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Influence of an Adaptogenic Complex on a State of Tissues of a Gingiva at Orthodontic Tooth Movement on the Background of an Experimental Goiter

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Abstract: An experiment goiter is modeled on 75 rats of Vistar's line by introduction of 1 % solution of potassium per-chlorate with drinking water within 20 days. At the second stage of experiment model of orthodontic tooth movement (OTM) within 21 days is reproduced. It was introduced intragastric solution of potassium iodide (PI) in a dose of 20 mkg/kg to rats of the 3rd - 5th groups since the 22nd day. Drug «Echinacea compositum C» was introduced to animals of the 3rd and 5th groups from the 22nd day during 5 days. After reproduction of OTM to rats of the 4th and 5th groups on the background of injection of PI Teraflex in a dose on glucosaminoglican 750 mg/kg was introduced. In homogenates of gingival tissue activity of acidic phosphatase (AP), elastase, catalase and maintenance of malonic dialdehide (MDA) is defined. At carrying out of orthodontic intervention in rats with an experimental goiter AP activity in a gingiva has increased on 58,9 % and elastase - on 71,4 %. Gross disturbances of work of an antioxidative-prooxidative system in the gingival tissues caused by OTM in the presence of a goiter that demands carrying out of the conforming correction are revealed. Stage-by-stage appointment of the developed complex of drugs to rats with a goiter and orthodontic intervention allows essentially reducing intensity of inflammation, a level of peroxide oxidation of lipids and to increase antioxidative protection in gingival tissues.

Key words: Experimental Goiter · Orthodontics · A Gingiva · Biochemistry

INTRODUCTION

It is determined that thyroid hormones have an antioxidative effect [1,2], reduce intensity of a stress-syndrome [3,4] and stimulate immune system of an organism [5,6]. Disturbances of a thyroid gland function are negatively reflected on a state of periodontal tissues [7,8]. Experimental stress on the background of a thyroid gland oppressing causes expressed disturbances of a state of periodontal tissues: increase of a degree of recession of a gingival, an atrophy of an alveolar process, teeth mobility [9]. Considering that the fixed orthodontic apparatus is multiplane stress, introduces theoretical and practical interest of studying of influence of dysfunction of a thyroid gland on a state of antioxidative-prooxidative system, inflammation markers in gingival tissues at orthodontic teeth movement.

MATERIALS AND METHODS

Animals: Experiment on 75 rats of Vistar's line of gregarious breeding (females, 5 months, 210±28 g) is carried out. Experimental researches are carried out by the rules provided by Council of the international medical organizations, which are presented in «the International references of carrying out of medical-biological researches on experimental animals» and «the Letter of the commission on problems of ethics concerning animals» and also were guided by the Law of Ukraine «About protection of animals against cruel treatment». The permission to carrying out of researches has received at session of Committee on bioethics of State Establishment «Institute of stomatology AMS of Ukraine». Experimental study design. At the first stage of experiment a goiter is modeled. At the second stage of experiment under

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Table 1: The schema of distribution of animals in experiment

Drug	Days of experiment	1st group	2nd group	3rd group	4th group	5th group
Sodium perchlorate	1- 21	-	+	+	+	+
Potassium Iodide	22-45	-	-	+	+	+
Echinacea compositum C	22-26	-	-	+	-	+
Orthodontic model of teeth movement	29		5 rats from each group			
Teraflex	30-45	-	-	-	+	+

thiopental narcosis (20 mg/kg) a closing nickel-titanic spring is fixed with the help of ligature wire on an incisor of the upper jaw (20mg/kg) and a molar tooth and reproduced model of orthodontic tooth movement (OMTM) within 21 days. 5 experimental groups have been distinguished. Allocation of rats on groups is reflected in Table 1. Animals were taken out from experiment under thiopental narcosis by opening of the great vessels of heart.

Drug: For modeling of an experimental goiter a standard technique of introduction of 1 % solution of potassium per-chlorate with drinking water to rats within 20 days is used [10]. Since the 22nd day to rats of the 3rd, 4th and 5th groups introduced solution Potassium iodide (PI) intragastric in a dose of 20 mkg/kg, the producer - VAT "Monfarm", Ukraine (Table 1). Besides it is introduced intragastric a drug «Echinacea compositum C» (Comb drug, the producer "Biologische Heilmittel Heel GmbH", Germany) to animals of the 3rd and 5th groups from the 22nd day during of 5 days. After fixation of orthodontic springs to rats of the 4th and 5th groups on the background of PI injection intragastric Teraflex is introduced in a dose on glucosamineglicane 750 mg/kg (producer Contract Pharmacal Corporation for "Bayer Konsyumer Ker AG", USA/Swissland).

Collection of Samples: Gingival homogenates are prepared from calculation of 20 mg/ml of 0,05 M tris-HCI pH 7,5. In gingival tissue activity of acidic phosphatase (AP), elastase, catalase and maintenances of malonic dealdegide (MDA) are defined.

