Research Article

Effects of Fixed Orthodontic Appliance with Antihypertensive Drugs on the Body Weight of Experimental Rats

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Abstract: Background: This study aims to investigate the effect of fixed orthodontic appliances and/or antihypertensive drugs on the weight of experimental rats. Materials and Methods: Thirty-six male Wistar albino rats were subjected to a split-mouth design study, in which an orthodontic appliance was inserted in one side to move the first molar mesially for 2 weeks while the other side acted as a control to tooth movement. The rats were allocated into three groups: group A (n = 12), without any pharmacological treatment; group B (n = 12), subcutaneous injection of bisoprolol fumarate (5 mg/kg) daily; and group C (n = 12), subcutaneous injection of valsartan (10 mg/kg) daily. A fixed orthodontic appliance with a closing coil spring delivering 50 gm of force was used to move the first molar mesially while the incisors served as an anchor unit. The weight of the rats was measured on days 1 (the day of appliance insertion), 7, and 14. Results: No significant ($P > 0.05$) difference in the body weight of rats was found among the three groups at each time point; however, significant ($P < 0.05$) weight loss was noted after the first and second week of the experiment in all study groups. Conclusion: The body weight loss of rats following the insertion of a fixed orthodontic appliance could be related to the effects of the appliance rather than the administered antihypertensive drugs.

Keywords: Fixed Orthodontic Appliance, Body Weight, Antihypertensive Drugs

Introduction

Orthodontic treatment procedures require applying continuous force on teeth to reposition them, which causes various cellular–molecular changes that lead to biological movement into a new position. In this procedure, the mechanical stimulant is distributed to the surrounding periodontium; several studies have found that a variety of medicines may affect this process [1, 2].

Hypertension is a common chronic medical condition characterized by an increase in arterial pressure [3]. Despite years of research on hypertension, no specific cause has been established. It is thought to be the result of a combination of genetics, nutrition, lifestyle, and age [4].

The study of diseases and biological behavior in people and animals as well as their causes, diagnoses, and treatments depends on the use of animals [5]. If an overestimation of results is required, then the

anatomy, microanatomy, and biology of an animal model should represent those of humans. The biology of tooth movement has frequently been studied using rat models [6].

Cardiovascular diseases are generally treated with different types of drugs, such as beta-blockers (BB) and angiotensin receptor blockers (ARB), a class of antihypertensive medications approved by FDA.

BBs are indicated for the treatment of hypertension. These drugs bind to beta-adrenoceptors and block the binding of norepinephrine and epinephrine to these receptors [7, 8].

Bisoprolol, a cardio-selective beta1-blocker (B1-blocker), is used to manage and treat hypertension. It may affect bone structure, metabolism, and fracture healing [9, 10].

The two common types of beta-adrenergic receptors in human osteoblastic and osteoclastic cells are types 1 and 2 β2-receptors. The effect of beta receptors on bone metabolism has been demonstrated in different in vitro and in vivo studies. Beta-receptor stimulation can induce osteoclastogenesis by promoting the production of IL-6, IL-11, and PGE2 and the expression of osteoclast differentiation factors in osteoblasts. The expression of RANKL and OPG on osteoblasts is regulated by the sympathetic nervous system, which is mediated by α- and β-adrenergic stimulation. The catabolic effect of the sympathetic nervous system on bone has been reported [11].

β-Adrenergic signaling in osteoblasts can suppress activity and promote osteoclastogenesis, thereby enhancing bone resorption. The nervous system plays a crucial role in bone mass regulation. Studies on rodents reported that the β2-adrenoreceptor subtype mediated the activation of β-adrenoceptors on bone-forming osteoblasts, which can induce bone loss [12].

The renin-angiotensin-aldosterone system (RAAS) is fundamental to blood pressure control and has long been a target of antihypertensive medicines. This category includes various types of medications that affect different portions of the RAAS axis, such as angiotensin-converting enzyme (ACE) inhibitors and ARB, which are commonly used for hypertension, heart failure, and cardiovascular and renal protection. The RAAS agent acts not only systemically but also locally in a variety of tissues, including bone [13].

The accidental withdrawal from ACE or ARB medication in patients who have hypertension or heart failure could result in clinical instability and negative health outcomes. This finding emphasizes the importance of continuing such medicine even for patients who are infected with COVID-19 [14].

Valsartan is an ARB that selectively blocks the binding of angiotensin II to the angiotensin II type 1 receptor. Many studies have demonstrated the efficacy of valsartan in lowering blood pressure [15].

