Original Article

The efficiency of propranolol on occurrence and development of 4-nitroquinoline 1-oxide-induced squamous cell carcinoma of the tongue in rats

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Abstract

Aims: The aim of this study to investigate the efficiency of propranolol on occurrence and development of 4-nitroquinoline 1-oxide (4NQO)-induced squamous cell carcinogenesis of the tongue in rats.

Subjects and Methods: The sample was composed of 27 male Sprague Dawley rats that received 50 ppm 4NQO for 20 weeks in drinking water. Group 1 ($n = 9$) was treated with 50 mg/kg/day propranolol for 20 weeks, Group 2 ($n = 9$), after carcinogenesis inducement for 20 weeks, received propranolol (50 mg/kg/day) for 2 weeks and Group 3 ($n = 9$) received no treatment. At the end of the experimental stage, the tongue specimens were evaluated under a light microscope and categorized as low- or high-risk lesions according to a binary system.

Statistical Analysis Used: The statistical comparison was performed with a likelihood ratio test.

Results: Histopathological analysis revealed the risk of malignant transformation rates as 33.3% in Group 1, 55.5% in Group 2 and 77.8% in Group 3; however, the difference between the groups was not statistically significant ($P > 0.05$).

Conclusion: The results of the study suggest that propranolol has a tendency to preventive effect against carcinogenesis.

Keywords: 4-nitroquinoline 1-oxide, carcinogenesis, chemoprevention, propranolol

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INTRODUCTION

Squamous cell carcinoma (SCC) is the most common type of oral carcinoma and is the eighth most common human malignancy. Although many studies have been conducted on treatment and chemopreventive techniques for SCC of the oral cavity, the 5-year survival rates for patients have been reported as approximately 56%.[1,2]

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Carcinogenesis is a multistep process in which accumulated genetic changes are induced by carcinogenic agents.[3] 4-nitroquinoline 1-oxide (4NQO) is widely used as a carcinogen for the development of oral SCC via damaging DNA similar to other carcinogens present in tobacco.[3] 4NQO can be applied to rats in drinking water or topically, and precancerous or cancerous lesions can

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be produced in this model. One of the advantages of this cancer model is that it simulates the development of human SCC within premalignant white oral lesions; thus, the developmental stages of carcinogenesis can be studied in this model.[4] The growth and metastasis of solid tumors depends on angiogenesis; therefore, suppression of tumor vascularization is one of the main targets for therapy.

Propranolol is a nonselective β-blocker drug widely used for the treatment of problematic infantile hemangiomas.[5-9] The mechanism of therapeutic effect of propranolol on hemangiomas is not known precisely, but may be due to vasoconstriction, inhibition of angiogenesis and induction of apoptosis in capillary epithelial cells.[5,10] Based on the agent's antiangiogenic effect, it has been reported that propranolol may inhibit tumor progression, metastasis and the secretion of angiogenic cytokines in several types of cancers.[11] Propranolol reduces head and neck SCC cell line viability, induces apoptosis and inhibits production of the proangiogenic protein vascular endothelial growth factor.[12] In addition, prostate carcinoma, lung carcinoma and growth of neuroblastoma in mice models were reduced by administration of propranolol.[13-17]

We hypothesized that since oral tissues and oral SCC are rich in vascularization, propranolol, an antiangiogenic agent, might have an inhibitory and/or therapeutic effect on the carcinogenesis stages of oral SCC. Therefore, the aim of this study was to investigate the efficiency of propranolol on occurrence and development of 4NQO-induced squamous cell carcinogenesis of the tongue in rats.

SUBJECTS AND METHODS

Animals and experimental design

All experimental protocols involving animals conformed to procedures describing in the Guiding Principles for the Use of Laboratory Animals. The study was approved by the Animal Committee of Baskent University of Ankara, Turkey (number D-DA 13/04). The power analysis revealed that 9 animals per group were needed to detect clinically meaningful differences between the groups at a power of 90% and at 0.05 significance level. A total of 27 male Sprague Dawley rats weighing approximately 350 g were obtained from Baskent University (Ankara, Turkey) and maintained under controlled temperature (24°C ± 2°C) and 12 h: 12 h light/dark period conditions and with free access to water and food.

The rats were distributed into three groups (n = 9 per group). All groups received 50 ppm 4NQO (Sigma Aldrich, St Louis, USA) for 20 weeks in drinking water. Group 1 was treated with 50 mg/kg/day propranolol by oral gavage for 20 weeks. Group 2 was treated with propranolol for 2 weeks after carcinogenesis inducement for 20 weeks, and Group 3 (the control group) received no treatment. The experimental periods were established to evaluate the effect of propranolol on all stages of carcinogenesis (from the initiation phase to 20 weeks) and after 20 weeks of the carcinogenesis process. The rats were euthanized with high-dose ketamine at the end of the experimental stage. The tongues were longitudinally cut into halves for histopathological examinations. The tissues were fixed in 10% buffered formalin, embedded in paraffin blocks and stained with hematoxylin and eosin.

