Age dependency of cyclosporin A-induced gingival overgrowth in rats

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Abstract

Effects of age on cyclosporin A- (CsA) induced gingival overgrowth were investigated in Fischer rats. Rats 15, 30, 45, and 60 days old were fed a diet containing cyclosporin A (120–200 μg/g) for 40 days. Gingival overgrowth was estimated by measuring the gingival sulcus depth with a thin color slide probe under a stereoscopic dissecting microscope. The youngest group (15 days old) of rats developed the most significant gingival overgrowth (buccal sulcus depth of mandibular first molar, CsA-treated rat/untreated rat: 875 ± 78/275 ± 25 μm, mean ± SD, $P < 0.001$), followed by those in which CsA treatment was initiated at age 30 days (505 ± 29/267 ± 56, $P < 0.001$) and 45 days (400 ± 45/267 ± 25, $P < 0.001$). Significant gingival overgrowth was not induced in rats when CsA treatment had been started at age 60 days (310 ± 38/292 ± 18). Average body weight gain of CsA-treated rats during this experiment period was not different from untreated rats of the same age group. These results suggest that CsA-induced gingival overgrowth in rats is age dependent. (Pediatr Dent 15:414–17, 1993)

Introduction

Cyclosporin A (CsA) is an immunosuppressive agent most widely used to control rejection in human organ transplant.[1–3] CsA selectively suppresses helper T-cell function without interfering with the B-cell function.[4] However, CsA is associated with several adverse side effects, such as nephrotoxicity, hepatotoxicity, and gingival overgrowth.[5–7] This gingival overgrowth does not develop in all the patients receiving CsA; incidence ranges from 25 to 81% in bone-marrow or kidney transplant patients.[7–12] This variable incidence of CsA-induced gingival overgrowth in humans may be due to individual differences of drug susceptibility. This may be dose related,[13] however, Wysocki[8] and Pernu[14] reported that there was no correlation between daily drug dose or serum level and the degree of gingival overgrowth. Hassell et al.[15] suggested the possible presence of two groups of fibroblasts depending upon responses to CsA, namely responder fibroblasts and nonresponder fibroblast. Although the mechanism of this gingival overgrowth is still not clear, it is considered from clinical findings that such drug-induced gingival overgrowth caused by CsA or phenytoin therapy appears more frequently in younger people than adults. This hypothesis is also supported by the experiment of Kitamura et al.[16] because they reported 100% incidence in young Fischer rats (Charles River Japan Inc. Osaka, Japan).

The purpose of this study is to clarify the relationship between induction of CsA gingival overgrowth and age in the rat model.

Methods and materials

Animals and diets

Forty male, specific pathogen-free Fischer rats of four different ages, 15 (Group I E and I C), 30 (Group II E and, II C), 45 (Group III E and III C) and 60 (Group IV E and IV C) days old, were used for the experiment. Ten rats of each age group were divided randomly into an experimental group (CsA treatment) and a control group. They were reared in a clean room at 25°C in an animal facility of Osaka University Faculty of Dentistry and fed an ordinary powdered diet (Diet CE-2, Clea Japan Inc., Osaka, Japan). Experimental groups (I E, II E, III E, and IV E) were fed the diet containing Cyclosporin A (120 μg/g weight; provided by Sandoz Pharmaceuticals Ltd., Basel, Switzerland: CsA Lot No. 8706601, pure powder with 0.8% moisture) for the first 10 days and 200 μg/g thereafter to the end of experiment, and the control groups (I C, II C, III C, and IV C) were fed the same diet without the CsA by following CsA treatment schedule used in a previous study.[16] The rats were weighed weekly and given the diet and drinking water *ad libitum* during the 40-day experimental period.

Estimation of gingival overgrowth

At the end of the experimental period, all the rats were sacrificed under Nembutal® anesthesia (Abbott Laboratories, North Chicago, IL), and mandibles with surrounding soft tissues were removed and then soaked in neutralized 10% formalin for two days. Gingival overgrowth was estimated by measuring the depth of buccal and lingual gingival sulcus with thin color slide probes under stereoscopic dissecting microscope.[17] This measurement was performed by only one examiner (IM) throughout the experiment, and the examiner was provided samples in a random and blind manner. The experimental protocol had been approved by Animal Experiment Committee of Osaka University.

