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Histometric Evaluation of Atenolol Effect on Developing Rat Dentition: Experimental Study

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الخلاصة

الأهداف: تهدف هذه الدراسة إلى تقييم التأثير القياسي لدواء الاتينولول على أسنان الجرذ النامية. **المواد وطرائق العمل:** قسمت الجرذان الحوامل إلى مجموعتين رئيستين، مجموعتي سيطرة ومعالجة. أعطيت مجموعة السيطرة (1 مل) ماء مقطر فمويا من يوم (12 إلى يوم 18) من الحمل ثم ضحى بـــ(30) حديث ولادة في أعمار (1,5,10) بعد الولادة. أما مجموعة المعالجة فأعطيت (10 ملغم) من باودر الاتينولول وحلل في (1 مل) ماء مقطر فمويا وللمدة نفسها من الحمل في مجموعة السيطرة، ثم ضحى أيضا بـــ(30) حديث ولادة في أعمار (1,5,10) بعد الولادة. حُضّرت النماذج وصبغت بصبغة الهيماتوكسلين والايوسين وفحصت بالمجهر الضوئي. وأخذت القياسات النسيجية بواسطة عدسة مخصصة للقياسات النسيجية(مقياس العينية). **النتائج:** نتائج الدراسة أثبتت أن لدواء الاتينولول تأثير واضح على أسنان الجرذ النامية حيث سبب تراجعا في تكوين طبقة العاج، وتناقص في الأبعاد فضلاً عن تأخير في ظهور الأسنان وزيادة في خلوية لب الأسنان المعالجة.

ABSTRACT

Aims: To investigate the histometric effect of atenolol drug on the developing dentition of rats. **Materials and Methods**: The pregnant rats were divided into two main groups; control and atenolol treated groups. The control pregnant rats were given 1 ml distilled water orally from 12 – 18 day of gestation period, after delivery of these rats, thirty newborn rats from six mothers were sacrificed at ages 1, 5, 10 days postnataly (p.n). The experimental group were given 10 mg atenolol powder suspended in 1 ml distilled water orally, daily as a single dose from 12 – 18 day of gestation, thirty newborn rats from each six mothers were sacrificed at ages 1, 5, 10 days postnataly. The specimens were prepared for processing and staining with Hematoxyline and Eosin stains, and then examined by Motic microscope. Histometric measurements were under taken for these specimens by graticule lense. The histometric parameters used are the dentin thickness, mesiodistal dimension of molar teeth, pulp density (cellularity) and eruption rate. **Results:** The results showed that atenolol affect on the developing rat dentition .This manifested by less dentin thickness, decreased mesiodistal dimension, increase in pulp cellularity and delay in eruption rate of lower incisor tooth in the atenolol treated groups in comparison to the control groups. These observed changes are proceeded or continued as development process until last expremintal age 10 p.n. **Conclusions:** Atenolol drug delay the tooth development and affect it.

Key Words: Atenolol, dental tissue development,tooth eruption.

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INTRODUCTION

Atenolol is a cardioselective beta – blocker,[1] it inhibits at high doses beta 2 – adrenoceptors, chiefly in the bronchial and vascular musculature.[2] Cardiac adrenergic receptors are blocked by cardioselective B – blocker, resulting in a decreased heart rate and force contraction, which lower blood pressure. Atenolol inhibits epinephrine, xanthine, and B – adrenergic bronchodilators,and increases hypotensive effect of calcium channel blockers.[3,4] Atenolol can cause fetal harm when administrated to a pregnant women. The drug crosses the placental barrier, and the ration of atenolol concentration in maternal to cord blood is about 1: 1. No studies have been performed on the use of atenolol in the first trimester and the possibility of fetal injury cannot be excluded.[5] Many teratologists believe that any chemical administered under appropriate conditions of dose and time of development can cause some disturbances in embryonic development in some laboratory species,[6]the most characteristic susceptibility of the embryo to chemical insult during the organogenesis period is the induction of structural birth defects, although these are often accompanied by embryo lethality.

Within the organogenesis period, individual organ systems possess highly specific periods of vulnerability to teratogenic insult.[7] Drugs are well known for their effect on developing dental tissue, the most common of these being: anxiolytics,[8] chemotherapy of cancer,[9] and hydrocortisone.[10] Atenolol proved teratogenic effect when exposure with atenolol during a critical time in neurogenesis ,alters the normal architecture of neuroepithelium, with loss of integrity at both basal and apical surfaces and affect ultrastructure of cellular elements, malformed embryo with neural tube defects, gross anomalies and growth retardation,[11,12]this drug may iduce hepatotoxicity[13] and many side effects are associated with its use.[14] The aim of this study, to determine and establish the histological measurement including (dentin thickness at two areas, mesiodistal dimensions,eruption rate and pulp cell density) on the teratogenic effects of atenolol on developing teeth.