The effects of ARB on bone structure are controversial. Angiotensin II can activate osteoclasts in mice, but this effect is reversed by an injection of ARB. Moreover, ARB therapy increases bone mass, which could be associated with increased osteoblastic and decreased osteoclastic activity. However, angiotensin II can promote osteoblast growth, indicating that blocking this enzyme may inhibit bone formation in vivo [16].

Other previous works reported that weight loss in experimental animals is multifactorial [17]. The present study aims to examine the effect of fixed orthodontic appliances and/or the administration of hypertension medication on the body weight of experimental rats.

Methods

The study was approved by the scientific research and ethics committee at the Collage of Dentistry, University of Baghdad (Ref. 630 in 2022).

Laboratory animals

Thirty-six 10-week-old male Wistar albino rats, weighing 250–350 g, were included in this study. Each animal was kept in temperature-controlled separate cages and exposed to 24 hours of light–dark cycle of equal time. The animals were fed with standard rat pellets and given free access to tap water [18, 19]. The rats were divided randomly into three groups: group A includes the control group (n =12) without any pharmacological treatment but received orthodontic appliance for 2 weeks; group B (n = 12) received subcutaneous injection of bisoprolol fumarate (5 mg/kg every day) and orthodontic treatment simultaneously for 2 weeks; and group C (n = 12) received subcutaneous injection of valsartan (10 mg/kg every day) and orthodontic appliance simultaneously for 2 weeks. All procedures on animals were carried out under general anesthesia by using intramuscular injections of a mixture of ketamine (50 mg/kg body weight) [20] and xylazine (5–12 mg/kg body weight).

Dosage and administration of antihypertensive drugs

Rats in group B were injected with 5 mg/kg bisoprolol subcutaneously every day for 2 weeks. The solution was freshly prepared daily by dilution in distilled water according to rat weight.

Rats in group C were injected with 10 mg/kg valsartan subcutaneously every day for (2 weeks). The solution was freshly prepared daily, and it was dissolved in 100% ethanol not in water. The acute dermal toxicity of ethanol is low in rats [21]. A toxic dose of approximately 0.8 g/kg (1 mL/kg) of pure ethanol, valsartan dissolved in 400 μL, and 1600 μL of distilled water were combined to form a homogeneous suspension that can be injected [22].

Placement of orthodontic appliance

All rats in this experiment were subjected to orthodontic tooth movement (OTM). Each rat was examined for a complete and intact set of teeth. The distance between the mesial surface of maxillary first molar to the distal surface of the maxillary third molar at the gingival level (M1–M3 distance) at both appliance (right) and non-appliance (left) sides were measured using Dental micro vernia (DENTART, China) with accuracy of ±0.01.

OTM was achieved using a fixed orthodontic appliance, which consist of a stainless steel ligature wire (0.009 in diameter and 4 mm length) inserted interdentally between the first and second maxillary molars, which looped around the cervical part of the first molar. It was ligated tightly to ensure maximum stabilization of the wire, to which a NiTi closing coil spring was attached. An angled handpiece with an

inverted cone bur was used to make grooves cervically on the labial, palatal, and distal surfaces of both maxillary incisors. Another preformed stainless steel ligature wire (diameter of 0.009 in and length of 5 mm) was looped around the grooves by using inverted cone bur on both incisors to compensate for the conical shape of the rats' incisors and subsequently prevent the slippage of the wire and appliance (Fig. 1).

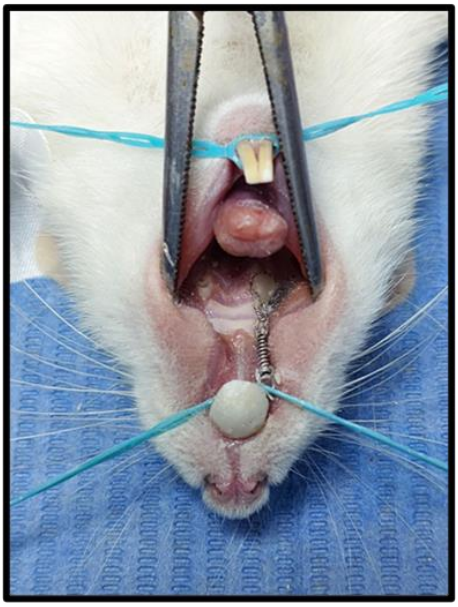

Figure 1: Active orthodontic appliance in the rat's oral cavity.