Histological examination

Two mandibles from each group of rats were decalcified in 10% formic acid for one week, then embedded in paraffin wax. Thin sections, 4 μm in thickness, were cut in buccolingual direction and stained with hematoxylin and eosin for a light microscopic examination.

Statistical analysis

Statistical differences of probing depth between CsA treatment and control group of the same age rats were analyzed in all experiments by Student's *t*-test. Also, the statistical analysis within CsA-treated groups or control groups was determined by an analysis of variance (ANOVA) and post-hoc tests.

Results

Gingival overgrowth

Average body weight gain of each group at the end of the experiment was 132 ± 8 g (Mean ± SE, Group I E), 157 ± 7 g (Gr. II E), 153 ± 3 g (Gr. III E), and 96 ± 4 g (Gr. IV E) in experimental groups, and 142 ± 6 g (Gr. I C), 166 ± 5 g (Gr. II C), 153 ± 4 g (Gr. III C), 101 ± 4 g (Gr. IV C) in control groups. No significant difference ($P > 0.05$) was found between CsA-treated and control rats of the same age group by Student's *t*-test, indicating that CsA did not affect the rat growth in this experiment.

Macroscopic gingival overgrowth was induced around mandibular molars of the rats treated with CsA as shown in the Figure. The most remarkable gingival overgrowth was found in buccal gingiva of Group I E and the top of buccal overgrown gingiva was sometimes beyond the level of occlusal plane. Gingival overgrowth was more severe in the buccal than in the lingual in all experimental groups except IV E. In Group IV E no marked gingival overgrowth was detected in comparison with control. On the other hand, gingival tissue around molars of the maxilla did not show apparent macroscopic changes in rats of both CsA-treated and untreated groups.

The Table shows the average depth of buccal and lingual gingival sulci around the first and second mandibular molars. In this study, gingival overgrowth was measured around the first and second molars; measurement on the third molar was technically difficult

