On Collagen Biosynthesis as Affected by Prednisolone Acetate and Fluoride in Rat Molar Teeth

DONG-KYUN CHEONG,* BONG-HO KIM and BYUNG-MOO MIN**

Departments of Dental Pharmacology and Oral Biochemistry***
College of Dentistry, Seoul National University, Seoul 110, Korea

[**Abstract**] Effects of prednisolone and fluoride on the collagen biosynthesis in molar teeth were studied with ovariectomized Sprague-Dawley rats. Effects of administration of prednisolone acetate and fluoride to the ovariectomized animals were studied, comparing with the control. Each group was injected with ^{3}H-proline and sacrificed, followed by removals of molar teeth by manual dissection. Separations and quantitative analyses of proline and hydroxyproline were performed by means of thin layer chromatography; and radioactivities of the separated amino acids were assayed by liquid scintillation counter. Normally the incorporation of ^{3}H-proline into collagen in 3rd molar crown was higher than that of 1st and 2nd molar crown, but very lower than that of 1st and 2nd molar root, and collagen turnover rate of 3rd molar crown was almost similar to that of 1st and 2nd molar crown. In the tissues tested from ovariectomized rats, the incorporation rate of ^{3}H-proline into hydroxyproline was markedly decreased than that of the former. Decreased in ^{3}H-proline incorporation into collagen in ovariectomized rats was markedly antagonized by fluoride, but not influenced by prednisolone acetate in the tissues tested.

Collagen serves as the principal structural protein in tissues with a diversity of functions and metabolic properties. It is presumed that collagen metabolism like this varies with the different tissues and functions in the same species, and will be affected various systemic factors: nutrition, hormones, infection and inorganic salts.

The teeth of rats are monophyodont. The dental formula is $\frac{1.0.0.3}{1.0.0.3}$. The molars erupt about the 19th(M_1), 22nd(M_2), and 35th to 40th(M_3) day postnatally (Schour& Massler, 1949; Mohr, 1952). First molar has five roots, 2nd molar four, and 3rd molar three in the upper jaw; in the lower jaw, four, three, and three, respectively (Schour & Massler, 1949; Schneider, 1970). The row of three tightly apposed molar teeth of each quadrant increases in both length and width by about 10% in older(from 300 days) rats (Donaldson & French, 1927).

The biosynthesis of collagen has been extensively studied in many tissues by following up the incorporation rate of ^{3}H-proline(Cohen *et al.*, 1977; Orlowski, 1977; Park & Cheong, 1980). Hydroxyproline of collagen is derived from the hydroxyration of peptide bound-proline during the synthesis or the release of the nascent chains of collagen peptides(Cardinale & Udenfriend, 1974). The conversion of proline into hydroxyproline is a measure of the collagen synthesis (Firschein, 1967).

Changes in collagen synthesis in many tissues following administration of prednisolone acetate and fluoride have been described (Chvapil, 1959; Goldhaber, 1967; Houck, 1968; Cohen *et al.*, 1977; Park & Cheong, 1980). However, it is rarely reported the collagen synthesis in rat molar teeth.

The following investigation were designed to elucidate the rate of the collagen synthesis by means of the incorporation of ^{3}H-proline into ^{3}H-hydroxyproline as a parameter of the collagen synthesis, and to determine the effects of prednisolone acetate and fluoride on the collagen turnover in rat molar teeth.

MATERIALS AND METHODS

Animals

The female Sprague-Dawley rats weighing 140 ±20 gm which were supplied from S. N. U. animal house were used in the present experiment. After the rats were treated with sodium pentobarbital (300 mg/kg of body weight) followed by ovariectomy, they were fed for seven days prior to subjecting the animals to the present experiment.

The animals were divided into normal and ovariectomized groups. The latter was subdivided into 3 groups; control, prednisolone acetate and fluoride administered groups, each being consisted of 12 rats.

The ovariectomized groups were injected intramuscularly prednisolone acetate and were fed fluoride for six days, starting from the 7th day after ovariectomy. Each group received intraperitoneally 0.7 μCi of ^{3}H-proline per gm of body weight. After 1 day, a half of rats of each group was sacrificed, the remaining half sacrificed 6 days later during which prednisolone acetate and fluoride were injected and fed daily, respectively. The molar teeth were removed and divided into 3 groups; lst and 2nd molar crown, lst and 2nd molar root, and 3rd molar crown, and their wet weights were measured immediately.

All the rats except those of normal group were ovariectomized, and one group was injected prednisolone acetate (Chong Keun Dang Pharm. Co., Korea, 20 mg/kg of body weight) once a day intramuscularly and the other group was fed sodium fluoride (Yoneyama Pharm. Co., 50ppm of fluoride) in the drinking water. Ovariectomized rats without any agents were used as control.

