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Sclerotic effect of bleomycin on the submandibular gland: An experimental model

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ABSTRACT

Objectives: To evaluate the sclerotic effect of bleomycin on the submandibular gland histopathologically and assess it as a possible alternative therapy for sialorrhea.
Methods: An experimental model was designed and 18 New Zealand white rabbits were used. The rabbits were divided into two groups: a bleomycin group ($n = 9$) and a sham group ($n = 9$). The submandibular glands of the bleomycin group were injected with 0.3 ml bleomycin (3 mg/ml) while the sham group received 0.3 ml saline. Four weeks after the procedure, the glands were removed. Histopathological studies including hematoxylin–eosin and Masson's trichrome stain were carried out. The glands were evaluated for tissue inflammation, fibrosis, edema, lipomatosis, atrophy and congestion. To investigate apoptosis, terminal deoxynucleotidyl transferase (TdT)-mediated digoxigenin-11-dUTP nick-end labeling (TUNEL) immunohistochemical staining was used.
Results: In the group injected with bleomycin, inflammation ($n = 8$), edema ($n = 4$), fibrosis ($n = 3$), congestion ($n = 4$) and lipomatosis ($n = 7$) were observed. In the sham group, only lipomatosis was observed. The TUNEL assay results were 5.06 ± 1.18 ($p < 0.05$) for acinar cells and 8.46 ± 0.82 ($p < 0.05$) for ductal cells in the bleomycin group. This was significantly different from the results in the sham group.
Conclusions: Apoptosis, inflammation, fibrosis, edema, lipomatosis and congestion were observed in the ductal and acinar cells of the bleomycin group. Bleomycin may be an alternative treatment for sialorrhea cases. However, more research is needed.

1. Introduction

Bleomycin is known for its sclerotic effects and it is a preferred sclerotherapy agent in numerous pathologies including malignant pleural effusions, renal cysts and lymphatic malformations of the head and neck [1–3]. It is an antibiotic derived from *Streptomyces verticillus* and is usually used for chemotherapy. It inhibits DNA and protein synthesis and it is thought that the sclerotic effect results from a separate inflammatory response with secondary fibrosis [4]. This sclerosant can be used effectively for some salivary gland pathologies such as sialorrhea.

Sialorrhea, or drooling, is the involuntary loss of saliva from the mouth after infancy or after oromotor maturation occurs. It is a problem for developmentally disabled children and adults [5]. Drooling usually occurs as a result of some form of neurological disturbance, either central (e.g. cerebral palsy) or peripheral (e.g. facial palsy) [6]. The prevalence of moderate or severe sialorrhea in the population is estimated to be between 10% and 37%. Factors contributing to sialorrhea include the integrity of oropharyngeal motor function, oral structures, orofacial sensory perception and feedback, rate of saliva secretion, and cognitive awareness of salivation [5]. Medical therapy, tympanic neurectomy, corda tympani section, ductal ligation, ductal rerouting, botulinum toxin injection and major salivary gland excision are some of the treatments for drooling.

Numerous approaches including ductal ligation, radiotherapy and neurectomy are designed to produce parenchymal atrophy which causes secretory ablation and will reduce symptoms. There is insufficient research in the literature about the effects of bleomycin on salivary gland pathologies. In the present study, we evaluated the sclerotic effect of bleomycin injections on rabbit submandibular gland tissue using histopathological methods. If bleomycin induces atrophic, inflammatory and hypofunctional effects on gland tissue, it may be an alternative treatment for sialorrhea.

2. Materials and methods

Eighteen New Zealand white rabbits (weighing 2.5–3 kg) were used in this study. Permission was obtained from Canakkale Onsekiz Mart University (COMU)-Animal Ethics Committee. All animals were kept under optimum conditions in the COMU

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laboratory in accordance with the guide for the care and use of laboratory animals-8th edition [7]. They were divided into two groups, a bleomycin group (group B, $n = 9$) and a sham group (group S, $n = 9$).

2.1. Surgical operation

Xylazine (12.5 mg/kg, intramuscular) and ketamine (100 mg/kg) anesthesia were applied before surgery. We made a midline incision on the neck. After passing through the skin and under the skin tissue, we identified the right submandibular gland under the superficial fascia and identified near the medial surface of the angulus mandibula. Then 0.3 ml of bleomycin (Bleocin-S, 3 mg/ml) was injected into the glands in group B. For the sham group (group S), all the surgical procedures discussed above were applied apart from the bleomycin injection. A saline injection (0.3 ml) was applied in this group.

We removed the submandibular glands for histopathological investigation 4 weeks after the initial procedure.