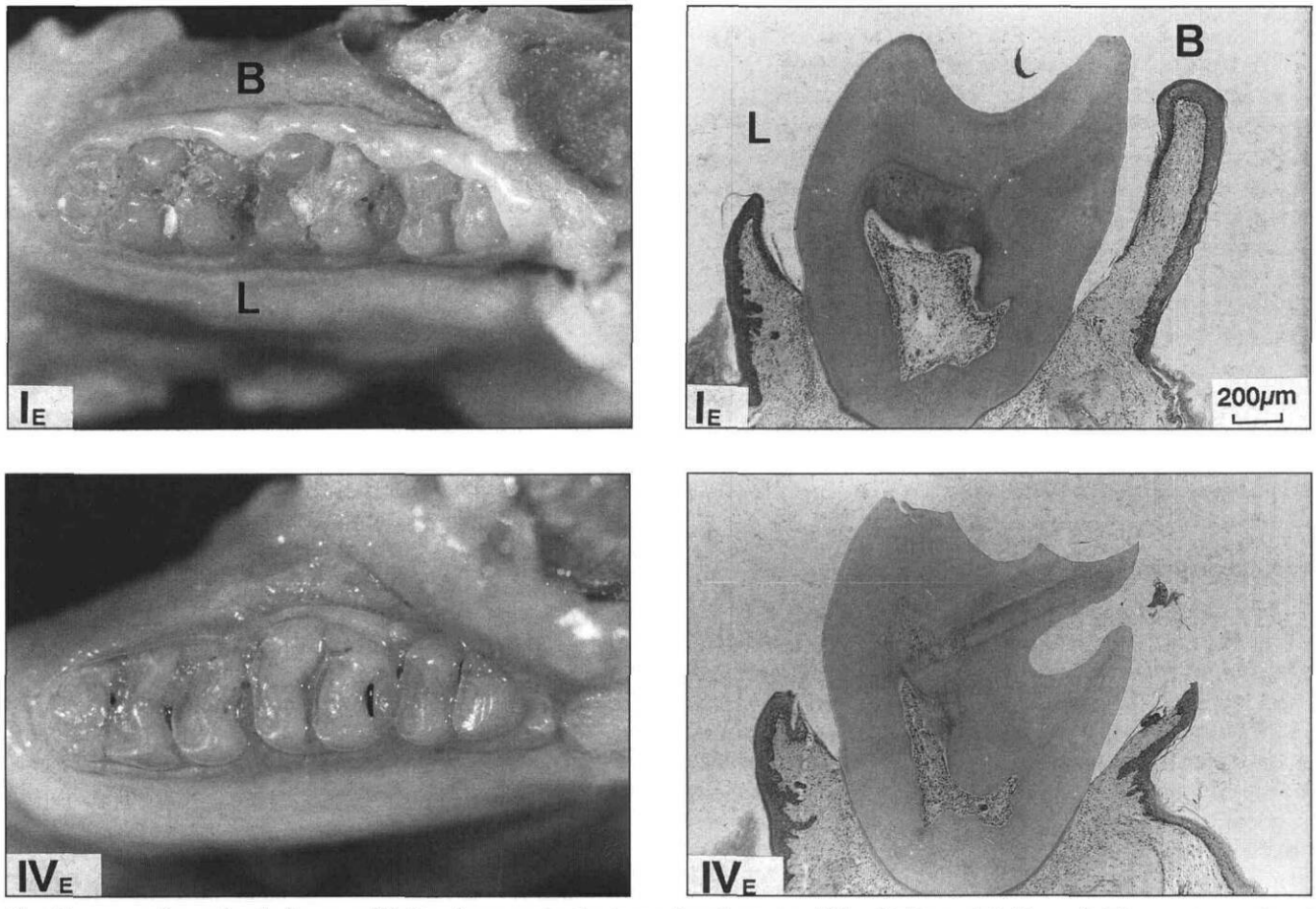

Fig. Macroscopic occlusal views and light microscopic photographs of rat mandibles (B: buccal, L: lingual). The most prominent gingival overgrowth is seen in group I E. Gingivae of Group IV E were not different from untreated control rats. Buccolingual sections of rat mandibles were stained with hematoxylin and eosin (original magnification 12.5x). Gingival overgrowth was more severe in buccal gingiva (B) than in lingual (L) in all samples.

Table. Effects of rat age on cyclosporin A-induced gingival overgrowth

Group	*Rat Age*‡	*Buccal*		*Lingual*	
		M_1	M_2	M_1	M_2
IE	15 days	875 ± 104§•••,†††,‡‡‡	800 ± 110•••,†††,‡‡‡	367 ± 41•••,†,‡‡‡	333 ± 52•••,†,‡‡‡
IIE	30	505 55•••,†††,‡‡‡	485 63•••,†††,‡‡‡	315 41•••,†,‡‡‡	285 41•••,†,‡‡‡
IIIE	45	400 52•••,†††,‡‡‡	370 42•••,†††,‡‡‡	260 46•,†,‡‡‡	240 46••,†,‡‡‡
IVE	60	310 57	245 55	215 34	205 37
IC	15	275 27	233 26	208 38	175 27
IIC	30	267 61	250 32	208 49	183 26
IIIC	45	267 26	225 27	200 32	175 27
IVC	60 days	292 ± 21	242 ± 20	208 ± 20	175 ± 27

‡ The number indicates the rat age (days old) that the CsA treatment was initiated.

§ The numbers are the arithmetic mean ± standard deviation of buccal or lingual depth (μm) of gingival sulcus around the first (M_1) and second (M_2) molars of rat mandibles.

•, ••, ••• Significant difference from each control was detected by Student's *t*-test at $P < 0.05$ (•), $P < 0.01$ (••), and $P < 0.001$ (•••).

†, ††, ††† Significant difference between groups at the same site was determined by ANOVA and post-hoc test at $P < 0.05$ (†), $P < 0.01$ (††), and $P < 0.001$ (†††).

due to interference by the mandibular ramus and was also quite unstable. In the control group, there was no effect of age on gingival sulcus depth at any site. By contrast, the depth of gingival sulcus in the experimental group was greater than that in control groups in all sites except for Group IV E. The most severe gingival overgrowth was induced in the rats of Group I E followed by groups II E, III E, and IV E (in order) at any site. Buccal sulci of Group I E rats were almost three times as deep as those of Group IV E. Lingual gingiva also showed the same tendency as the buccal. As shown in the Table, sulcus depths of these four groups were significantly different from each other irrespective of measuring sites by ANOVA analysis. It is clear that rats treated with CsA earlier in their life developed more severe gingival overgrowth. No significant difference ($P > 0.05$) was found in sulcus depths between the rats of CsA-treated (IV E) and nontreated (IV C) groups since those rats were 60 days old at the beginning of the experiment.