Detection of proline and hydroxyproline by T.L.C.

The methods used have been described in detail (Min *et al.*, 1981). The samples were collected by manual dissection and stored in a desiccator at −20°C until they were analyzed. The samples from each group were pooled, and duplicate analyses were performed and averaged.

The two amino acids were separated by two-dimentional thin layer chromatography and analyzed quantitatively using Thin Layer Chromatogram Scanner (Aloka Japan Radio. Co.)

To detect the radioactivities of proline and hydroxyproline separated by T.L.C., their spots were collected and subjected to counting by Liquid Scintillation Counter (Nuclear Enterprise Co.).

RESULTS AND DISCUSSION

It was shown that incorporation of ^{3}H-proline into collagen of 3rd molar crown in the non-treated normal rats was higher than that of lst and 2nd molar crown, but very lower than that of lst and 2nd molar root. And collagen turnover rate of 3rd molar was observed slightly rapid as well than that of lst and 2nd molar crown (Table 1).

It is presumed that collagen synthesis and turnover rate varies not only in species, but also at different stage of development and in different tissues in the same species. The rapid rates of collagen biosynthesis and turnover may be directly related to the rate of growth of the rat tissues

(Kao *et al.*, 1961; Orlowski, 1977; Park & Cheong, 1980). Some workers reported in the study of the changes in collagen biosynthesis with upper and lower incisors of the young female rats that incorporation rate of ^{3}H-proline into collagen was greater in root than in crown, and collagen turnover rate in root was observed rapid as well than that of crown (Park & Cheong, 1980). Other studies found also that incorporation and collagen turnover in pulp is higher than in gingiva or periodontal ligament of the rat incisor. This rapid turnover may be directly related to the rate of eruption of the rat incisor(Orlowski 1977).

Therefore, if we are considering that the molars erupt about 19th(M_1), 22nd(M_2), and 35th to 40th(M_3) day postnatally, it was understanding that the difference in rate of incorporation and turnover between lst and 2nd, and 3rd molar was due to the divergence of maturity. Also, we presumed that the migration and accumulation of ^{3}H-hydroxyproline from pulp tissues to crown increased the specific activity in hydroxyproline of the growing dental hard tissues at the 6th day.

In ovariectomized rats, the incorporation of ^{3}H-proline into collagen was markedly decreased than that of the non-treated normal rats in all the tissues (Table 1).

On the other hand, in ovariectomized rats injected with prednisolone acetate, the specific activity of ^{3}H-hydroxyproline in lst and 2nd molar crown and root was moderately higher and lower than that of control, respectively. But, the specific activity of ^{3}H-hydroxyproline in 3rd molar crown was almost similar to that of control (Table 2).

Some investigaters recently reported that the decreased rate of ^{3}H-proline incorporation into collagen in ovariectomized rats was not affected by prednisolone acetate in the rat incisors(Park & Cheong, 1980). It was shown that diminished hydroxyproline formation in systemically cortisone treated rats and chick embryos when measuring ^{14}C-proline incorporation (Chvapil, 1959). On the other hand, several studies showed that corticosteroids enhaned collagenase activity(Houck, 1968). Other studies strongly suggested in the study of corticosteroids usually did not alter

Table 1. *Incorporation of ^{3}H-proline into non-ovariectomized and-ovariectomized group*

+Normal; Non-ovariectomized group. Control; Ovariectomized group

*Specific Activity, cpm/μg of amino acids; – ⟨0.05

Groups+	Tissue	Days after ^{3}H-Pro inj.	Specific Activity* Pro	Hyp
Normal	lst and 2nd molar crown	1	2.78	0.84
		6	2.05	0.75
	3rd molar crown	1	4.75	0.98
		6	2.92	0.52
	lst and 2nd molar root	1	7.89	3.38
		6	5.98	5.41
Control	lst and 2nd molar crown	1	1.56	—
		6	1.40	—
	3rd molar crown	1	1.35	—
		6	4.64	0.13
	lst and 2nd molar root	1	6.54	2.25
		6	5.07	2.47

Table 2. *Incorporation of ^{3}H-proline into prednisolone acetate administered group in ovariectomized rats*

+Control; Ectomy+Predni.; Ovariectomized group injected with prednisolone acetate.

Groups+	Tissues	Days after ^{3}H-Pro inj.	Specific Activity* Pro	Hyp
Control	lst and 2nd molr crown	1	1.56	—
		6	1.40	—
	3rd molar crown	1	1.35	—
		6	4.64	0.13
	lst and 2nd molar root	1	6.54	2.25
		6	5.07	2.47
Ectomy + Predni.	lst and 2nd molar crown	1	3.76	0.47
		6	3.00	1.08
	3rd molar crown	1	1.72	0.22
		6	6.74	0.19
	lst and 2nd molar root	1	5.28	0.11
		6	1.58	1.31

collagen synthesis but enhanced collagen degradation (Cohen *et al.*, 1977).

