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Effects of Amoxicillin on the Structure and Mineralization of Dental Enamel and Dentin in Wistar Rats

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A B S T R A C T

Objectives: The development of teeth is affected by genetic and environmental factors. Amoxicillin is a widely prescribed semi-synthetic antibiotic. Its most frequent side effects are gastrointestinal disorders and hypersensitivity reactions. The purpose of this study was to determine the effect produced by amoxicillin administration on dental enamel and dentin in Wistar rats.

Materials and Methods: Twelve pregnant adult Wistar rats were equally divided into four different groups. Negative controls were prescribed with a saline solution. Positive controls were prescribed with tetracycline (130 mg/kg). The other two groups were treated with amoxicillin doses of 50 and 100 mg/kg (every 8 hours), respectively. The treatments were daily administered by oral gavage from the 13th gestation day to the end of gestation. After birth, the offspring also received the same treatment as their mothers from day one to day twelve. After 24 hours, the newborns were sacrificed, the jaws were dissected, and the first molar teeth were collected. The samples were fixed in 10% formaldehyde solution and were histomorphologically and histopathologically observed to determine enamel and dentin abnormalities.

Results: The mean ameloblastic layer thickness, enamel thickness, odontoblastic layer thickness, and dentin thickness were significantly different in the tetracycline group and the amoxicillin 50 and 100 mg/kg groups compared to the control group. Also, dentin hypomineralization and vacuolization of the odontoblastic layer were observed in the tetracycline- and amoxicillin- treated groups.

Conclusion: This study showed that amoxicillin interferes with amelogenesis and dentinogenesis and reduces enamel and dentin thickness.

Keywords: Amoxicillin; Dental Enamel; Dentin; Hypomineralization; Wistar Rats

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INTRODUCTION

The development of teeth is affected by genetic and environmental factors. In fact, factors interfering with tooth development may not only impair the number of teeth developed but can also affect the structure and quality of enamel and dentin formation [1]. Since hard dental tissues cannot be remodeled, these effects can be observed in enamel secretion and deposition in later years [1]. Amoxicillin is an extended-spectrum semi-synthetic antibiotic with a bactericidal action against gram-positive

and gram-negative bacteria. It is therefore administered as the first choice of antibiotics for respiratory, gastrointestinal, neuronal, and skin infections [2]. In dentistry, amoxicillin is administered to fight complex microorganisms associated with dentoalveolar and soft tissue abscess and complex maxillary sinus infections [2]. It is also the first choice of medication for preventing endocarditis infections [3]. The most frequently reported side effects of amoxicillin include gastrointestinal problems and increased sensitivity [2].

According to previous studies, amoxicillin may have pathologic effects such as fluorosis on the enamel structure as a result of excessive fluoride absorption during the formation of enamel structure [4,5]. The hypomineralization of teeth involves specific qualitative defects in the enamel caused by impaired decalcification or the maturation of enamel prisms, creating enamels that are morphologically normal but contain structural or qualitative defects [6]. The hypomineralization of teeth is a dentistry challenge that can be caused by genetic and environmental factors during the development of dental enamel [7,8]. Although epidemiological studies have not identified the definite effect of amoxicillin on the hypomineralization of teeth, they suggest that children treated with this antibiotic exhibit more impaired tooth development [9,10].

Amoxicillin is administered for pregnant women just as it is for children. Given the lack of information on the effect of amoxicillin on dental enamel and dentin development and the widespread administration of this antibiotic in children and pregnant women, further studies to ascertain the effect of this antibiotic on dental disorders seem necessary [10].

Since there are no definitive results on this subject [11], the present study was conducted to investigate the administration of two different doses of amoxicillin before and after birth on enamel and dentin structure in Wistar rats.

MATERIALS AND METHODS

This research was done in 2017 at the Nervous System Stem Cells Research Center and at the Research Center of Physiology of Semnan University of Medical Sciences, Semnan, Iran (ethical approval code: IR.SEMUMS.REC.1394.234).

