and in dentine in mesiohistol sections of both molars in nine-month-old-treatol rabs pulp atraphy

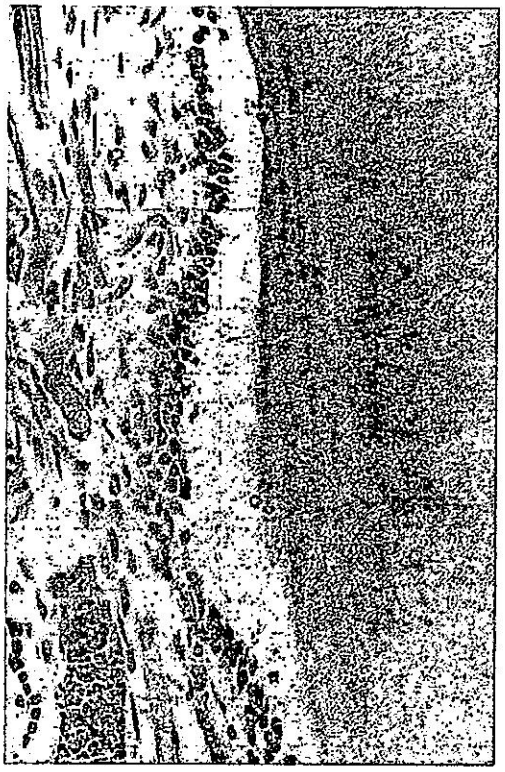

Figure 3. Dental pulp and dentine in the second molar root of a six-month-old treated rat. Incremental lines in dentine. Predentine of uneven width. Disturbed odontoblast layer. Significant hyperemia in blood vessels. H. E. x 100.

was dominant in the histologicsl picture. Hyalinized connective fibres and unhomogeneous cellularity could be noticed (Figure 6). Blood vessels were dilated and overfilled with blood elements. At some parts, damaged blood vessels and extravasacy of blood cells could be noticed (Figure 6, Figure 7). Pulp tissue edema was also present (Figure 6, Figure 7). The changes in the odontoblast layer were the same as those in the younger experimental group. The number of odontoblasts was reduced and their arrangement disturbed (Figure 7). Some odontoblast nuclei were pycnotic (Figure 6). The odontoblast layer change was accompanied by uneven predentine width (Figure 6, Figure 8). Dentine of irregular formation often invaginated towards the pulp. The inconsistent formation

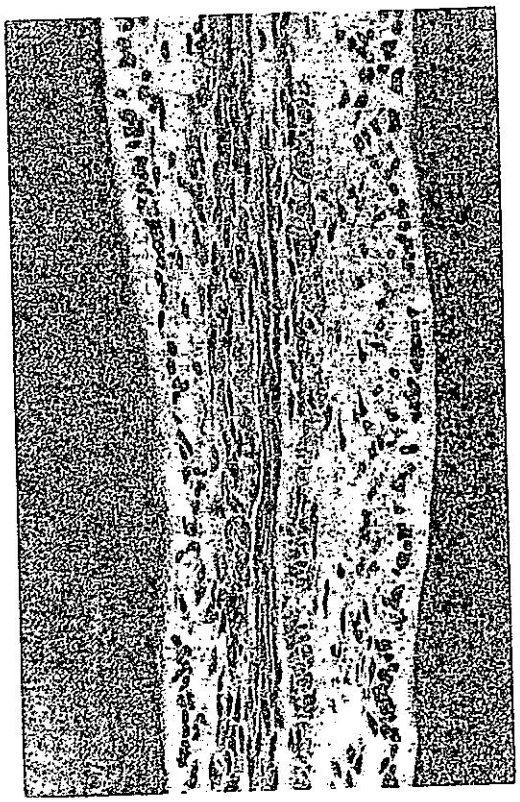

Figure 4. Reduced number of odontoblast cells and blood vessel hyperemia in the first molar root pulp of a six-month-old treated rat. H. E. x 100.

and calcification of dentine went together with cross fissures and the loss of canalicular structure. The dentine changes were clearly significant as unhomogenous lines in dental cusps and in dental fissures in both experimental groups of rats (Figure 8).

