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Research Article

Effects of Valsartan on the Gingival Architecture of Rats: Histological Study

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Abstract

Background: Valsartan is an angiotensin II receptor inhibitor prescribed to regulate heart pressure. The bulk of studies documented the impact of antihypertensive drugs on the gingival tissues; however, none have investigated the effect of valsartan on the gingival architecture. ***Objective***: To investigate the histological effect of valsartan administration on the gingival tissue architecture in rats. ***Methods***: Twenty Wistar male rats (2–3 months old) were randomly assigned to the control and valsartan groups. 10mg/kg/day valsartan was administered subcutaneously for two weeks. Then all the animals were sacrificed, and full-thickness samples of gingival tissue were harvested from the palatal aspect of the rat's molar teeth and prepared for examination under a microscope. ***Results***: Although the results revealed that the valsartan group showed thicker epithelium (mean=0.1280 mm) compared to the control group, the difference did not reach a statistical significance. In the lamina propria, the thickness change was statistically significant (0.07400±0.0143 mm) compared to the controls (0.0573±0.00696). The submucosa thickness measures were equivalent for both groups. The count of blood vessels showed no variation between the two experimental groups. ***Conclusions***: Treatment with valsartan resulted in a significant increase in lamina propria thickness with no influence on epithelial thickness, submucosal thickness, or blood vessel count in rat gingival tissue.
Keywords: Angiotensin II receptor blockers, Anti-hypertensive, Gingiva, Histology, Valsartan.

تأثير فالسارتان على بنية اللثة في الجرذان: دراسة نسيجية

الخلاصة

الخلفية: فالسارتان هو مثبط لمستقبلات الأنجيوتنسين II يوصف لتنظيم ضغط القلب. وثق الجزء الأكبر من الدراسات تأثير الأدوية الخافضة للضغط على أنسجة اللثة. ومع ذلك، لم يحقق أي منهم في تأثير فالسارتان على بنية اللثة. **الهدف**: التحقيق في التأثير النسيجي لإعطاء فالسارتان على بنية أنسجة اللثة في الفئران.**الطرائق**: تم تقسيم عشرين جرذا من ذكور ويستر (2-3 أشهر) بشكل عشوائي إلى مجموعات التحكم والفالسارتان. تم إعطاء 10 مجم/كجم/ يوم فالسارتان تحت الجلد لمدة أسبوعين. تم التضحية بجميع الجرذان، وتم أخذ طبقة كاملة من أنسجة اللثة من داخل اللثة الخلفية للفئران وإعدادها للفحص تحت المجهر. **النتائج**: على الرغم من أن النتائج بينت أن مجموعة الفالسارتان أظهرت ظهارة أكثر سمكا (المتوسط = 0.1280 مم) مقارنة بالمجموعة الضابطة، إلا أن الفرق لم يصل إلى دلالة إحصائية. في الصفيحة الخاصة، كان تغير السماكة معتدا به إحصائيا (0.07400±0.0143 مم) مقارنة بعناصر التحكم (0.0573±0.00696). كانت مقاييس سمك تحت الغشاء المخاطي متكافئة لكلا المجموعتين. لم يظهر تعداد الأوعية الدموية أي اختلاف بين المجموعتين التجريبيتين. **الاستنتاجات**: أدى العلاج بالفالسارتان إلى زيادة كبيرة في سمك الصفيحة المخصوصة دون أي تأثير على سمك الظهارة أو السماكة تحت المخاطية أو عدد الأوعية الدموية في أنسجة اللثة في الجرذان.

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INTRODUCTION

Considered as a basic part of the periodontal apparatus, the gingival tissue shields against mechanical, bacterial, and chemical damage, therefore promoting oral health. Structurally, it comprises lamina propria, submucosa, and a stratified squamous epithelial layer, all of which have different roles in general functional maintenance. Whereas the internal lamina propria and submucosa help in tissue healing and coordinate the immune response to external assaults, the outer compact epithelial layer serves mostly as a physical barrier [1]. Systemic medications may change the thickness and vascularization of these layers, which could affect how the gingival structure changes and possibly the physiological or pathological response [2]. Commonly used as an antihypertensive medicine, valsartan, an angiotensin II receptor blocker (ARB), was used to treat essential hypertension, heart failure, and kidney diseases. ARBs work by blocking receptors that the angiotensin II (Ang II) hormone acts on, specifically angiotensin II type 1 receptor (AT1) receptors, which are expressed in the heart, blood vessels, and kidneys and recently were found to be expressed in gingival tissue, as confirmed by a rodent study [3]. Blocking the action of angiotensin II helps to lower blood pressure and prevent damage to the heart and kidneys [4]. New information suggests that ARBs may have effects on the body other than their main cardiovascular function, which means they may change the structure and function of tissues that are not their target [5].

