Proliferation of epithelial rests in periodontal ligament of rats induced by 1-butyl-1-nitrosourea

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Odontogenic tumor is one of the tumors of great significance in the oral region. The process of its development remains obscure. It seems very advantageous in the investigation of odontogenic tumor to establish experimental models in laboratory animals. However, experimental attempts of induction of odontogenic tumor have not been very successful. In recent years, the carcinogenic property of nitroso derivatives has been confirmed in various organs including oral apparatus, and induced odontogenic tumor and its related lesions have been reported in several papers during carcinogenic experiments with this chemical. It is generally thought that odontogenic epithelium (reduced enamel epithelium, fragmented dental lamina, epithelial rests in the periodontal ligament) may well play a role in the pathogenesis of odontogenic tumors. And the epithelial rests in the periodontal ligament are known to proliferate under certain stimuli. However, the initial stage of epithelial proliferation in odontogenic tumor has not been clarified as yet. The present study was intended to make further detailed observations in this respect.

Material and Method

The rats used in this experiment were Long-Evans strain obtained from the National Institute of Genetics. The animals were 1 month old and both sex were used. 1-butyl-1-nitrosourea (BNU) was administered by gastric intubation (300 mg/kg) 4 times at biweekly intervals to each rat. Total doses of BNU per individual rat were 1200 mg/kg. Complete autopsies were performed on all animals killed or found dead and paraffin embedded sections were made in a routine manner and stained with hematoxylin and eosin. The present report describes only the proliferative changes of the epithelial rests in the periodontal ligament that were induced in Long-Evans rats by BNU.

Result and Discussion

Generally, 2 main types of epithelial masses were observed in the periodontal ligament. There was one small resting type present as small clusters of cells. In addition, a proliferating type was present characterized by more voluminous cell masses. In control rats, a few foci of the resting type were found in the periodontal ligament but there was no proliferating type. In experimental rats, scattering proliferative epithelial masses in various degree in the periodontal ligament were found. These proliferative epithelial masses consisted of columnar epithelial cells with palisade-arrangement located in the peripheral portion and stellate cells located in the central region. The structures had striking resemblance to a tooth germ and several of them consisted of squamous metaplastic cells with or without slight parakeratosis. The epithelial masses were classified by degree of proliferation, i.e. (−): resting epithelial mass without proliferation, (+): epithelial mass with slight proliferation,
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(++) with moderate proliferation, and (###) with marked proliferation. The findings of each grade are shown in Figs. 1, 2, 3, and 4. The proliferating epithelial masses were round to ovoid in shape and surrounded by thin basement membrane-like layer. The larger voluminous proliferating epithelial masses were polyhedral in shape. Cystic degeneration was frequent in the epithelial mass. In most cases with marked epithelial proliferation graded as (###), many epithelial masses were found scattered in the same areas. In accordance with the classification of epithelial proliferation their frequency was recorded. 122 to 305 days after administration of BNU, proliferative changes of epithelial cells showing more than (+) in periodontal ligament were found in 34 of 54 rats (62.3 per cent). Although moderate to marked proliferative changes had a tendency to be more frequent in rats with a long life span, there was no statistical significance between frequency of epithelial proliferative changes and the life span. Most of the proliferative changes of the epithelial rests were found in the molar areas and only occasionally in the incisor areas.

The frequency and morphologic characteristics of the epithelial rests in the rat molar periodontal ligament have been reported by Wentz et al.7), Gilhuus-Moe and Kvan6). They pointed out that the epithelial rests

Photomicrographs showing proliferation of epithelial rests in the molar periodontal ligament (Fig. 1: slight proliferation, Fig. 2: moderate proliferation, Fig. 3 and 4: marked proliferation). Magnification of figures: ×200. Hematoxylin and eosin stain.
were found in 40 to 50 per cent of the rat molar periodontal ligament. However, proliferative changes of more than (+) observed in the present study were not found in their reports. Furthermore, Herrold stated that the epithelial rests were found in the periodontal ligament of hamsters treated with diethylnitrosamine but the epithelial rests were not found in untreated hamsters. In the present study using Long-Evans rats, a few small epithelial rests consisting of pyknotic cells (up to several) were observed in the periodontal ligament of control rats but the larger epithelial masses showing proliferative activity were found in only in BNU administered rats. Therefore, it is thought that the proliferative changes of the epithelial rests in the periodontal ligament are induced by BNU. Many nitroso derivatives are known to be powerful mutagenic and carcinogenic compounds. BNU, one of the nitrosamides, has long been reported to induce tumors in various organs. Although reports of carcinogenesis by BNU in the oral region are not so numerous as in the other organs, the effects induced by BNU include disturbance in odontogenesis, odontogenic tumor and epidermoid carcinoma. The detailed mechanism by which BNU exerts its carcinogenic action and biologic effects are unknown. Tumorigenesis in the oral apparatus by nitroso derivatives was first attempted by Herrold, who reported various types of odontogenic tumors and epidermoid carcinoma by N-methyl-N-nitrosourea. Then, Kohgo, Jurgelsky, Wang et al., and Suzuki reported similar experiments. Herrold concluded that one of the target tissues of nitroso derivatives in oral apparatus is primarily the periodontal ligament and that odontogenic tumors are definitely of epithelial rather than connective tissue origin. The results of our experiment supported Herrold's opinion. In the reports by other investigators, most of the proliferative changes of the odontogenic epithelium in the periodontal ligament were observed in the incisor areas. On the other hand, most of the proliferative epithelial masses were found in the molar periodontal ligament and proliferative findings were more demonstrable than other reports in the present experiment using Long-Evans rats. Discrepancies among the results in each experiment may have originated in the difference of the animal species and strains, or other unknown factors.

Our experiment would not be a complete model for the initial stage of odontogenic tumor but the mode of epithelial proliferative changes in the periodontal ligament may offer some suggestions in interpreting the development of odontogenic tumor. The results and significance of this experiment will be reported further in detail.

References