Experimental animals:

Twelve adult female Wistar rats (weighing 250±20 g) were used in the present study. The animals were kept under controlled temperature (22±2°C) and humidity of 55±5% in a 12-hour light-dark cycle with free access to food and water. The rats were in proestrus or estrus stage (diagnosed by vaginal smear). Female rats were allowed to mate with male rats overnight (two females with one male). Male rats were removed from the cages early in the morning, and vaginal smears were taken from female rats to be analyzed under an optical microscope (Reichert Jung DiaStar®; Cambridge Instruments, Buffalo, NY, USA). The detection of sperm in the stained vaginal smears was taken to indicate day zero of pregnancy. The pregnant rats were then randomly divided into four groups of three each (Table 1), and each group was kept in a separate Plexiglas cage [12].

Experimental procedures:

The pregnant rats in the experimental groups were treated with a daily oral dose using a metal esophageal catheter from day 13 to day 22 of their pregnancy (Table 1).

Table 1. Description of the studied groups (n=3)

	Groups	Treatment type/Medication dose
1	Negative Control	Normal saline (1 ml/kg)
2	Positive Control: using tetracycline, which its toxic effect on enamel development has been demonstrated both in humans and animals	Tetracycline (130 mg/kg)
3	Experimental 1: using the prescribed dose for the treatment of acute otitis media in children	Amoxicillin (50 mg/kg, every 8 hours)
4	Experimental 2: using experimental doses double the treatment dose (experimental doses for surveying of the effect of amoxicillin)	Amoxicillin (100 mg/kg, every 8 hours)

After birth, the newborns also received the same treatment as their mothers from day one to twelve. The reason for starting the treatment on day 13 of pregnancy was because amoxicillin can pass through the placenta and the umbilical cord during this period and also because this period marks the beginning of the development of the first maxillary molar in rats (these animals only have a permanent dental system) [12]. In 12-day-old babies, the first molars usually show their last secretion stage in the cervical region and begin enamel mineralization in the cusp. In addition, at the age of 12 days, the first molars undergo active eruption which is a process characterized by mucus perforation [12].

Histological processing:

The Twelve-day rats in each group were sacrificed with sodium pentobarbital overdose, and their heads were immediately submerged in 10% formaldehyde fixative solution at room temperature for 48 to 72 hours. Their molar teeth were then removed from the jawbones for decalcification and were placed in 7% disodium ethylene-diamine-tetra-acetic acid (EDTA) containing 5% formalin in 0.1 M of sodium phosphate for 3 to 7 days. Next, using a tissue preparation device, the samples were dehydrated, made transparent, and submerged in paraffin to form paraffin blocks of the teeth [12,13]. Then, 60 sections with a 5-micrometer (μm) thickness were carefully prepared from each sample using a microtome at low speed in the frontal plane (parallel to the molars' axial plane). During preparation, the angle of the sections was carefully analyzed, and the sections were stained using Hematoxylin-Eosin (H&E) and Alizarin red. The stained sections underwent histomorphometric and histo-pathological examinations using a light microscope (Reichert Jung DiaStar®; Cambridge Instruments, Buffalo, NY, USA; ×200 magnification) with a colored digital camera (Sony, Tokyo, Japan) and equipped with a computer containing Motic Images Plus 2.0 ML software (Motic China Group Co. Ltd., Xiamen, Fujian, China) for morphometric analysis of tissue images.

Morphological analysis and measurement of dental parameters:

First, using the microscope equipped with a colored digital camera, images were taken from the samples, and the tissue images underwent histomorphometric examination using the morphometric analysis software. The enamel and dentin thickness and the thickness of the ameloblastic and odontoblastic layers, which are organized as a single layer of cylindrical cells, were measured in at least ten regions per sample [13]. Data were analyzed by an expert histologist blinded to the group allocations, and the results were recorded.

Statistical analysis:

After data collection, we analyzed the data using the Shapiro-Wilk test. This test was used to normalize the data in each of the groups, and the result was that the distribution of all the variables was normal in all groups. Therefore, we used one-way analysis of variance (ANOVA) and Tukey's post-hoc test in SPSS 23.0 software (SPSS Inc., Chicago, IL, USA). Also, the variance between the groups (test of homogeneity of variances) was not significantly different for all the variables. Accordingly, $P<0.05$ was considered statistically significant.

