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Effects of Adenosine A_{2A} Receptor Agonist on Histopathology of Some Oral Tissues in Rabbits

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ABSTRACT

Adenosine is a protective regulator that act endogenously to restore equilibrium of cellular energy in response to tissue trauma. It can perform such function of different systems in the body by activation of adenosine receptors. Study the effects of systemic administration of the adenosine on tongue and salivary glands tissues in the rabbit model. Thirty male rabbits of body weight of 1.5 ± 0.25kg were included in the study. In control group (15 animals), one ml of distilled water was injected intraperitoneally while in treatment group (15 animals) were injected by adenosine intraperitoneally at a dose of one mg/ml, All animals were sacrificed after 30 days. Serum samples were separated and used for analysis of adenosine deaminase (ADA)and glutathione(GSH). Tissue samples sections from tongue and salivary glands were stained with hematoxylin-eosin (H&E) and examined under a light microscope for histological changes by a blinded pathologist. Histological sections in treatment group showed congestion of blood vessels and infiltration of inflammatory cells with mild hemorrhage among acini of salivary glands. Increased level of adenosine in the body microenvironment may affect tongue and salivary glands tissues by modulating some processes including inflammation and blood vessels.

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INTRODUCTION

It is well recognized that adenosine acts to restore energy balance in cells during exposure to stress or trauma. It has protective effects in a wide spectrum of normal physiological and abnormal pathological conditions including inflammation, neuronal hyper excitability, various toxicities, seizures, and pain. These protective functions of adenosine result in its classification as a "retaliatory" or "homeostatic" cellular modulator (Jarvis, 2019). Adenosine (C10H13N5O4) endogenous purine nucleoside molecule is formed intra- and extracellularly by dephosphorylation of ATP (Kazemzadeh-Narbat *et al.*, 2015), with firmly controlled concentration that regulate its intense and diverse biological abilities (Köröskényi *et al.*, 2017; Palmer and Trevethick, 2009). It induces vasodilation, increases oxygen supply and improves blood flow in the heart, skeletal muscle, brain, oral mucosa, gingival, tongue and salivary glands. So, adenosine provide a negative feedback signal to preserve normal tissue oxygenation (Adair, 2005; Sun *et al.*, 2011; Koizumi *et al.*, 2009). Much facts has been accumulated on anti-inflammatory effects of adenosine molecule that is mainly mediated by A_2a receptor activation; this receptor has an important role in matrix deposition and wound healing in a damaged tissue, and acting as a guard to mucosal and dermal tissues serving in

Table 1: Comparison in Serum Biochemical Markers AmongThe Study Sampled Groups

Parameters	Adenosine Group Mean ± SD	Control group Mean ± SD	P-value*
No. of rabbits	15	15	—
ADA (U/ml)	12.16 ± 2.89 A	0.00 ± 0.00 B	0.000
GSH (μg/ml)	18.44 ± 5.83 A	4.22 ± 1.47	0.009

* One-wayANOVA-test with Tukey's Pair wise comparisons was used.
Means with the different letters are statistically significant (p<0.05).

protection and repair (Ialenti *et al.*, 2018). Adenosine signaling is controlled by stimulation of molecular signaling events which results in physiological responses by activation of one or more of the four transmembrane adenosine receptors (four distinct extracellular G protein-coupled adenosine receptors called A1, A2a, A2b and A3) (Lee and Yilmaz, 2018). Such evidence support the participation of adenosine and mostly of its receptor A_2a in the regulation of various tissues (Adair, 2005; Colella *et al.*, 2018). A promising concept is that adenosine stimulate the growth of blood vessels (angiogenesis') by poorly understood mechanism, a lot of studies have shown that the administration of adenosine as well as the up regulation of endogenous adenosine can promote angiogenesis by the action of vascular endothelial growth factor (VEGF) in a variety of cell types (Adair, 2005). Adenosine and its receptors have been studied at site of inflammation and infection and used in medicine but little is known about adenosine in relation to dentistry and oral environment. it can be involved in the modulation of inflammatory responses like in periodontitis which is an inflammatory disease of oral tissue including tongue and salivary glands (Lee and Yilmaz, 2018; Al-Mashhadane *et al.*, 2019). Adenosine and its receptor agonists have been used in medicine effectively but little is known about it in context to dentistry (Sun *et al.*, 2011) and this deserve further studies. This work was to study the effects of the adenosine administration on tongue and salivary glands in the rabbit.

MATERIALS AND METHODS

Table 2: Correlation Between ADA And GSH In Adenosine Group

Parameters	r - value	P - value
ADA and GSH	0.152	0.588

*Pearson correlation method (r) was used. P- value <0.005 is significant.

RESULTS AND DISCUSSION

Statistical analysis for ADA serum level and GSH showed significant differences between adenosine and control groups (Table 1). Serum ADA levels have non significant positive correlation to GSH levels(Table 2).