DISCUSSION

The histological analysis showed changes in the dental pulp and dentine of both molars in rats successively treated with the cytostatic Vincristine and

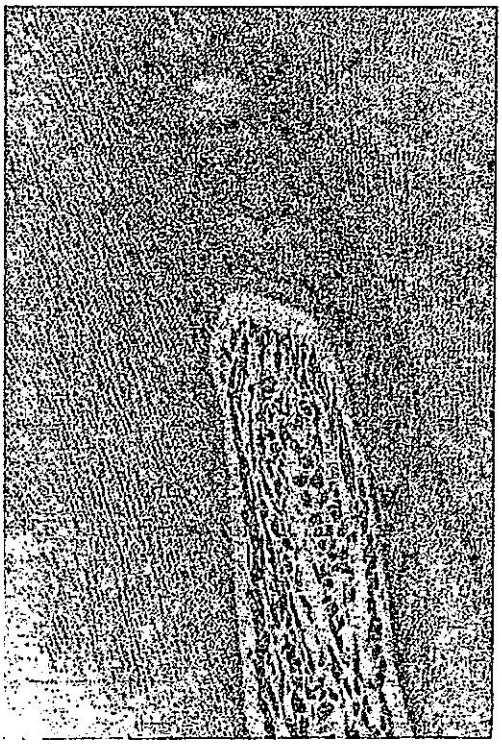

Figure 5. Uneven predentine width and tertiary cusp dentine of the second molar in a six-month-old treated rat. H. E. x 80.

decapitated at 6 or 9 months old. At both experimental periods the pulp changes were of similar quality; whereas the intensity of the changes was more significant in the second experimental period.

Pulp tissue atrophy is dominat in the histological picture of both molars; although the pulp chamber size decreases as animals grow old because of the secondary dentine apposition (Pinzon, et al., 1967). Once-a week administration of Vincristine during five-weeks, was a chronic attack on the intensively developing tissues and repeatedly inhibited their recovery (Jakovljević, 1996). The cumulative influence of this cytostatic (five doses of 0,1 mg/kg b.w.) probably

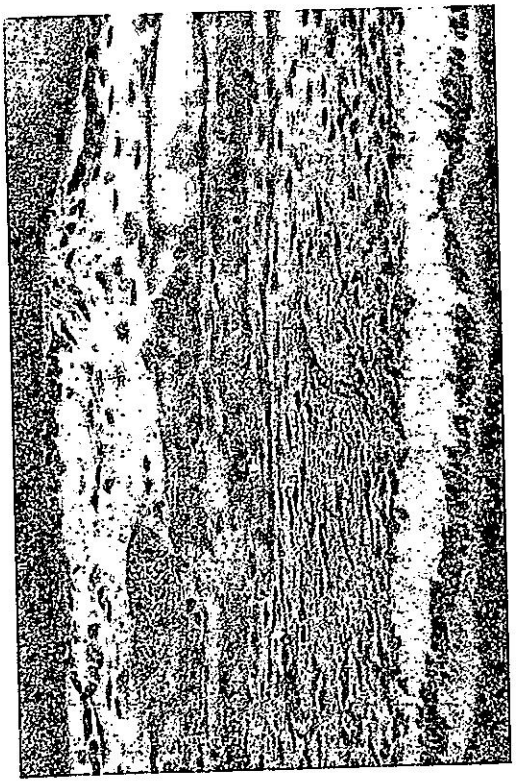

Figure 6. The first molar root pulp of a nine-month-old treated rat. Hyperemia in blood vessels. Hyalinisation of pulp connective fibres. Interrupted odontoblast layer with pycnotic nuclei and edema. H. E. x 100.

caused the changes in molar pulp in experimental rats and dentine consequently. These explanations agree with the results of Washkinel et al. (1991) who, by electro - microscopic and histochemical methods, demonstrated that the negative effect of a single dose of Vincristine lasts eight days which was confirmed by the state of the regenerated microtubule structure.

Our earlier experimental research showed that a single dose of Vincristine at 0,1 mg/kg b. w. injected in intensively growing rats decapitated before sexual maturity caused changes in blood vessels accompanid with a significant hyperemia and haemorrhagia, as well as changes in the odontoblast layer with reduced

Figure 7. The first molar root pulp of a nine-month-old treated rat. Disrupted odontoblast arrangement. Pulp edema and blood vessel hyperemia overfilled with blood cells. H. E. x 100.

odontoblast number, and disturbed structure. All the noticed changes are more significant when related to time (Jakovljević, et al., 1992).

Moreover, our previous researche on successive dose effects of Vincristine on the molar pulp-dentine complex in rats decapitated at the age of 3 months, showed that the reduced number of cell elements and enlarged mass of connective fibres, as well as the disturbed odontoblast structure, are dominant in the histological picture. Predentine widening and interglobular dentine occurrence, as well as discontinued incremental lines were noticed (Jakovljević and Sedlecki, 1993). These experiments showed similar qualitative changes in pulp and den-

Figure 8. Changed dentine and pulp in the second molar cusp and pulp horn of a nine-month-old treated rat. H. E. x 50.

tine, while the changed intensity was more significant when related to time, which was noticed especially on the dentine of both molars.