The ligature wire was ligated tightly and attached to the other end of the closing coil spring was attached so that the spring of the fixed orthodontic appliance delivers a total orthodontic force of 50g [23] for mesial traction of the maxillary first molar. Measurement was conducted by a digital hand-held force gauge (Sr-1kg Gray Digital Hanging Scale, American Weigh Scales, GA, USA).

The labial surface of the maxillary incisors was etched with acid etch gel (37%) for 1 minute, rinsed, and dried. A small amount of light-curing composite filling material was applied to the occlusal surface of the first molar and around the maxillary incisors to cover the ligature wire and cured for 20 seconds according to the manufacturer's instructions. The appliance was checked daily to ensure any loss or damage. The weight of the rats was measured on days 1 (the day of appliance insertion), 7, and 14 (the end of the experiment) using a Four-digit digital scale (China).

Statistical analysis

Shapiro–Wilk test was performed to evaluate data distribution. Descriptive statistics including mean, standard deviation, minimum, and maximum were analyzed for each group. One-way ANOVA was used to compare the mean of body weight among the three groups on days 1, 7, and 14.

Repeated-measure ANOVA was performed to compare the mean of body weight for each group at different time points (days 1, 7, and 14). Bonferroni test was used to determine significant differences among groups. A value of $P \leq 0.05$ was considered significant.

Results

A normality test was done to check the data distribution, the Shapiro-Wilk test showed that data of all variables was normally distributed.

Table 1 and Figure 2 shows the Descriptive statistics of body weight of rats in all groups.

One-way ANOVA showed no significant difference in the mean body weight among the three groups at each time point (days 1, 7, and 14; Table 2).

Repeated-measure ANOVA showed a significant difference in the mean body weight between the three time points within each group (control, valsartan, and bisoprolol, with P=0.001, P=0.009, and P=0.001 respectively) (Table 3).

Bonferroni test was performed to compare the mean difference within each group at different time points. Significant difference (P≤0.05) was found between each pair of time points in the control and bisoprolol groups; however, in the valsartan group, significant difference (P≤0.05) was observed only between days 1 and 14 (Table 4).

Table 1: Descriptive statistics and comparison of the body weight of rats among different groups at three time intervals.

Intervals	Groups	N	Mean	S.D.	95% CI		Min.	Max.
					Lower Bound	Upper Bound		
Day 1	Control	12	308.750	40.906	282.760	334.740	250	400
	Valsartan	12	319.583	34.737	297.513	341.654	275	390
	Bisoprolol	12	327.500	41.148	301.356	353.644	250	400
Day 7	Control	12	287.917	38.580	263.404	312.429	250	385
	Valsartan	12	287.917	38.580	263.404	312.429	250	385
	Bisoprolol	12	298.750	45.733	269.693	327.807	240	400
Day 14	Control	12	270.417	39.223	245.496	295.338	200	350
	Valsartan	12	257.917	39.912	232.558	283.276	200	325
	Bisoprolol	12	269.583	43.456	241.973	297.194	200	380

Table 2: One-way ANOVA for comparison of the mean body weights between groups and within groups at different time intervals.

Intervals		Sum of Squares	d.f.	Mean Square	F-test	p-value
	Between Groups	2126.389	2	1063.194		
Day 1	Within Groups	50304.167	33	1524.369	0.697	0.505
	Total	52430.556	35			
	Between Groups	938.889	2	469.444		
Day 7	Within Groups	55752.083	33	1689.457	0.278	0.759
	Total	56690.972	35			
	Between Groups	1172.222	2	586.111		
Day 14	Within Groups	55218.750	33	1673.295	0.350	0.707
	Total	56390.972	35			

Table 3: Repeated-measure ANOVA for comparison of the mean body weights at different time intervals within each group.

Groups	Intervals	Descriptive statistics					Comparison	
		N	Mean	S.D.	Min.	Max.	F-test	p-value
Control	Day 1	12	308.750	40.906	250	400	24.443	0.000
	Day 7	12	287.917	38.580	250	385		
	Day 14	12	270.417	39.223	200	350		
Valsartan	Day 1	12	319.583	34.737	275	390	7.288	0.009
	Day 7	12	287.917	38.580	250	385		
	Day 14	12	257.917	39.912	200	325		
Bisoprolol	Day 1	12	327.500	41.148	250	400	33.341	0.000
	Day 7	12	298.750	45.733	240	400		
	Day 14	12	269.583	43.456	200	380		

Table 4: Multiple comparisons using Bonferroni test within each group at different time points.