In present investigation, incorporation of ^{3}H-proline into collagen in the rats which were fed 50 ppm of fluoride in the drinking water was higher than that of control in all the tissues, and collagen turnover rates in these tissues were observed rapid as well that of control(Table 3).

Table 3. *Incorporation of ^{3}H-proline into fluoride administered group in ovariectomized rats*

+Control, Ectomy+Fluoride; Ovariectomized group fed with 50 ppm of fluoride in the drinking water.

Groups+	Tissues	Days after ^{3}H-Pro inj.	Specific Pro	Activity* Hyp
Control	lst and 2nd molar crown	1	1.56	—
		6	1.40	—
	3rd molar crown	1	1.35	—
		6	4.64	0.13
	lst and 2nd molar root	1	6.54	2.25
		6	5.07	2.47
Ectomy + Fluoride	lst and 2nd molar crown	1	1.92	1.09
		6	0.55	—
	3rd molar crown	1	0.92	1.10
		6	2.78	0.31
	lst and 2nd molar root	1	5.72	4.42
		6	3.69	1.48

Some workers reported in the study of bone collagen synthesis in tissue culture that the effect of fluoride at lower concentrations either does not significantly change the rate of bone collagen synthesis, or actually increase it slightly. And bone collagen synthesis was reduced at the higher concentrations of fluoride, bone collagen resorption was much more affected than bone collagen synthesis, and there was little or no effect on the rate at which the newly synthesized collagen was degraded (Goldhaber, 1967). Also as previously reported that the first effect of fluoride tends to decrease intracellular uptake of ^{3}H-proline, with the result that collagen synthesis would be eventually decreased.

In the view of the point, the results of this investigation was similar to that of others.

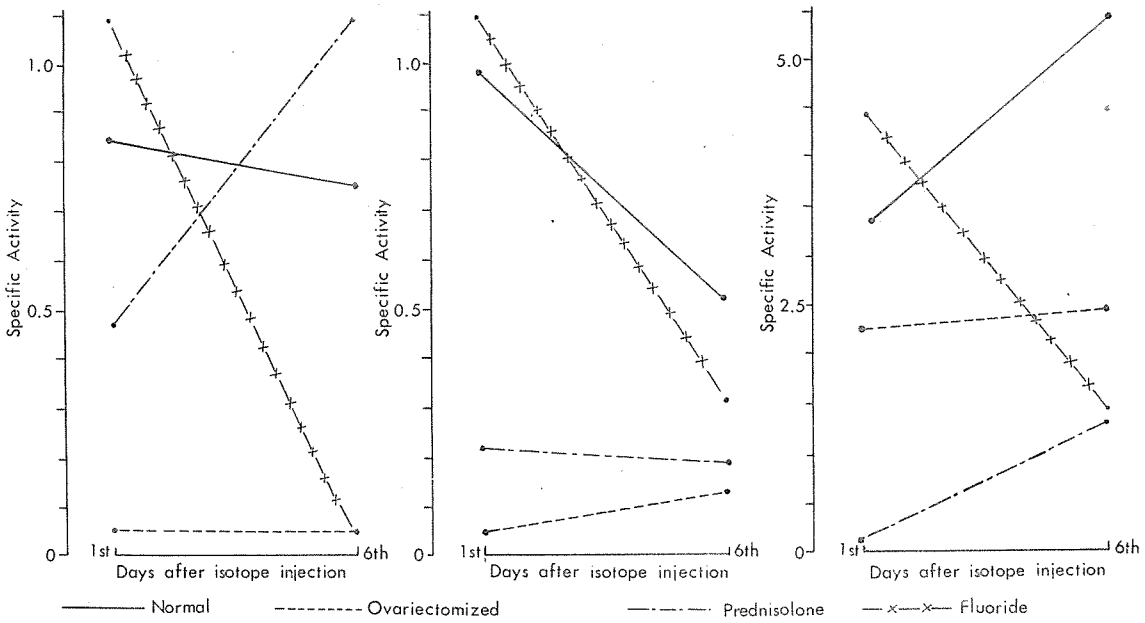

Fig. 1 *Specific Activity in hydroxyproline of the lst and 2nd molar crown*

Fig. 2 *Specific Activity in hydroxyproline of the 3rd molar crown*

Fig. 3 *Specific Activity in hydroxyproline of the lst and 2nd molar root*

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