Light microscopic examination of the tissue samples of tongue showed that presence of normal, well organized, homogenous histology of tongue tissues with mucous membrane covered by keratinized stratified squamous epithelium in the control group (Figure 1)while the section of adenosine group showed congestion of blood vessels, infiltration of inflammatory cells among muscle fibers with mild serous exudates in the muscles(Figure 2). For salivary gland, histological examination showed, Normal histological structure of skeletal muscles(A) and normal submucosal connective tissues(B) but there are mild congestion of blood vessels(arrow). H&E. 100x

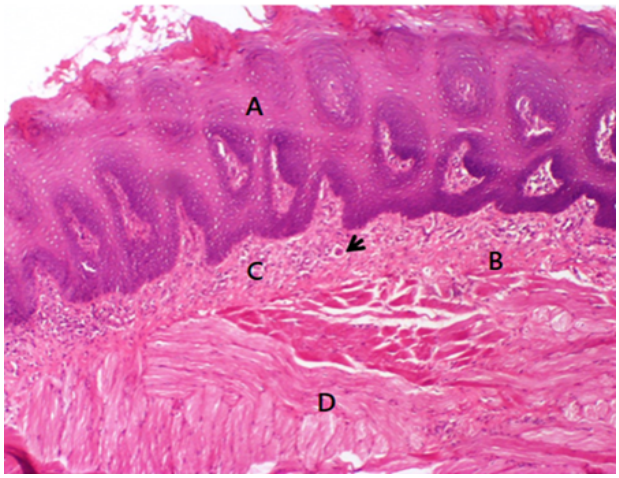

Figure 1: Histological section of tongue of control group. There is normal architecture of the tongue tissues

normal architecture of serous acini, the intralobular duct (intercalated duct) and the interlobular duct in the control group (Figure 3), There is normal

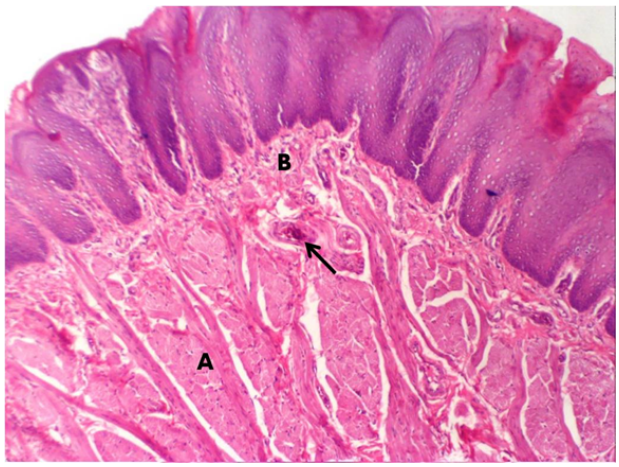

Figure 2: Histological section of tongue of adenosine group.

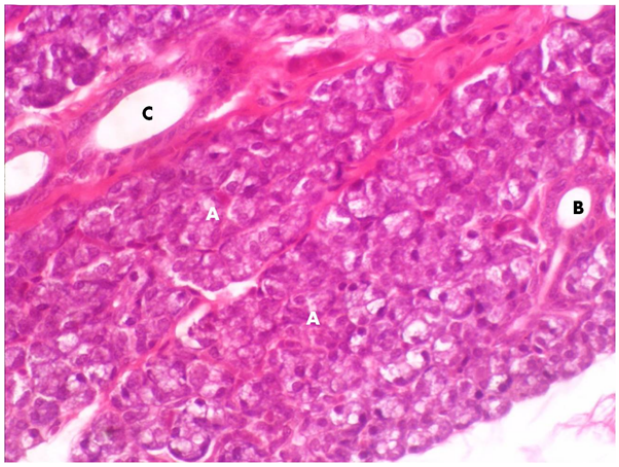

Figure 3: Histological section of salivary glands of control group.

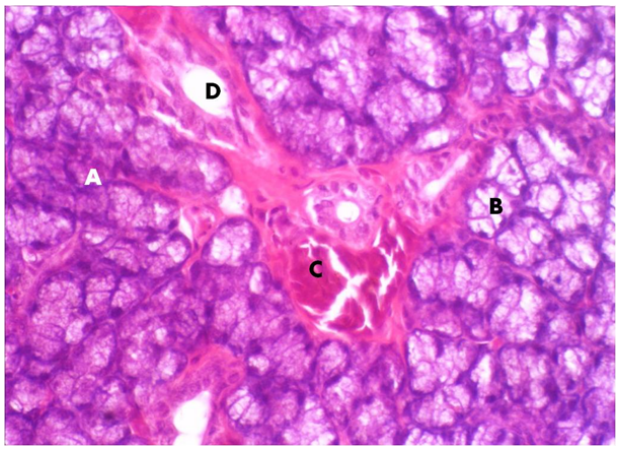

Figure 4: Histological section of salivary glands of adenosine group.