Histological findings

Histological examination of gingival tissue in the experimental groups (Fig) showed the marked buccal gingival overgrowth of Group I E rat in a striking contrast to those of Group IV E. A few inflammatory cells were found in some tissue sections, however, the overgrown gingival tissue consisted mainly of connective tissue components such as fibroblasts, capillaries, and intercellular fibers. Remarkable change was not found histologically in the lingual gingival tissue from any group.

Discussion

There have been only a few comprehensive epidemiological studies on the relationship between incidence of drug-induced gingival overgrowth and the age of man or animal. In the human, it is reported that the incidence of CsA-induced gingival overgrowth has variably been in the range of 25 to 81%.[6,10] On the other hand, the average incidence of PHT-induced gingival overgrowth is about 50% in patients taking this anticonvulsant.[18] From the present experiment and our previous studies of CsA[16] and phenytoin,[17] the incidence of drug-induced gingival overgrowth was 100% in young Fischer rats. Hassell described in his review article[6] that "phenytoin-induced gingival overgrowth is a lesion primarily of young individuals." On the other hand, from Tyldesley's observation,[9] it is more frequent in females (38%) than males (17%), but is not age dependent.

In humans, it has been suggested that phenytoin-[19] or CsA-[4,11,20] induced gingival overgrowth appears in younger subjects more frequently. This study also suggests that the development of gingival overgrowth induced in rats treated with CsA may be age dependent. Since rats become adult about 8 weeks after birth,[21] our study indicates that CsA could not induce the significant gingival overgrowth in adult Fischer rats. Though the exact amount of CsA intake of each age rat was unclear in this experiment because of the method of CsA administration, weight gain of all four groups was not different from untreated control rats. It is suggested a similar result of age dependency of CsA-induced gingival overgrowth using beagle dogs without showing the drug level in their serum.[6] As to blood level of CsA and the gingival overgrowth, several reports showed no correlation in humans,[9,11,14] though Seymour et al.[10] reported opposite results. In our additional experiment we confirmed that CsA serum concentration showed no significant difference among several aged rats fed a diet containing the same dose of CsA (101 ±

33.8 ng/ml, 55 days old; 86.0 ± 19.2 ng/ml, 75 days old; 95.0 ± 19.2 ng/ml, 95 days old; 108 ± 19.4 ng/ml, 115 days old). These data suggest CsA serum level is maintained equally by our CsA administration method regardless of rat body weight or age. Our results suggest that the drug-induced gingival overgrowth may be influenced not only by blood level of CsA but also by age.

Recently Weinstein et al.[22] suggested that glycosaminoglycan in rat gingival proteoglycans decreased with age. Therefore, the results presented here might reflect such changes of gingival tissue components if gingival overgrowth was induced as a result of accumulation of intercellular glycosaminoglycans or fibroblasts and collagen.[12] Gingival overgrowth might not be dependent on serum CsA level or daily oral dose, but "responder" and "nonresponder" subjects to CsA in terms of gingival overgrowth might be present as McGaw suggested.[23] Another possibility of this low incidence of CsA-induced gingival overgrowth in humans compared to inbred rats may be due to the genetic heterogeneity. As to rats, we have induced the gingival overgrowth in young rats of two different inbred strains, Fischer and Sprague-Dawley™ (Charles River Laboratories) rats at the same level.[24] Guggenheim et al.[25] studied the effects of CsA on periodontal tissue in rats infected with *Actinomyces viscosus* and induced alveolar bone loss in rats (28 days old, Sprague-Dawley) irrespective of the CsA treatment. However, no information concerning drug-induced gingival overgrowth was given in this report.

Further controlled animal experiments are required to elucidate a more precise mechanism of CsA-induced gingival overgrowth.

Conclusions

1. Gingival overgrowth (increased sulcus depth) was induced in 15-, 30- and 45-day-old rats after CsA treatment for 40 days. However, significant change was not found in 60-day-old rats by CsA treatment.

2. The most severe gingival overgrowth was found in rats that had the CsA treatment started at 15 days of age, followed by 30- and then 45-day-old rats.

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