2.2. Histopathology

Submandibular gland samples were fixed in neutral buffered formalin (10% formaldehyde). Following the fixation procedure, the tissue was trimmed and processed using standard paraffin-embedding methods. Sections were cut at 4 μm and then stained with hematoxylin–eosin and Masson's trichrome stain. Submandibular gland histopathology was examined using a light microscope (Carl Zeiss Axio Scope photomicroscope, Germany) at 4–40× magnification in a double-blind procedure. Histologic features (Fig. 1) such as inflamed ductal and acinar cells, fibrosis, edema, lipomatosis, and atrophy were evaluated using a semiquantitative scale: (N) negative or absent, (+) low, (++) moderate and (+++) strong.

2.2.1. Immunohistochemistry

To examine apoptotic cells, the modified terminal deoxynucleotidyl transferase (TdT)-mediated digoxigenin-11-dUTP nick end labeling (TUNEL) assay method [8] was performed using the ApopTag Plus Peroxidase In Situ Apoptosis Detection Kit (Chemicon, USA). Approximately 500 acinar cells and 500 ductal cells in four randomly chosen fields were counted at magnifications of 400× (CX41 microscope, Olympus, Tokyo, Japan) on sections stained with TUNEL. The TUNEL labeling index was calculated for each cell type. The mean of the labeling index from four fields was taken as the representative value for that animal [9].

2.2.2. Statistical analysis

SPSS 20 software was used for statistical analysis of data. The means and standard deviations of the groups were calculated. The Mann–Whitney *U*-test was used to determine statistically significant differences between the groups and the results were evaluated at a significance level of $p < 0.05$.

Table 1
Semiquantitative scale results of the histopathological investigation. "N" negative or absent, "+" low, "++" moderate and "+++" strong.

	Inflammation*	Edema	Fibrosis	Lipomatosis	Congestion
Bleomycin group	++	+	+	N	+
	++	+	N	N	+
	+	+	N	+	+
	+	N	N	+	N
	+	N	N	+	+
	+	N	N	+	N
	+	N	+	+	N
	N	N	N	+	N
	+	+	+	+	N
Sham group	N	N	N	+	N
	N	N	N	N	N
	N	N	N	+	N
	N	N	N	N	N
	N	N	N	+	N
	N	N	N	+	N
	N	N	N	N	N
	N	N	N	+	N
	N	N	N	N	N

* Significant statistically difference between groups ($p < 0.05$).

3. Results

Dissection of the necks of the animals revealed larger edematous submandibular glands and fibrotic mesenchymal tissue around the glands in the bleomycin group (group B). These findings were not present in the sham group (group S).

Samples from groups B and S were examined histopathologically and immunohistochemically. In the bleomycin group, the following was observed: inflammation ($n = 8$, $p < 0.001$), edema ($n = 4$, $p = 0.113$), fibrosis ($n = 3$, $p = 0.258$), lipomatosis ($n = 7$, $p = 0.436$) and congestion ($n = 4$, $p = 0.113$) (Table 1 and Fig. 1). The sham group only displayed lipomatosis. Atrophy was not detected in the samples from either group. We detected low or moderate degrees of inflammation in the gland samples from group B. Similarly, low degree edema, congestion and lipomatosis were found in this group. Additionally, we detected periductal fibrosis in group B.

TUNEL assays were performed on ductal and acinar cells to investigate apoptosis (Table 2). Statistically significantly higher labeling was found in ductal cells ($p < 0.001$) and acinar cells ($p < 0.001$) in the bleomycin group compared with the sham group (Fig. 2). In the bleomycin group, the mean TUNEL labeling indices

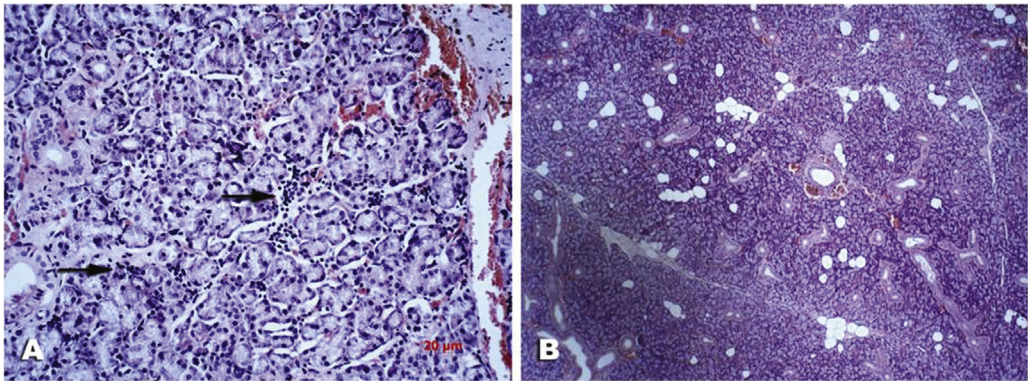

Fig. 1. Mononuclear inflammatory cell infiltration (A) (arrow, HE 200×), and lipomatosis (B) (HE 50×) in salivary gland.