Confirmed in an animal investigation is a gingival expression of angiotensin II receptor [3]. Concerning the histological characteristics of gingival tissue, especially epithelial thickness, lamina propria thickness, submucosa thickness, and vascular density, nothing is known to date about how valsartan affects them. Therefore, the aim of this work is to investigate the histological variations in gingival tissue of healthy and valsartan-treated rats, analyzing differences in epithelial layer thickness, lamina propria thickness, submucosa thickness, and the number of blood vessels, so clarifying the possible extracardiac pharmaceutical effects of valsartan on gingival tissue. By closing this disparity, the results of the study would enable dentists to better understand how hypertension medications affect oral health, thereby enabling them to modify and tailor dental treatment to the patient's systemic health and drug intake.

METHODS

Study design

The experimental animal study was authorized by the Ethical Committee of Department of Dentistry, Al-Rafidain University College (Certificate no. 42525 on 25-3-2025). According to the principles of Animal Research: Reporting of In Vivo Experiments (ARRIVE), all animals were treated humanely [6].

Sample selection

The samples comprised a total of 20 male Wistar albino rats 2 months old, diseases free and weighing in average 250-300g. The animals were maintained in animal facility under standardized conditions, including a 12-hour light/dark cycle, a temperature controlled at 22.2°C, and a humidity of 60%. Rats were acclimated for one week prior to experimental procedure. Animals were kept in plastic cages identified by their number, group, and date. Animal monitoring was conducted daily throughout the entire experimental period. Animals were allocated into control group (10 rats) and valsartan-treated group (10 rats). Valsartan group animals received a subcutaneous daily injection of 10mg/kg of valsartan raw powder (Hyper Chem, China) for 2 weeks duration.

Preparation of valsartan solution

Provided that the valsartan dose is 10 mg/kg and the rat weight is ≅ 250 gm, the valsartan dose was calculated according to the following equation:

The dose for each rat = *Valsartan dose* × *rat weight/1000*

Valsartan powder is insoluble in water but soluble in 100% ethanol. The acute dermal toxicity of ethanol in rats was reported to be minimal, and the toxic dose of pure ethanol was roughly 0.8 g/kg (1 mL/kg). To formulate the daily dosage of valsartan 2.5 mg powder, 100 µl of 100% pure ethanol were added. Additionally, 400 µl of distilled water was required for every 0.5 ml of pure drug suspension to create a homogeneous injectable suspension [7]. At the end of the experiment, all rats were euthanized ethically by general anesthesia overdose administration with an intramuscular injection of ketamine (87 mg/kg) (Ketamine 10%, Alfasan, Woerden, Holland) and xylazine muscle relaxant (10 mg/kg) (Xylazine 2%, Alfasan, Woerden) [8].

Sample harvesting

A horizontal shallow sulcular incision was made around the right maxillary molars with a No.11 scalpel blade to sample gingival tissue surrounding accused teeth from the resected maxillae. Next, close to the palatal midline, a second horizontal incision was made parallel to the first. Later, a surgical blunt dissection is used to carefully remove the whole gingival band [9].

Histological evaluation

The collected samples were immediately stored in the specified plastic containers with formalin after being rinsed with tap water to avert clot blood. Fixation with 10% neutral buffered formalin solution for 24 hours and prepared for histological processing. The specimens were dehydrated by passing them through a series of increasing alcohol concentrations, then the specimens were passed through two jars of xylene, each jar for half an hour. The specimens were placed in a dish of melted embedding paraffin, then the specimen was poured in the centre of the block paraffin. Sectioning: Five µm-thick semi-serial longitudinal sections were prepared. H&E slides were examined for histological changes using a light microscope (LEICA DM750, Germany) equipped with a digital video camera (LEICA ICC50 E, Germany). Microphotographs of the sections were taken using the LEICA company application (LAS EZ). The region of interest (ROI) was defined as a rectangular area of 0.18 mm². ImageJ software (NIH, United States) measures dimensions in microphotographs in pixels. To change pixels to mm, the set scale option was used in the program by measuring the known length with a scale of 1492 pixel\mm at 20x power, as shown in Figure 1 [10].