Histopathological analysis

Histopathological evaluation was performed under a light microscope. The tongue sections were evaluated as normal, hyperplasia/hyperkeratinization, dysplasia or carcinoma. The scoring was based on the architectural and cytological changes described in the World Health Organization classification, and precancerous lesions were classified as low- or high-risk lesions according to the binary system [Table 1].[18] If there were at least four architectural changes and five cytological changes, the lesion was considered as high-risk lesion for malignant transformation. If there were fewer than four architectural changes or five cytological changes, the lesion was considered as low-risk lesion for malignant transformation.

Statistical analysis

Data analysis was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA). Nominal data were analyzed with the likelihood ratio test. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical view of the lesions

All lesions were localized at the posterior dorsal tongue, and most were characterized as white lesions with a straight or papillary/nodular surface [Table 2]. There was 1 tumor occurrence in each group. The diameter of the mass was 0.6 cm, 0.9 cm and 1.8 cm in Groups 1, 2 and 3, respectively [Figures 1-3].

Histopathological evaluation

The histopathological analysis showed that the SCC had spread into the submucosa and underlying muscle layer, forming small islands with typical keratin pearl formation in the tumor samples from Group 2 and Group 3. In contrast, the malignant transformation in the tumor

sample from Group 1 was limited to the epithelial layers, and a histopathological diagnosis of carcinoma *in situ* was determined for this sample [Figure 4].

Decreased risk of malignant transformation in Group 1 (33.3%) compared with Group 2 (55.5%) and 3 (77.8%) was detected based on the binary system of oral precancerous lesions. There were slight differences between the three groups regarding the high-risk category (Group 1, 11.1%; Groups 2 and 3, 22.2%). The rates of low risk were 22.2% in Group 1, 33.3% in Group 2 and 55.5% in Group 3. The difference between the groups was not statistically significant ($P > 0.05$).

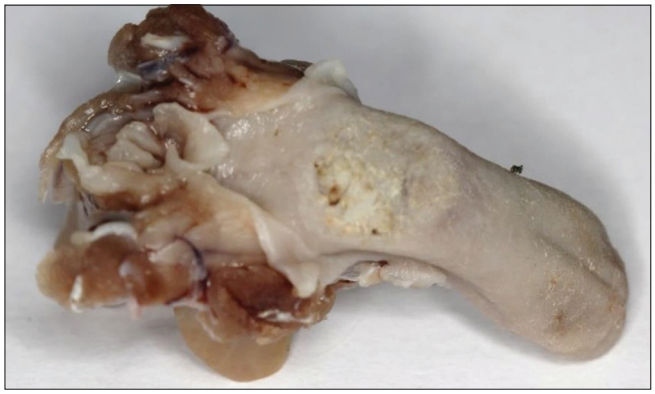

Figure 1: Tumor sample from Group 1. The maximum width of the tumor surface is measured as 0.6 cm

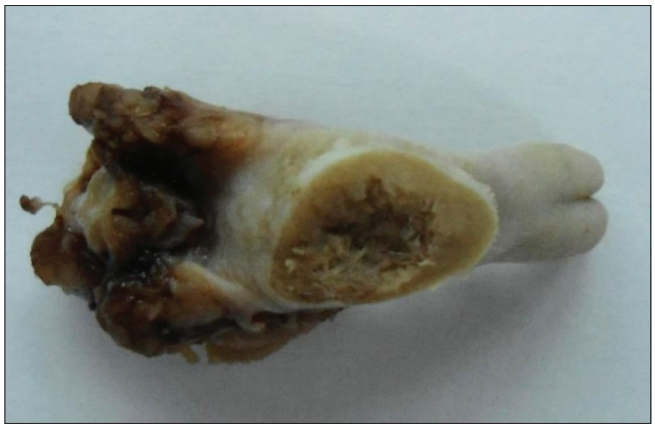

Figure 3: Tumor sample from Group 3. The maximum width of the tumor surface is measured as 1.8 cm

DISCUSSION

Although many data have been published about treatment approaches to and diagnosis methods for oral SCC, the survival rates of patients have not improved significantly during the last 40–50 years.[19] Consequently, investigations related to chemoprevention that can extend the latent period of carcinogenesis or prevent the development of carcinoma have received attention.