Groups	Intervals		Mean Difference	p-value	95% CI for Difference	
					Upper Bound	Lower Bound
Control	Day 1	Day 1	20.833	0.001	9.743	31.924
		Day 14	38.333	0.001	17.627	59.040
	Day 7	Day 14	17.5	0.009	4.570	30.430
Valsartan	Day 1	Day 1	31.667	0.278	-16.848	80.181
		Day 14	61.667	0.000	32.246	91.087
	Day 7	Day 14	30	0.453	-24.830	84.830
Bisoprolol	Day 1	Day 1	28.750	0.002	11.733	45.767
		Day 14	57.917	0.000	32.738	83.095
	Day 7	Day 14	29.167	0.001	12.534	45.799

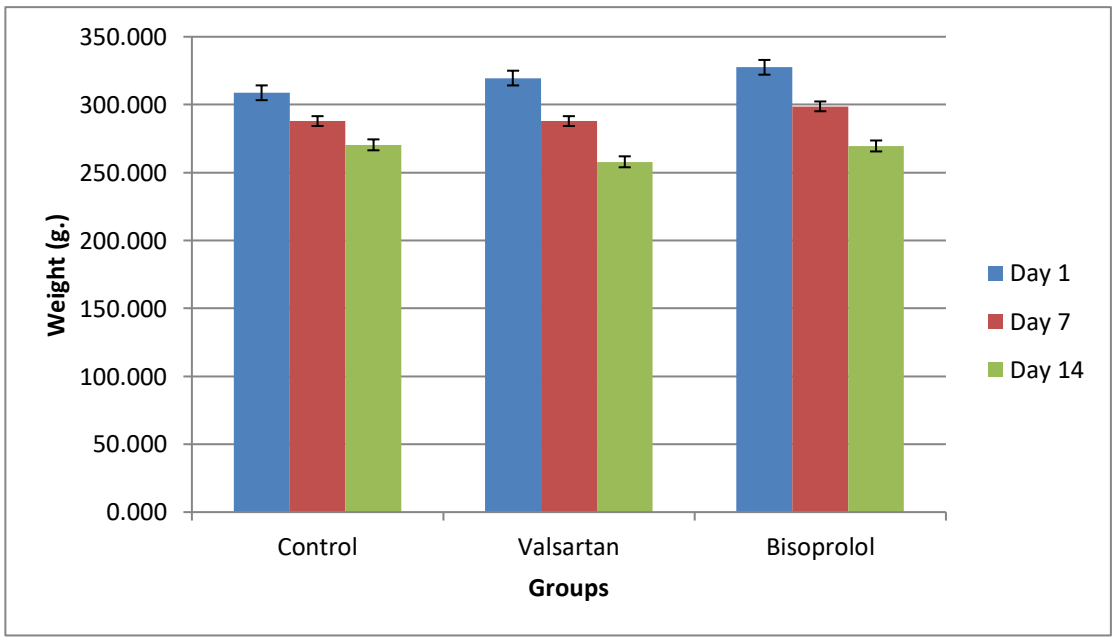

Figure 2: Mean body weight of rats in the three groups at different time intervals.

Discussion

Rats were used in this study because they can be considered a good experimental model to understand biological and cellular mechanisms and develop new orthodontic treatment options [24].

This study evaluated changes in the body weights of experimental rats during OTM using a fixed orthodontic appliance (closing coil spring) with or without subcutaneous administration of antihypertensive drugs (valsartan and bisoprolol) for 2 weeks. The body weight was significantly reduced weekly between the control group and the two other antihypertensive drug groups.

Drugs and nutrients given to patients on a regular basis can enter the mechanically stressed paradental tissues and interact with local target cells by circulation. Mechanical forces mixed with one or more of these chemicals may have inhibitory, additive, or synergistic effects [25].

Strong evidence indicates that the autonomic nervous system controls bone remodeling through beta-adrenergic receptors. These receptors have specific effects on cortical and trabecular bone and can be inhibited to increase the trabecular bone volume. BB can affect bone metabolism [10], and antihypertensive drugs could influence the body weight, bone metabolism, and OTM. Some of the metabolic consequences of beta-blocker medication include reduced energy usage, lipolysis inhibition, impaired insulin sensitivity, and weight gain [26]; this report contradicted the present findings that the weight of rats decreased with time after administration of bisoprolol. Another study conducted by Watanabe and coworkers reported that bisoprolol had no effect on body weight [27]. This finding could explain that the reduction in the body weight of rats in the bisoprolol group could be related to the presence of orthodontic appliance rather than the effect of drug, as supported by the insignificant differences in the body weight of rats between the bisoprolol and control groups at each time point.

