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AUTORADIOGRAPHIC INVESTIGATION OF THE EFFECTS OF LOW-DOSE COLCHICINE ON DENTINOGENESIS IN RAT INCISORS

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ABSTRACT: Twelve wistar rats received 0.5 mg/kg colchicine (CLC) i.v. Three animals were sacrificed 5 h, 24 h, 3 days and 7 days after the injection. Ninety minutes before sacrifice all animals received tritiated proline intraperitoneally. Autoradiograms of sections from the maxillary incisor were subjected to quantitative, and statistical analysis. These results revealed an increased secretory activity in the odontoblasts in the more incisal parts of the tooth, indicating a stimulatory effect of CLC on collagen production and secretion after 3 days.

KEYWORDS: Dentinogenesis; colchicine; collagen.

INTRODUCTION

It has been shown that colchicine (CLC) causes depolymerization and/or inhibition of polymerization of microtubules[3, 4]. However, low, non-toxic doses of the drug seem to have a stimulatory effect on synthesis and secretion of several substances, including collagen[2, 19, 26].

In a previous study NOGUEIRA et al[22] showed that CLC in a dose of 0.5 mg/kg b.w. produced an incremental line in the dentin of rat incisors, in addition to some irregular, globular dentin, indicating a mild functional derangement of the odontoblasts. The purpose of the present study was to evaluate the functional alterations in-

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duced in the odontoblasts by this low dose of CLC by utilizing statistical analysis of autoradiograms from incisors. Tritiated proline was used as a marker for collagen and its precursors.

MATERIAL AND METHODS

Twenty-four rats, approximately 60 days old and with an average weight of 160 g, were divided into one experimental (A) and one control group (C) of 12 animals each.

Group A was injected in the tail vein with 0.5 mg/kg of CLC dissolved in isotonic saline solution, under ether anesthesia. The injected volume was 0.5 ml/200 g b. w. The control group received the equivalent volume of pure saline solution. Three animals of each group were sacrificed 5 h, 24 h, 3 and 7 days after the injection. All animals (group A and C) received an intraperitoneal injection of 1.5 u Ci/g b. w. of tritiated proline (L-5-3H proline, s. a. 24 Ci/mmol, The Radiochemical Centre, Amersham, England) 90 min. before sacrifice. The maxillae were freed from soft tissue and subjected to fixation and demineralization as previously described[22]. The section mounted on glass slides were dipped in liquid photographic emulsion (Ilford K-5) and developed according the liquid emulsion technique described by KOPRIWA, LEBLON[15].

After development, fixation and washing, the sections were stained with hematoxylin-eosin and studied in the light microscope.

The odontoblast population, as seen in longitudinal sections of the incisors, was divided into 5 labial (la_{1-5}) and 3 lingual (li_{1-4}) sectors, as previously described by STENE[28]. The quantitative analysis of the autoradiograms was based in the incorporation of tritiated proline in odontoblasts and predentin evaluated by counting the silver grains over this structure both in experimental and control animals. A grid delimitating squares of 81 μm^2 was used with an oil immersion (loox) objective. Twenty squares in each of the 9 sectors of the incisors were included.

The statistical analysis was performed using the quantity of silver grains in the 5 labial and 4 lingual sectors of the incisor. The descriptive statistical analysis indicated anassymetrical distribution and non-parametric analysis using the signal test was performed. The basis for these analysis were the proportional diferences obtained in the distribution when cutted in the median point[27]. The analysis were performed in two stages: a) a study of the control groups using median values and testing equality between the observation times, and b) a comparison between the control groups and the experimental groups. Five per cent was used as significante level.

RESULTS

This and previous experiments have shown that tritiated proline administered 90 minutes before sacrifice is incorporated into the pulpal two-thirds of the labial pre-

dentin and in entire predentin layer on the lingual side of the incisors. The odontoblasts showed a weak but definite labelling. The following description refers to differences as regards the quantity of granules over the odontoblasts in the control and experimental groups. The initial study of the control groups indicated some variation, when the observation-time was used as main variable and for this reason, comparisons between the differents experimental groups and the control groups were done. Table 1 presents the general median values for the control and the experimental groups together. Table 2 shows the values of signal test obtained from comparisons between each of the control and the respective experimental groups. Table 3 was designed to show the "trend" (+ or -) in each of the incisors sectors from the experimental animals, to reveal either more or less granules than the corresponding control animals.