MATERIALS AND METHODS

Pregnant rats (250--300 gm) were separated and housed individually in plastic cages.

In control group, eighteen pregnant rats, each rat was given distilled water (1 cc) at the 12th day of gestation untill 18th day. After delivery of these rats, roughly 5 new born of each mother were selected randomly, 30 newborn rats sacrificed at (1,5,10) days old.

The experimental group, is consisted of eighteen pregnant rats, each rat was given 10 mg atenolol powder suspended in 1 cc distilled water to obtain a concentration of 10mg/1ml orally as a single dose, daily from 12 – 18 day of pregnancy. After delivery of 18 pregnant rats treated by atenolol drug, roughly 5 new born of each mother were selected randomly, 30 newborn rats sacrificed at (1,5,10) days old (Table 1).

Table (1): Experimental design

Groups	1 day old	5 days old	10 days old	
Control group (18) pregnant rats¤	30 *	30 *	30 *	90
Treated group (18) pregnant rats •	30 *	30 *	30 *	90
Total	60	60	60	180

¤ distilled water given.; • Atenolol treated; * Sacrificed (30 newborn rats from each 6 mothers, 5 newborn from each mother)

One hundred and eighty newborn rats were examined through this study. Set of serial saggittal sections were prepared for processing and staining with heamatoxyline and eosin staining and examined under light microscopes,all the midsaggital sections were examined under Motic microscope.

Histological measurements:

Using an eye piece graticule lens with 4 times and 8 times magnification to measure the mid saggital sections

1. Dentin Thickness: Dentin thickness is measured at the tip of the mesial cusps of molar teeth in 1, 5, 10 p.n (at mid saggital section only) and also dentin thickness at the labial surface at middle area of the incisor teeth were measured in 1, 5, 10 p.n of the control and treated groups.
2. Mesiodistal Dimensions: The distance between the mesial and distal sides were measured for first molar tooth (from cemento-enamel junction CEJ at mesial side to CEJ at distal side), at 10 p.n in the control and treated groups.
3. Eruption Rate: The distance between cemento – enamel junction (CEJ) and the tip of incisor edge. The cemento – enamel junction (CEJ) was used as the fixed point of the tooth. This point was chosen because it was easily recognized and was not lost with age.
4. Density: Circumscribed standard circle is projected on the slide which is considered the area of the pulp evaluated, then counting of cells inside this circle. Results indicated the cell density, i.e., cell per unit area (area of this circle) of the pulp in erupting, middle and developing portions of the pulp of the first molar teeth.

Statistical analyses were done using SPSS version 7.5 computer software (statistical package for social science). The statistical significance of the difference in dentin thickness mean, mesiodistal dimension mean, cell density and eruption rate, between 1 p.n, 5 p.n and 10 p.n, control group and atenolol treated group were tested by t – test.

RESULTS

Gross Observations:

The atenolol treated animals appeared less active and smaller in their size.

Morphological Profile of the Experimented Groups:

There is a decrease in the tooth size at 1st day p.n in atenolol treated group and disorganization of pulp cells, abnormal dentin thickness delay in apposition of dentin layer in comparison to the control group. The growth continues but there are some abnormalities at 5 day p.n like disorganization of odontoblast in some area, increase in vascularity globular dentin, in comparison to the control group, while at 10th day p.n there is increase in pulp size, decrease in ameloblast layer and decrease in eruption rate of lower incisor, decrease in eruption rate (not completely appeared) in the oral cavity, dentin thickness is abnormal and less dentin deposition than control group (Figures 1 and 2).

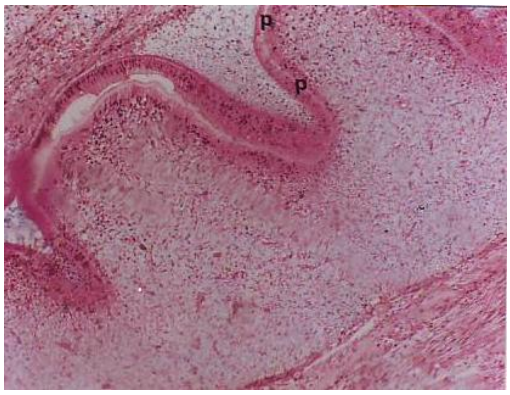

Figure (1): Midsaggital section of mesial cusp of lower first molar in the control group at 1 day old. Notice there is thin predentin (p) at tip of the cusp.(X 20)