RESULTS

The mean thickness of the ameloblastic layer was significantly different in the four groups ($F(3.56)=23.6$, $P<0.001$; Fig. 2A). Accordingly, the mean thickness of the ameloblastic layer was lower in the tetracycline group and the amoxicillin 50 and 100 mg/kg groups compared to the control group ($P<0.001$). The mean thickness of the ameloblastic layer in the amoxicillin 100 mg/kg group was less than that in the tetracycline group ($P=0.02$; Fig. 1a-d).

The mean enamel thickness was significantly different in the four groups ($F(3.56)=3.12$, $P=0.033$; Fig. 2B). The mean enamel thickness was significantly lower in the amoxicillin 100 mg/kg group compared to the control group ($P=0.033$) but there were no significant differences between the other groups ($P>0.05$; Fig. 1a-d). The mean thickness of the odontoblastic layer was significantly different in the four groups ($F(3.56)=6.51$, $P=0.001$; Fig. 2C). The odontoblastic layer thickness was significantly lower in the tetracycline group ($P=0.031$), amoxicillin 50 mg/kg group ($P=0.004$), and amoxicillin 100 mg/kg group ($P=0.001$) than in the control group; however, the mean thickness of the odontoblastic layer was not significantly different in the other three groups ($P>0.05$; Fig. 1a-d).

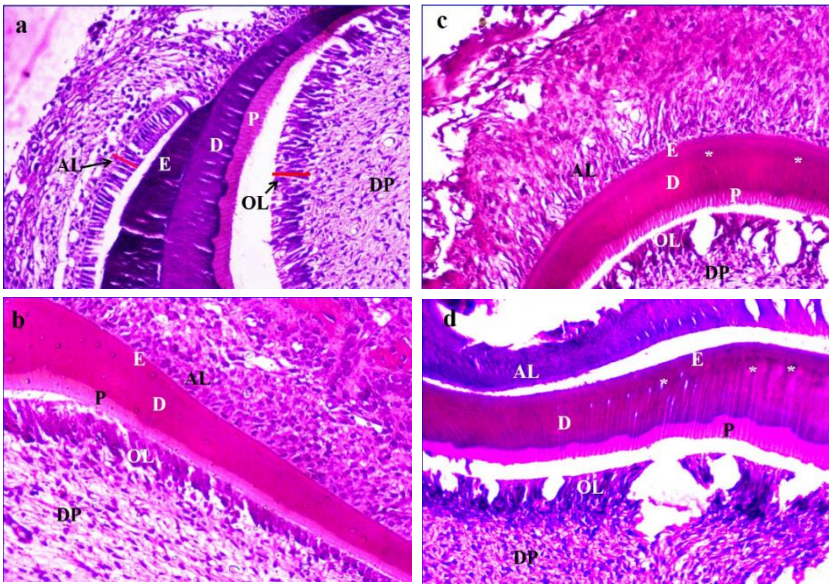

Fig. 1: (a) Frontal photomicrography of a tooth from the negative control group; AL=Ameloblastic Layer, E=Enamel, D=Dentin, P=Predentin, OL=Odontoblastic Layer, DP=Dental Pulp; hematoxylin and eosin (H&E) staining; ×200 magnification. (b) Frontal photomicrography of a tooth from the tetracycline group; AL=Ameloblastic Layer, E=Enamel, D=Dentin, P=Predentin, OL=Odontoblastic Layer, DP=Dental Pulp; H&E staining; ×200 magnification. (c) Frontal photomicrography of a tooth from the amoxicillin 50 mg/kg group; AL=Ameloblastic Layer, E=Enamel, D=Dentin, P=Predentin, OL=Odontoblastic Layer, DP=Dental Pulp, *Hypomineralization; H&E staining; ×200 magnification. Disorder (the presence of several vacuoles) is clearly evident, especially in the odontoblastic layer. (d) Frontal photomicrography of a tooth from the amoxicillin 100 mg/kg group; AL=Ameloblastic Layer, E=Enamel, D=Dentin, P=Predentin, OL=Odontoblastic Layer, DP=Dental Pulp, *Hypomineralization; H&E staining; ×200 magnification. Disorder (the presence of several vacuoles) is clearly evident, especially in the odontoblastic layer