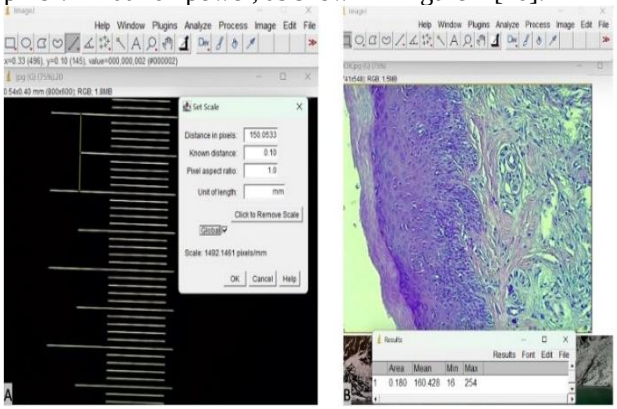

Figure 1: Set scale function to convert pixels to mm in ImageJ (**A**). Region of interest ROI measurement (**B**).

As illustrated in Figure 2 four measurements per ROI were taken for each sample of the following parameters: Epithelium thickness measured from the outer surface stratum corneum to the basement membrane, lamina propria thickness, submucosa thickness, and number of blood vessels.

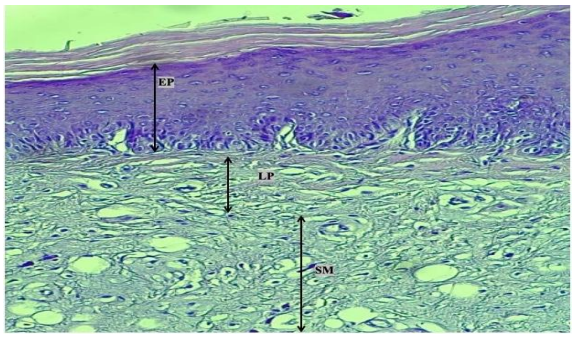

Figure 2: Photomicrograph illustrating gingival architecture parameters measured histologically: epithelium thickness (**EP**), lamina propria (**LP**) and submucosa (**SM**) at 20X.

RESULTS

The results of the histological analysis were presented in (Table 1). The average epithelial thickness was 0.1280±0.01135 mm for the control group and 0.1386±0.01914 mm for the valsartan group, respectively. Despite the valsartan group exhibiting greater epithelial thickness, the difference was not statistically significant, with a *p*-value of 0.1493. With valsartan-treated rats measuring 0.074±0.0143 mm compared to the control group (0.0573±0.00696), the thickness of the lamina propria exhibited a statistically significant difference (*p*= 0.0038). Recorded at 0.098±0.01135 mm and 0.0977±0.012, respectively, the submucosa thickness measures for the control and valsartan groups were equivalent.

Table 1: Descriptive statistics and comparative analysis for means of epithelial thickness, lamina propria thickness, submucosa thickness, and blood vessels count.

Variables	Control (n=10)	Valsartan (n=10)	*p*-value
Epithelial thickness (mm)	0.128±0.01135	0.1386±0.01914	0.1493
Lamina Propria thickness (mm)	0.0573±0.00696	0.074±0.0143	0.0038
Submucosa thickness (mm)	0.098±0.01135	0.0977±0.012	0.9548
Blood vessels count	5.4±0.8433	5.4±0.6992	>0.9999

Values were expressed as mean±SD.

In line with this trend, the count of blood vessels showed no variation between the two experimental groups; the recorded count of blood vessels for the control group was 5.4±0.8433 and for the valsartan-treated group was 5.4±0.6992 (Figures 3 and 4).

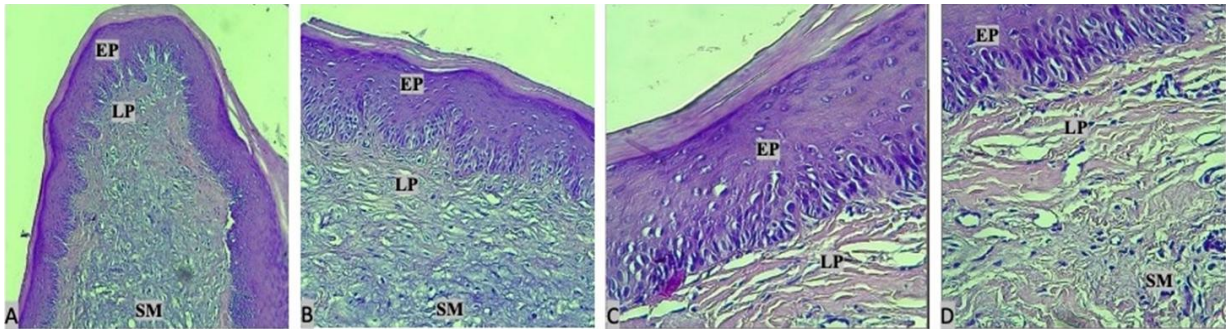

Figure 3: Photomicrographs of the gingiva from the control group with regular thickness of epithelium (**EP**), lamina propria (**LP**), and submucosa (**SM**). **A**) Free gingival margin 10X; **B**) Gingival tissue 20X; **C**) Gingival tissue (40X); and **D**) Lamina propria and submucosa of gingiva.