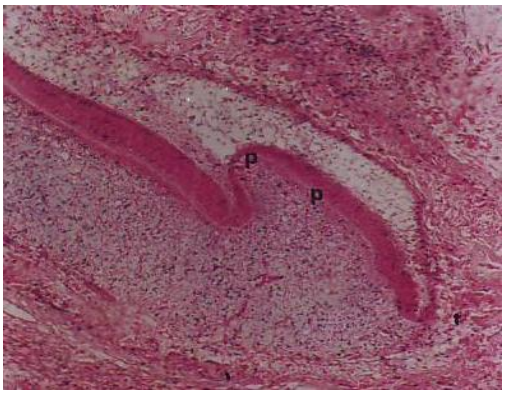

Figure (2): Midsaggital section of mesial cusp of lower first molar in the atenolol treated group at 1 day old. Notice there is thin very very thin predentin (p) at tip of the cusp,smaller tooth germ size in comparison to control group.(X 20)

Numerical Profile:

The results presented were based on the analysis of data on control and atenolol treated groups using t – test.

Dentin Thickness it is shown in Tables (2) and (3) at ages 1 p.n, 5 p.n, 10 p.n, the dentin thickness of first molar tooth and dentin thickness at labial side of incisor tooth of the treated groups were significantly lower than that of the control group. Mesiodistal Measurement of First Molar as shown in Table (4), at ages of 1 p.n, 5 p.n, 10 p.n in atenolol treated group, the mesiodistal dimension of first molar teeth were significantly lower than that of the control group.

Eruption Rate:

In Table (5), the eruption rate of lower incisor of 10 p.n in atenolol treated group was significantly lower than that of the control groups.

Regarding the Cellularity of the Pulp. It is shown that atenolol have significant direct effect on the celularity (increasing) of the pulp in all three regions erupting, middle and developing areas (Table 6).

Table (2): The mean measurement of the dentin thickness of first molar teeth (at tip of the cusp) of the control and treated groups at 1, 5 and 10 post natal.

Period	Group	No.	Mean-µm	Standard deviation	T - value	*P* - value
1 day	Control	30	1.503	0.076	107.65	0.000
	Treated	30	0.990	0.088		
5 day	Control	30	3.000	0.695	23.65	0.000
	Treated	30	2.000	0.643		
10 day	Control	30	4.000	0.643	34.06	0.000
	Treated	30	3.033	0.809		

Table (3): The mean measurement of the dentin thickness at the labial surface of incisor tooth (at mid region) of the control and treated groups at 1, 5 and 10 post natal.

Period	Group	No.	Mean-µm	Standard deviation	T - value	*P* - value
1 day	Control	30	2.496	0.089	153.66	00.000
	Treated	30	1.5133	0.077		
5 day	Control	30	3.967	0.669	32.49	0.000
	Treated	30	2.833	0.747		
10 day	Control	30	5.833	0.747	42.79	0.000
	Treated	30	4.000	0.587		

Table (4): The mean measurement of the mesiodistal dimension of first molar teeth of the control and treated groups at 1, 5 and 10 post natal.

Period	Group	No.	Mean-µm	Standard deviation	T - value	*P* - value
1 day	Control	30	49.967	0.928	294.96	00.000
	Treated	30	35.067	0.785		
5 day	Control	30	64.933	0.691	514.33	0.000
	Treated	30	50.067	0.944		
10 day	Control	30	120.200	1.031	638.84	0.000
	Treated	30	74.967	0.718		

Table (5): The mean measurement of the eruption rate of incisor tooth at 10 p.n in the control and treated groups.

Group	No.	Mean	Standard deviation	T - value	*P* - value
Control	30	1.983	0.245	44.33	0.000
Treated	30	1.000	0.233		

Table (6): The mean measurement of pulp cell density in molar teeth at erupting or upper, middle and developing areas at 1, 5 and 10 post natal in central and treated groups.