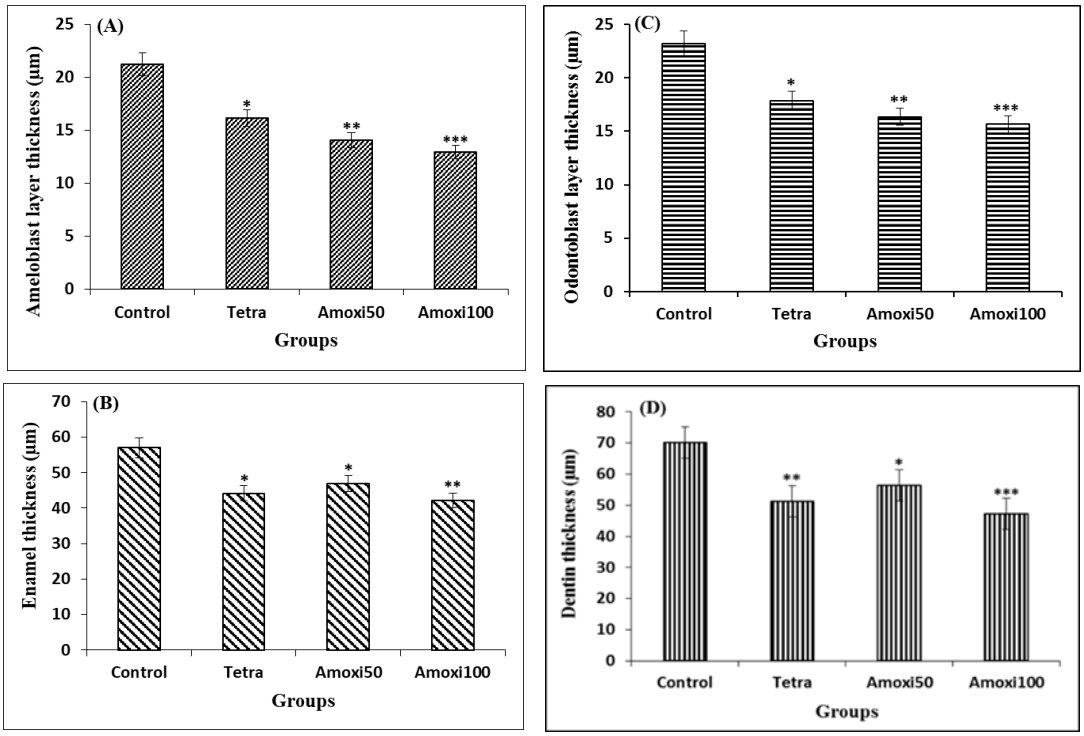

Fig. 2: The error bar of mean and standard errors of (A) ameloblastic layer thickness, (B) enamel thickness, (C) odontoblastic layer thickness, and (D) dentin thickness in the rat neonates. Negative control group (control), positive control group (Tetracycline group, Tetra), amoxicillin 50 mg/kg group (Amoxi50), and amoxicillin 100 mg/kg group (Amoxi100). * P<0.05, ** P<0.01, and *** P<0.001 compared to the control group. All data are presented as Mean±Standard Error (SE).

The mean dentin thickness was significantly different in the four groups (F (3.56)=9.10, P<0.001; Fig. 2D). The mean thickness of dentin was lower in the tetracycline group (P=0.001), amoxicillin 50 mg/kg group (P=0.024), and amoxicillin 100 mg/kg group (P<0.001) than in the control group; however, the mean dentin thickness was not significantly different in the other three groups (P>0.05; Fig. 1a-d).
Hypomineralization (star sign) was observed in both enamel and dentinal layers of the amoxicillin-treated groups (50 and 100 mg/kg). Also, abnormality (the presence of several vacuoles in this layer) is clearly evident, especially in the odontoblastic layer in the amoxicillin-treated groups (50 and 100 mg/kg; Fig. 1c and 1d).