TABLE 1 – General median values of radioisotope granules in odontoblasts for control and experimental groups

Tooth	Observation-time			
sectors	5 h	24 h	3 days	7 days
La_1	20	18.5	18	14.5
La_2	21	20	18	15
La_3	19	18	16	11
La_4	21	16	14	9
La_5	15	14	12.5	9
Li_1	17	14	13	9
Li_2	25	15	15	12
Li_3	23	16	15	11
Li_4	16	15	14	10.5

TABLE 2 – Values of X^2 in signal tests, using the total number of radioisotope granules of the odontoblasts, obtained from comparison between control and experimental groups

Tooth	Observation-time			
sectors	5 h	24 h	3 days	7 days
La_1	0.01	0.33	3.92**	7.87*
La_2	7.75*	0.31	2.25	8.36*
La_3	2.52	0.91	12.67*	1.78
La_4	4.86*	2.06	12.30*	0.72
La_5	0.46	0.73	1.92	2.61
Li_1	0.72	0.41	0.006	0
Li_2	0.41	0.08	7.33*	0
Li_3	1.96	0.01	11.38*	2.73
Li_4	5.12*	0.27	11.16*	9.84*

* $p < 0.01$
** $p < 0.05$

TABLE 3 – Increase (+) or decrease (-) of the radioisotope granules numbers in odontoblasts of experimental groups when compared with controls confirmed by signal test. Significance level included as p ⟨

Rat incisors Tooth sectors	Observation-time							
	5 h		24 h		3 days		7 days	
	trend	p	trend	p	trend	p	trend	p
La_1	0	n.s.	0	n.s.	-	0.05	+	0.01
La_2	+	0.05	0	n.s.	-	0.05	+	0.01
La_3	-	0.05	0	n.s.	-	0.01	+	0.05
La_4	-	0.05	0	n.s.	-	0.01	0	n.s.
La_5	0	n.s.	0	n.s.	-	0.05	-	0.05
Li_1	0	n.s.	0	n.s.	0	n.s.	0	n.s.
Li_2	0	n.s.	0	n.s.	-	0.01	0	n.s.
Li_3	+	0.05	0	n.s.	-	0.01	-	0.05
Li_4	+	0.05	0	n.s.	-	0.01	-	0.01
				n.s.				

n.s. = not significant

After 5 h observation-time the odontoblasts in sectors la_2, li_3 and li_4 in the experimental animals showed a tendency to incorporate more tritiated proline than those in the corresponding controls. The difference was statistically significant. On the other hand, the odontoblasts in sectors la_3 and la_4 in the experimental animals incorporated significantly less of the radioisotope than those in the control group (Table 2 and 3). After 24 h observation-time the incorporation of tritiated proline in the odontoblasts showed no difference between the control and the experimental groups (Table 2 and 3). After 3 days observation-time the odontoblasts of the experimental group incorporated less radioisotope than those of the corresponding controls in all sectors of the incisors, except the li_1. However, the level of significance was higher in the incisal sectors of the tooth ($p < 0.01$). After 7 days observation-time the odontoblasts of sectors la_1, la_2 and la_3 showed a tendency to incorporate more radioisotope than controls. In sectors la_5, li_3 and li_4 the opposite was seen, with a tendency to weaker labelling of the odontoblasts in the experimental than in the control group.

DISCUSSION

The main site of CLC is the microtubules, a citoplasmic component known to be involved in cellular secretory mechanisms[1, 2, 5, 9, 12, 14, 17, 18, 20, 30, 31] including the secretion of collagen[6, 7, 8, 11, 13, 19, 20, 22, 23, 25]. We have previously observed that CLC in doses of 0.5 mg/kg produces no morphological alterations in the dentin[21, 22]. However, arrested metaphase cells were present in the germinative part of the pulp,

indicating that the microtubules involved in the formation of the mitotic spindle are more susceptible to the effect of CLC than those involved in secretory processes, in the more mature dentin-producing odontoblasts. This is in agreement with other reports[10].

The fact that the odontoblasts 5 h after administration of CLC showed partly more, and partly less, incorporation of proline than the corresponding controls, could be due to the fact that the binding of CLC to microtubules apparently takes more than 4 hours[24], sharply contrasting with the properties of other antimicrotubular agents such as vincristine and vinblastine. The latter two, bind to tubulin, the subunit of microtubules, in less than 5 minutes. In addition, one should take into consideration that the odontoblasts in the different sectors of the incisors probably vary in their susceptibility to antimicrotubular agents, as indicated after administration of vincristine[28] and CLC[21].

After three days the odontoblasts in the experimental animals were less intensely labelled than the corresponding controls, but the results after 7 days are inconclusive. As no difference was seen after 24 hours, this might indicate that low doses of CLC could exert a stimulatory effect on collagen secretion after 3 days, depleting odontoblasts of tritiated proline, concurring with other observations[2, 19, 26]. An alternative explanation is that this difference reflects a compensatory increase in collagen production, after an initial inibitory effect of CLC, as held by KUDO[16], who suggested the duration of CLC's effects in the body to be less than 14 hours.

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RESUMO: Doze ratos receberam 0,5 mg/kg de colchicina (CLC) por via endovenosa. Cada três animais foram sacrificados após 5 h, 24 h, 3 dias e 7 dias após a administração de CLC. Noventa minutos antes do sacrifício, todos os animais receberam prolina triciada por via peritoneal. Os radioautogramas dos incisivos superiores foram submetidos a contagem e, posteriormente, foram analisados estatisticamente. Os resultados revelaram aumento na atividade secretora dos odontoblastos na porção mais incisal do dente, indicando um efeito estimulatório de CLC sobre a produção e secreção de colágeno após 3 dias de sua aplicação.

UNITERMOS: Dentinogênese; colchicina; colágeno.

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