architecture of serous acini(A).Note the intralobular duct (intercalated duct)(B),and the interlobular duct (C) H&E. 100x, while the section of adenosine group showed moderate congestion of blood vessels with moderate hemorrhage among acini (Figure 4). Serousacini(A) and mucous acini (B) of submandibular salivary glands. There ismoderate congestion of blood vessels(C). Note the interlobular duct(D) H&E. 400x

Adenosine is a multifunctional molecule dynamically involved in different tissues and responses in the body. It has been associated largely with the pathogenesis of vascular problems (Kam *et al.*, 2015). One of the present study conduction was to evaluate ADA and GSH levels in adenosine treated rabbits, the results showed highly significant mean levels of ADA in adenosine group when compared to controls, this can be explained by fact that ADA is one of the enzymes that are responsible for adenosine activity. Adenosine degraded by ADA, so more extracellular adenosine lead to increase ADA levels (Jarvis, 2019; Liu *et al.*, 2010). Also statistically significant increase in serum GSH is observed in adenosine group when compared to controls which could provide evidence that adenosine via a novel mechanism-activate some cellular antioxidants like GSH (Maggirwar *et al.*, 1994). Also in the present study, ADA serum levels were positively correlated to GSH which is in agreement with data propose that oxidative stress can raise the expression of the adenosine receptors by activating NFκB regulatory site(s) on this gene and thereby enhance the role of ADA on adenosine which will increase the level of GSH as antioxidant (Nie *et al.*, 1998). In disagreement with our results some studies showed that ADA serum level is negatively correlated with antioxidants, which indicates that ADA serum levels increases as antioxidant capacity reduced (Dasegowda *et al.*, 2015; Mehde *et al.*, 2013).

Adenosine is a key regulator of angiogenesis mainly by proangiogenic properties of A2a AR. This A2a AR angiogenic function is regulated by hypoxia. By additional investigation of the A2a AR role in angiogenesis, Liu *et al* (2010) demonstrated that inactivation of A2a AR attenuates retinopathy angiogenesis which is oxygen-induced only. Normal retinal vascularization not involved, supporting the therapeutic potency of A2a AR antagonists for retinopathy (Sun *et al.*, 2011; Bahreyni *et al.*, 2018). This angiogenic ability of adenosine could explain the results of the present study manifested by increased vascularity, congestion of blood vessels and hemorrhage. Although the mechanism by which adenosine induces angiogenesis is poorly understood, numer-

ous studies have shown that the exogenous adenosine as well as endogenous adenosine can modulate VEGF expression and other angiogenic factors (Kam *et al.*, 2015; Maugeri *et al.*, 2019). in a different cell types. Histological section of tongue in adenosine group of the present study show infiltration of inflammatory cells among muscle fibers suggesting that adenosine enhances inflammatory response (Meng *et al.*, 2019).

Pei *et al.* (2018) suggest that A2AR plays a role in tissue inflammation, which is attributable to A2AR proinflammatory activation. The significant high level of ADA serum level in adenosine group compared to control one can support this role of adenosine since that ADA released principally by inflammatory cell (Lee *et al.*, 2020) and its activity has important role as inflammatory modulators (Fávero *et al.*, 2018). Adenosine is closely connected to the A2AR activation (Al–Moula *et al.*, 2012) an increased extracellular adenosine concentration promotes the up regulation of A2AR signaling that enhance the synthesis of inflammatory mediators like IL-1b (Meng *et al.*, 2019), suggesting that adenosine enhances inflammatory response via A2AR-mediated signaling. So, the use of adenosine antagonists is a promising therapeutic strategy for intervening inflammatory diseases including inflammation of oral tissues. In this study irregularities of muscle fibers and hyaline necrosis that was founded in tongue tissues of rabbits injected by adenosine could be explained by the fact that numerous adenosine receptors were detected in the posterior lingual taste fields of the tongue suggesting the existence of an adenosine signaling system. Adenosine plays a role in signaling transmission via its receptors in tongue tissues of rabbits (Kataoka *et al.*, 2012; Nishida *et al.*, 2014; Choo *et al.*, 2017). The irregularities of muscle fibers that was noticed in all tissues could be due to direct stimulation of collagen matrix formation induced by adenosine through activation of its receptors (Feoktistov *et al.*, 2009; Parasuraman *et al.*, 2010; Ibrahim *et al.*, 2019).

CONCLUSIONS

Adenosine is involved in different processes inside the tissues. Increased level of adenosine in the body microenvironment may regulates growth of oral tissue by modulating processes like angiogenesis, which support the clinical importance of such molecules in treatment of certain oral diseases. Researches are needed to be done to release adenosine in a targeted areas without affecting other organ.

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Conflict of interest

Author states that she has no known competing financial attention or personal relations that could influence the work of this manuscript.

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