DISCUSSION

The results showed the reduced thickness of the ameloblastic layer, odontoblastic layer, enamel, and dentin in the amoxicillin 50 and 100 mg/kg and tetracycline groups compared to the control group. Using animal models, previous studies have shown that changes in the pH can lead to changes in transmitting molecules such as Cx43, ATPase, and H+-ATPase canals in the cell cytoplasm [13]. These transmitters have a role in the secretion and integration of ameloblasts, and subsequently, in the formation of a homogeneous morphology in the organic part of dental enamel [13]. The mechanism by which antibiotics affect dental enamel has been attributed to changes in protein synthesis [14]. Sahlberg et al [15] have shown that amoxicillin reduces the expression of matrix metallo-peptidase-20 (MMP20) protein-coding gene, which is a metalloproteinase that has a major role in degradation and extraction of enamel proteins [15]. The reduction in ameloblasts and enamel thickness in the present study might be due to the interference of amoxicillin with the transmitting molecule medium and the subsequent impairment of ameloblasts and reduction in enamel secretion [8]. De Souza et al [13] observed vacuole-like structures in the ameloblastic layer in rats treated with amoxicillin and concluded that these structures might have been formed by the interference of amoxicillin with the transmitting molecule medium, which ultimately reduced protein secretion and transmission.

Similar results were obtained in an in-vitro study, where the rats' erupted teeth were exposed to amoxicillin to ultimately show a reduction in their enamel thickness due to the effectiveness of amoxicillin in preventing ameloblastic differentiation [15]. Impaired dentin formation has been reported in the incisors of rats treated with amoxicillin [16]. One study showed that a single dose of amoxicillin significantly increases the interlobular dentin; it pointed out that amoxicillin reduces the ameloblastic activity [16]. Given that dentin secretion precedes enamel formation and is essential to ameloblastic differentiation, amoxicillin can cause delays in the differentiation of this dental epithelium, which results in the reduced secretion of enamel matrix. As also suggested by Sahlberg et al [15], chronic exposure to amoxicillin can interfere with ameloblastic differentiation. Although the precise mechanism of amoxicillin action on amelogenesis is still unknown [11,15], amoxicillin has been shown to prevent the deposition and mineralization of the mantle dentin matrix, causing delays in ameloblastic differentiation [15].
Previous studies have shown that many factors and diseases interfere with amelogenesis, including hormonal, nutritional, systemic, genetic, and environmental factors. Depending on the stage in the life cycle of ameloblasts, these factors affect amelogenesis, leading to a variety of enamel defects [15-19]. Changes have also been reported in ameloblasts and enamel matrix due to treatment with tetracycline [20]. Further studies, including the ultrastructural analysis of in-vivo and in-vitro studies, are required for the clarification of the mechanism of the effect of amoxicillin on ameloblasts and odontoblasts.

CONCLUSION

The present study showed that amoxicillin interferes with amelogenesis and dentino-genesis and reduces enamel and dentin thickness. It is hypothesized that amoxicillin intake (50 mg/kg and 100 mg/kg, every 8 hours) in the early stages of enamel and dentin formation may cause delays in the differen-tiation of ameloblasts and odontoblasts into secreting and functional cells.