Previous studies stated that valsartan can be well tolerated and has the potential to control blood pressure and promote weight loss to improve obesity-related disorders [28, 29]; however, the weight loss was also observed in the control group, which did not receive any medication. Thus, the presence of orthodontic appliance and its effect on intraoral interference with eating in all the three groups could be the main reason for weight loss rather than the used drugs. This explanation can be confirmed by the findings of a previous study, which showed that in the absence of changes in motor activity, valsartan had no effect on weight gain and promoted decline in daily energy expenditure [30].

Numerous studies on humans support that body weight loss is a side effect following the placement of orthodontic appliances because patients are in pain and discomfort [31-35]. In animals, orthodontic treatment contributes significantly to weight loss, especially during the first month of orthodontic treatment; in this period, the animal's mouth contains a fixed orthodontic appliance that may interfere with eating and swallowing and cause them to eat less because chewing and swallowing hard food can be challenging and the ability to masticate is decreased after the insertion of the appliance [36, 37].

Conclusion

The body weight loss of animals following the insertion of a fixed appliance by using closing coil spring to move teeth could be related to the effects of the appliance rather than the administered anti-hypertensive drugs.

Conflict of interest

The authors have no conflicts of interest to declare.

Author contributions

H.A.M. conceived of the presented idea, developed the theory of research ,carried out the laboratory animal model experiments and processed the experimental data. wrote the manuscript in consultation with H.F.S. and M.H.K. H.F.S. were involved in planning, wrote the manuscript with input from all authors. supervised the findings of this work. M.H.K., supervised the work and, performed the analysis, drafted the manuscript and designed the figures. All authors discussed the results and contributed to the final manuscript. The research, analysis, and manuscript were improved by all authors, who also offered constructive criticism.

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Informed consent

Informed consent was obtained from all individuals, or their guardians included in this study.

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تاثيرجهاز تقويم الأسنان الثابت مع الأدوية الخافضة للضغط على وزن جسم الجرذ التجريبي

طبيبة الاسنان هديل علي مهدي ، الاستاذ الدكتور حيدر فاضل سلوم ، الاستاذ المساعد الدكتور مهند علي حمودي كشمولة

المستخلص:

الخلفية : تهدف هذه الدراسة إلى معرفة تأثير أجهزة تقويم الأسنان الثابتة و / أو الأدوية الخافضة للضغط على وزن الفئران التجريبية.

المواد والطرق: تم إخضاع 36 من ذكور الجرذان البيضاء للدراسة بتصميم الفم المنقسم حيث تم إدخال جهاز تقويم الأسنان في جانب واحد لتحريك الضرس الأول للامام لمدة أسبوعين ، بينما عمل الجانب الآخر كعنصر تحكم في حركة الأسنان. تم تقسيم الجرذان إلى ثلاث مجموعات: المجموعة أ (عددها = 12) بدون أي علاج دوائي. المجموعة ب (عددها = 12) حقن بيسوبرولول فومارات تحت الجلد (5 مجم / كجم) يوميًا تلقت المجموعة ج (عددها = 12) حقنًا تحت الجلد من فالسارتان (10 مجم / كجم) يوميًا. تم استخدام جهاز تقويم الأسنان الثابت الحلزون الضاغط الذي يوفر قوة تقويمية (50 جرام) لتحريك الضرس الأول في حين أن القواطع تعمل كوحدة للتثبيت. تم قياس وزن الفئران في اليوم الأول (يوم إدخال الجهاز) ؛ اليوم السابع واليوم الرابع عشر. النتائج: أظهرت النتائج عدم وجود فرق معنوي بين المجموعات الثلاث في كل نقطة زمنية لكن لوحظ فقدان وزن معنوي بعد الأسبوعين الأول والثاني من التجربة في جمي مجموعات الدراسة. الخاتمة: خلاصة الدراسة إلى أن وزن الفئران المفقودة بعد إدخال جهاز تقويم الأسنان الثابت يمكن أن يكون مرتبطًا بتأثيرات الجهاز الثابت بدلاً من الأدوية الخافضة للضغط.