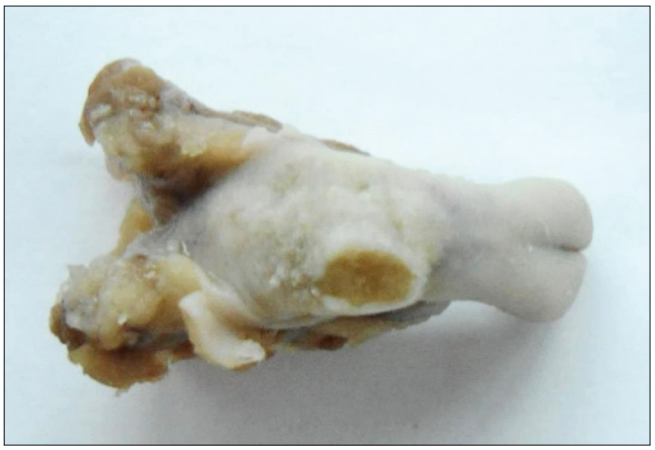

Figure 2: Tumor sample from Group 2. The maximum width of the tumor surface is measured as 1.2 cm

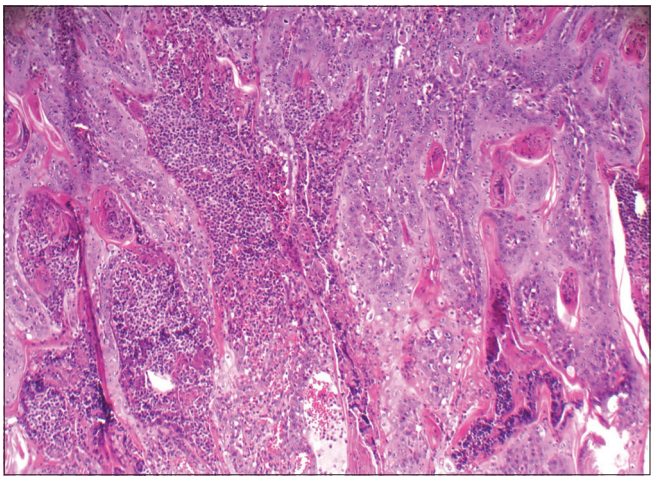

Figure 4: Histopathologic views of the tumor sample from Group 3. The invasive keratin pearls and tumor islands were seen (H&E, 10 × 10)

Table 1: Architectural and cytological changes described in the World Health Organization

	Structural changes	Cellular changes
1	Stratification	Abnormal variation in nuclear size
2	Loss of polarity	Abnormal variation in nuclear shape
3	Drop shaped rete ridges	Abnormal variation in cell size
4	Mitoses increased	Abnormal variation in cell shape
5	Abnormally superficial mitoses	Increased nuclear-cytoplasmic ratio
6	Premature keratinization	Increased nuclear size
7	Keratin pearls within rete ridges	Atypical mitotic figures
8	Basal cell hyperplasia	Increased nucleoli
9		Hyperchromatism

The 4NQO cancer model is one of the most frequently used methods in these types of studies. One of the advantages of the 4NQO model has been reported that it can be used for studying the early stages of oral cancer progression.[19] Although there are differences in the dose and application periods of 4NQO, general histopathological alterations are associated with carcinogenesis such as progression from early hyperplasia to dysplasia and then to SCC occur within 28 weeks.[20,21]

The binary system introduces to evaluate the prediction of malignant potential of oral premalignant lesions using quantitative morphological characteristics scoring system.[18] The binary system was used for classification of the lesions which occurred during carcinogenesis stages in this study. According to our experience, this technique is also feasible for animal studies considering that sample size is generally limited in comparison with clinical studies.

Ever since suppression of hemangiomas after the administration of propranolol was shown, investigators have been concerned about propranolol's ability to reduce the development of a malignancy. Several clinical studies were reported that propranolol might impact tumor development and decrease the cancer-specific mortality in breast cancer.[1] Pasquier *et al.* showed that propranolol can potentiate the antiangiogenic effects and antitumor efficacy of chemotherapy in breast cancer treatment.[11] Several *in vivo* studies have demonstrated that propranolol can reduce metastasis of PC-3 prostate cancer cells and avoid tumor growth in ovarian carcinoma.[14,16] Wolter *et al.* demonstrated that propranolol reduced head and neck SCC cell line viability, induced apoptosis and inhibited production of the proangiogenic protein. The authors also recommended propranolol as a useful adjuvant in head and neck SCC that can aid in reversing chemotherapy and radiation resistance.[12]

In our knowledge, this study was the first *in vivo* study that evaluated the prevention and curative effect of propranolol on oral SCC. At the end of the experimental stage, the tumor diameter was three times that of the control group compared with Group 1 (1.8 cm vs. 0.6 cm) and the risk of malignant transformation of precancerous lesion was almost two times for the control group compared with Group 1 (78% vs. 34%).

CONCLUSION

These results suggest that propranolol has potential to reduce the development of SCC in tongue. This is important for the determination of beneficial drug effects on patients who are treated for cardiac disease with propranolol. Furthermore, this study will inspire the investigators for future studies on curative effect of propranolol on SCC.

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Conflicts of interest

There are no conflicts of interest.

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