REFERENCES

1. Beentjes VE, Weerheijm KL, Groen HJ. Factors involved in the aetiology of molar-incisor hypomineralisation (MIH). Eur J Paediatr Dent. 2002 Mar;3(1):9-13.
2. Bascones Martínez A, Aguirre Urizar JM, Bermejo Fenoll A, Blanco Carrión A, Gay-Escoda C, González-Moles MA, et al. Consensus statement on antimicrobial treatment of odontogenic bacterial infections. Med Oral Patol Oral Cir Bucal. 2004 Nov-Dec;9(5):369-76, 363-9.
3. Dajani AS, Bawdon RE, Berry MC. Oral amoxicillin as prophylaxis for endocarditis: what is the optimal dose? Clin Infect Dis. 1994 Feb;18(2):157-60.
4. Levy SM, Kiritsy MC, Slager SL, Warren JJ. Patterns of dietary fluoride supplement use during infancy. J Public Health Dent. 1998 Summer;58(3):228-33.
5. Levy SM, Warren JJ, Davis CS, Kirchner HL, Kanellis MJ, Wefel JS. Patterns of fluoride intake from birth to 36 months. J Public Health Dent. 2001 Spring; 61(2):70-7.
6. Crombie F, Manton D, Kilpatrick N. Aetiology of molar-incisor hypomineralization: a critical review. Int J Paediatr Dent. 2009 Mar;19(2):73-83.
7. Hong L, Levy SM, Warren JJ, Bergus GR, Dawson DV, Wefel JS, et al. Primary tooth fluorosis and amoxicillin use during infancy. J Public Health Dent. 2004 Winter;64(1):38-44.
8. Laisi S, Ess A, Sahlberg C, Arvio P, Lukinmaa PL, Alaluusua S. Amoxicillin may cause molar incisor hypomineralization. J Dent Res. 2009 Feb;88(2):132-6.
9. Hong L, Levy SM, Warren JJ, Broffitt B. Amoxicillin Use during Early Childhood and Fluorosis of Later Developing Tooth Zones. J Public Health Dent. 2011 Summer;71(3):229-35.
10. Hong L, Levy SM, Warren JJ, Dawson DV, Bergus GR, Wefel JS. Association of amoxicillin use during early childhood with developmental tooth enamel defects. Arch Pediatr Adolesc Med. 2005 Oct;159(10):943-8.
11. Phipps KR. No evidence to support the claim that amoxicillin causes molar-incisor hypomineralization. J Evid Based Dent Pract. 2012 Sep;12(3 Suppl):73-5.
12. Gottberg B, Berné J, Quiñónez B, Solórzano E. Prenatal Effects by Exposing to Amoxicillin on Dental Enamel in Wistar Rats. Med Oral Patol Oral Cir Bucal. 2014 Jan 1;19(1):e38-43.
13. De Souza JF, Gramasco M, Jeremias F, Santos-Pinto L, Giovanini AF, Cerri PS, et al. Amoxicillin Diminishes the Thickness of the Enamel Matrix that is Deposited During the Secretory Stage in Rats. Int J Paediatr Dent. 2016 May;26(3):199-210.
14. Bread NS Jr, Armentrout SA, Weisberger AS. Inhibition of mammalian protein synthesis by antibiotics. Pharmacol Rev. 1969 Sep;21(3):213-45.
15. Sahlberg C, Pavlic A, Ess A, Lukinmaa PL, Salmela E, Alaluusua S. Combined effect of amoxicillin and sodium fluoride on the structure of developing mouse enamel in vitro. Arch Oral Biol. 2013 Sep;58(9):1155-64.
16. Kumazawa K, Sawada T, Yanagisawa T, Shintani S. Effect of single-dose amoxicillin on rat incisor odontogenesis: a morphological study. Clin Oral Investig. 2012 Jun;16(3):835-42.
17. Kuscu OO, Sandalli N, Dikmen S, Ersoy O, Tatar I, Turkmen I, et al. Association of amoxicillin use and molar incisor hypomineralization in piglets: visual and mineral density evaluation. Arch Oral Biol. 2013 Oct;58(10):1422-33.
18. Bronckers AL, Lyaruu DM, DenBesten PK. The impact of fluoride on ameloblasts and the mechanisms of enamel fluorosis. J Dent Res. 2009 Oct;88(10):877-893.
19. Abe T, Miyajima H, Okada K. Effects of a macrolide antibiotic on enamel formation in rat incisors--primary lesion of ameloblast at the transition stage. J Vet Med Sci. 2003 Sep;65(9):985-8.
20. Westergaard J. Structural changes induced by tetracycline in secretory ameloblasts in young rats. Scand J Dent Res. 1980 Dec;88(6):481-95.