This experiment demonstrates that the uneven predentine width, the reduced dentine canalicular number and their unhomogenous appearance in the newly - formed dentine are caused by reduced and disturbed odontoblast function, ontoblasts being the basic cells forming the dentine matrix. In addition. In addition to this, the experiments of Maclead et al. (1987) explain the reduced collagen secretion as caused by cytotoxic chemotherapy influence. As vascular changes are evident in the pulp tissue, the oxygen flow is reduced in both pulp tissue and odontoblasts. Takei et al. (1988) state that the pulp vascularity, i. e.

enough oxygen, is a prerequisite for a quick and accurate phenotype expression of tubular dentine.

The dentine apposition happens successively and rhythmically. The newly-formed dentine deposition followed by organic matrix mineralization may be noticed as regular incremental lines. The intensity of incremental striations was more significant with time, in both the control group and the treated one. In one study dealing with the forming of rhythmical lines in dentine, Watanabe (1978) found that the rhythm of dentine formation occurred as a response of the dentine forming cells - odontoblasts and their well - kept activity of precollagen secretion. The changes noticed in molar - dentine intensive and unhomogenous incremental lines in rats successively treated with Vincristine, are probably related to the reduced number and disturbed structure, as well as, the changed odontoblast morphology. The Vincristine cytostatic effect causes the formation of crystals in odontoblast microtubules and, in that way, disturbs intracellular transport processes (Hirose, Takiguchi, 1995). That makes calcium transport difficult, and disturbs dentinogenesis (Lundgren, Linde, 1992; Kortelainen, 1995).

Similarly, Maclead et al. (1987) noticed the presence of incremental lines in dentine on histological sections of human teeth in patients treated with cytostatics. The number and structure of these lines corresponded to the periods of intravenous chemotherapy, and the authors concluded that the incremental lines were the result of Vincristine effect, i. e. a temporary microtubule structure and function disturbance in odontoblasts.

There are some opinions that the rhythmic dentine apposition is conducted from the hypothalamus (suprachiasmatic nucleus) and corresponds to the biological circadian rhythm, with a daily dentine apposition of 16 μm in a rt molar cusp, and 2 μm at the root within 24 hours (Ashida, 1983). If this thesis were accepted as one of the possible causes of incremental line formation disturbance, a systemic cytostatic activity would cause disturbance of the "biological clock" as a result of hypothalamus damage.

Long-term effects of successive doses of Vincristine on dental pulp and dentine of both molars in experimental rats result in dental pulp and dentine changes. In dental pulp, the dominant dental pulp atrophy is accompanied with vascular changes and perivascular edema. Hyalinisation of connective fibres and reduction of the odontoblast layer are also present. As for predentine, it is of uneven width; while dentine shows inconsistent formation and calcification. Dentine does not remodel and it forms a permanent record of "patterns" made of incremental lines. These lines represent phases of laying down of the organic matrix and metabolic processes in dentine as a response to the changes in pulp after this cytostatic - activity.

All changes in dental pulp and dentine became more intense in relation to time.

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POZNI EFEKTI VIŠEKRATNIH DOZA VINCRISTINA NA ZUBNU PULPU I DENTIN PACOVA

ANKICA JAKOVLJEVIĆ

SADRŽAJ

Cilj ovih ispitivanja je da se u funkciji vremena prate pozni efekti višekratnih doza citostatika Vincristina i promene na zubnoj pulpi i dentinu oba molarna zuba pacova. Kao model ispitivanja korišćeni su pacovi Wistar soja oba pola. Životinjama je subkutano injiciran Vincristin u pet doza od po 0,1 mg/kg TM. Prva doza je injicirana 7-8 dana po koćenju, a sledeće četiri u razmacima od nedelju dana. Kontrolnu grupu su činile intaktne životinje. Životinje obe grupe su žrtvovane dekapitacijom šest i devet meseci po koćenju. Materijal je uobičajenom tehnikom pripremljen za histološku analizu.

Rezultati pokazuju promene u zubnoj pulpi i dentinu prvog i drugog molara životinja tretirane grupe u odnosu na kontrole. Histološkom slikom zubne pulpe dominira atrofija sa promenama na krvnim sudovima i perivaskularnim edemima, zatim redukcija odontoblastnog sloja i hijalinizacija vezivnih vlakana. Dentin je neujednačenog formiranja i kalcifikacije, sa izraženim nehomogenim inkrementnim linijama i gubitkom strukture. Sve promene u zubnoj pulpi i dentinu su u funkciji vremena jače izražene.