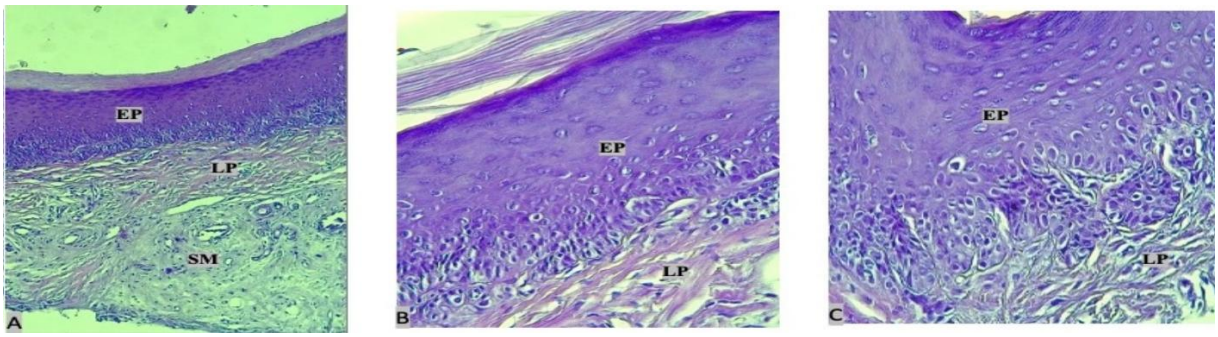

Figure 4: Photomicrographs of the gingiva from the valsartan group with regular thickness of epithelium (**EP**) (**A**), lamina propria (**LP**) **B**), and submucosa (**SM**) (**C**),10X, 20X, and 40X respectively.

DISCUSSION

To the best of our knowledge, this in vivo study is the first to investigate the effects of valsartan treatment on the gingival tissue of rats in terms of epithelial thickness, lamina propria thickness, submucosa thickness, and blood vessel count variations when compared to healthy controls. Histomorphometric analysis revealed valsartan-treated rats displayed a thicker epithelium compared to the control group. However, the absence of statistical significance indicates a small effect of the medicine on the

proliferative activity of gingival epithelial keratinocytes. No prior literature has established the influence of ARBs on oral keratinocyte kinetics. Nonetheless, numerous animal models established the anti-inflammatory impact of ARBs, suggesting that ARB-induced reduction in pro-inflammatory cytokine production, such as IL-6, may confer protective benefits to keratinocytes against inflammatory-induced damage [11,12]. A notable rise in lamina propria thickness observed in rats treated with valsartan ($p= 0.0038$) suggests a possible effect on the structural components of connective tissue. Increased thickness could be attributed to a variety of factors, including enhanced fibroblast activity, extracellular matrix production, altered cytokine profile, and vascular permeability [13]. However, Research on skin epithelium reported that the reduction of fibrosis and excessive extracellular matrix deposition were induced by Ang II. The profibrotic effects of Ang II are mediated via AT1receptor in cultured human and mouse skin fibroblasts, potentially preserving normal lamina propria thickness [14]. Accordingly, administration of ARBs such as valsartan should counteract the profibrotic effect of the angiotensin II receptor. To understand how valsartan administration results in the increased thickness of the lamina propria, more thorough molecular studies are required. Rats treated with valsartan and control rats exhibited no variation in submucosal thickness. This indicates that deeper gingival tissue may be unaffected by valsartan-induced changes, suggesting a more targeted influence on the lamina propria rather than encompassing the entire gingival connective tissue. Furthermore, a comparable blood vessel count between the control and valsartan-treated groups indicates that valsartan exerts no direct quantitative influence on gingival vascularization under normal physiological conditions. It is noteworthy to emphasize that valsartan, as an angiotensin receptor blocker (ARB) with established vasodilatory effects, may exert a greater functional than structural influence on blood flow or tissue perfusion [15].

Study limitations

A limited sample size, a brief duration of valsartan medication, and the absence of immunohistochemistry analysis constrain this study. The study presented novel in vivo findings that address a gap in the literature by exploring the peripheral effects of valsartan on oral gingival tissue in rats.

Conclusion

Valsartan treatment made the lamina propria thicker but had no effect on the thickness of the epithelium, the thickness of the submucosa, or the number of blood vessels in rat gingival tissue. These data suggest that, despite maintaining overall gingival structural integrity, valsartan may influence certain elements of connective tissue. Further work is required to elucidate the molecular processes underlying these changes and their potential therapeutic implications.

Conflict of interests

The authors declared no conflict of interest.

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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