Period	Area	Group	No.	Mean	Standard deviation	T - value	*P* - value
1 day	Erupting	Control	30	3.000	0.743	22.12	00.000
		Treated	30	5.034	0.421		
	Middle	Control	30	2.000	0.695	15.77	0.000
		Treated	30	3.000	0.587		
	Developing	Control	30	2.433	1.104	12.07	0.000
		Treated	30	4.000	0.743		
5 day	Erupting	Control	30	4.200	0.551	41.76	0.000
		Treated	30	6.000	0.378		
	Middle	Control	30	3.500	0.777	24.68	0.000
		Treated	30	4.000	0.655		
	Developing	Control	30	4.100	0.402	55.78	0.000
		Treated	30	6.000	0.454		
10 day	Erupting	Control	30	6.000	0.587	55.96	0.000
		Treated	30	8.000	0.371		
	Middle	Control	30	4.933	0.583	46.32	0.000
		Treated	30	7.000	0.788		
	Developing	Control	30	6.000	0.454	72.25	0.000
		Treated	30	7.000	0.743		

DISCUSSION

Many dental researchers have done investigated concentrating on what effects atenolol would have on developing tissue.The Previous experiments confirmed that atenlol was capable of causing obvious changes in the developing tissues.[12, 15, 16]

The literature have shown that there is a lack on the investigated effects of atenolol on the developing dental tissue, the present experiments were designed originally to study the histometric effects of atenolol on developing dentition of the rat. The control groups gave a normal histological appearance,while the atenolol treated groups gave obvious histological abnormalities in their developing teeth; so this confirmed that atenolol cross the placenta,[12] reaching to the featus via fetal blood carrying it from their treated mothers,also it can cause temporary or permanent problems to the unborn child and breast feeding child.[17] In this study the drug was given to pregnant rats at day 12th of gestation, just before the beginning of the development process of the rat dentition.

Irregular thickness and formation of dentin were observed in this investigation and due to effect of atenolol, these findings are in agreement with the other studies used other drug or other teratogen factors.[5, 11]

From the histopathological point of view, our histometric data corroborates the histopathological feature.There is a significant difference between control and atenolol treated group in dentin thickness, because of the effect of atenolol on the odontoblast cells, on its histo – differentitation and maturation. Many factors could effect on the dentin thickness in the treated group ,one of this is delay in dentinogenesis. This may be due to effect on the work of odontoblast cells, that secrete dentin matrix ,but at a less amount, or presence of functionless odontoblast or not very well differentiated to secrete enough dentin matrix. Most probably that atenolol affect the metabolic process of dentinogenesis.[18]

In the present study the odontoblast layer was thinner in the treated group than in normal group.This will give support to the above suggestions and give explanations of the delay in the dentinogenesis in the treated group. The mesiodistal dimensions of the first molar in 1, 5 and 10 p.n in control groups were significantly higher than that of the treated groups. This indicated that the tooth crown size is larger in the control than that of the atenolol treated groups. Diner *et al*[19] who reported that many alterations could be observed in the cortisone treated rat's third molar dimension as crown morphology and reduction in size of the tooth. The atenolol treated rats were markedly smaller in their dimensions than control due to effect of atenolol on the growth of developing tissue[12] So atenolol may affect on the growth of the developing bone – thus neonatal administration of atenolol would affect on the incisor and molar teeth crown size in rats because onset of histodifferentiation and matrix secretion for these teeth is initiated prenatally. As far as known that process of tooth eruption is affected by many factors.These factors include bone remodeling, root growth, vascular pressure and ligament traction, if one of the above factors or all will be disturbed, the eruption process is affected.The treated groups histologically shown an obvious delay in the eruption of incisor teeth at age 10 p.n. This delay was statistically significant p – value. This could be explained the effect of atenolol drug on the developing teeth. This indicated that the developing teeth during stages of development and eruption are sensitive to any alteration in environment of their eruption route, whether as atenolol or cortisone or other effect. Atenolol has greater influence on the primary odontogenic matrix, dental maturation and may be has effect on normal resorptive mechanism of alveolar bone (physical alteration of the surrounding tissue). Delay in the eruption of the tooth was also reported by Diner *et al*[19] in single dose cortisone acetate treated rats and by Al Douri.[11] The pulp showed an increased cellularity (density) in the incisor and molar teeth, this was due to effect of atenolol on the cell proliferation.This increase of cells is more evident at the region of development where the cells are crowded and mitosis is active. These findings are in agreement with other studies[20-22] who observed that the density of the pulp at (cortisone

treated) developing area is more than middle area of the pulp.Also a greater cell density was observed at erupting area because in the control group this subodontoblastic zone is rich with bipolar cells.[23] So, when these teeth are affected by this drug, the density at erupting area increase and become higher, So the statistical analysis confirmed that atenolol have an influence (direct effect) on the density of the pulp.

CONCLUSSION

The developing teeth are affected by atenolol drug (delay in tooth growth, decrease in dentin thickness, mesiodistal dimension and density of the pulp).

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