Biochemical Analysis: Activity of acidic (pH 4,8) phosphatase is estimated with the help of reaction with p-nitrophenylphosphate as a result of hydrolysis p-nitrophenol of yellow color is formed. Intensity of coloring is proportional to enzyme activity. The maintenance of MDA is defined with the help of thiobarbituric acid as a result of interaction with which a tinctured trimethyl complex is formed. Concentration of MDA is proportional to intensity of colouring. Level of MDA testified about a degree of peroxide oxidation of lipids (POL). Catalase level is defined with the help of a method based on an ability of hydrogen dioxide is formed in the presence of enzyme to be combined with molybdenum moles in a proof orange complex. On intensity of a complex colouring they judged about catalase activity. Activity of elastase is estimated on hydrolysis of N-t-BOC-L-alanin-p-nitrophenyl ester. Under the influence of elastase from substrate the p-nitrophenol giving yellow staining, which intensity is proportional to activity of enzyme is split off [11].

Statistical Analysis: Statistical analysis was carried out by one way analysis of variance (ANOVA). Comparisons between the experimental groups were done using the t-Student U and Mann-Whitney test (p.asymp.sig <0.05 was considered significant).

RESULTS

Activity AP and Elastase in Gingival Homogenates: In Table 2 results of definition of inflammation markers (activity AP and elastase) in gingival homogenates of experimental animals are resulted. The present tables testify that modeling of teeth movement in healthy rats has led to increase only to activity of AP (on 29, 6 %, P<0, 01) and essentially has not changed activity of elastase in gingival tissues (P<0, 1). Modeling of a goiter has caused authentic increase on the average on 33, 2 % of activity of both markers of inflammation in gingival tissues (P<0,001). And at carrying out of orthodontic intervention in rats with an experimental goiter AP activity in a gingiva has increased on 58,9 % and elastase - on 71,4 %. It is important to underline that a level of these markers was authentically higher of values in healthy rats with OMTM (P_1<0,01 and P_1<0,001) and indexes in rats with a goiter and without orthodontic intervention (P_2<0,01 and P_2<0,05). Injection of «Echinacea compositum C» in a combination with potassium iodide to animals of the 3rd group has led to certain reduction of AP activity in the gingival tissues which level was intermediate between an index in healthy animals and with pathology (P>0,2 and P_2>0,25). Appointment of Teraflex has made about the same influence on activity of this marker of inflammation (P<0,05 and P_3<0,01). The most essential reduction of AP activity in the gingival homogenates of rats in the presence of a goiter and also at additional orthodontic intervention has been registered at injection of a full

Table 2: Influence of an experimental goiter, orthodontic intervention and prophylaxis on inflammation markers in a gingiva of rats

No	Groups of animals		Activity of acidic phosphatase mk-kat/g	Activity of elastase, mk-kat/G
1	The intact	without OMTM n = 10	20,11 ± 1,27	0,042 ± 0,002
		OMTM n = 5	26,06 ± 1,37[a]	0,047 ± 0,002
2	Experimental goiter (EG)	without OMTM n = 10	25,82 ± 1,26[a]	0,058 ± 0,003a
		OMTM n = 5	31,97 ± 1,65[abc]	0,072 ± 0,006[ab]
3	EG + PI+ echinacea compositum C	without OMTM n = 10	23,21 ± 2,10	0,052 ± 0,005[a]
		OMTM n = 5	24,79 ± 1,84[ad]	0,064 ± 0,007[a]
4	EG + PI+ Teraflex	without OMTM n = 10	22,63 ± 2,04	0,050 ± 0,004[q]
		OMTM n = 5	25,06 ± 1,89[ad]	0,056 ± 0,005[ad]
5	EG + PI + echinacea compositum			
	C + Teraflex	without OMTM n = 10	21,32 ± 1,21[c]	0,045 ± 0,002[c]
		OMTM n = 5	23,47 ± 1,56[d]	0,049 ± 0,004[d]

*a - statistically significant differences from values in an intact group; b - statistically significant differences from indexes in rats with OMTM; c -statistically significant differences from indexes in rats with a goiter without OMTM; d - statistically significant differences from indexes in rats with a combination of a goiter and OMTM. (Values are Mean ±SE, p.asymp.sig t-Student and p.asymp.sig. U Mann-Whitney <0,05)

Table 3: Influence of an experimental goiter, orthodontic intervention and prophylaxis on indexes of antioxidative-prooxidative system in a gingiva of rats

No	Groups of animals		Maintenance of malonic dialdehyde, mkmol/g	Activity of catalase, mk-kat/g
1	The intact	without OMTM n = 10	16,58 ± 1,34	7,89 ± 0,29
		OMTM n = 5	25,42 ± 2,17[a]	7,93 ± 0,36
2	Experimental goiter (EG)	without OMTM n = 10	18,38 ± 1,87	6,34 ± 0,28[a]
		OMTM n = 5	23,67 ± 2,10[ac]	5,48 ± 0,26[abc]
3	EG + PI + echinacea compositum C	without OMTM n = 10	15,08 ± 1,17	7,56 ± 0,53[c]
		OMTM n = 5	20,13 ± 1,43	7,27 ± 0,61[d]
4	EG + PI + Teraflex	without OMTM n = 10	15,16 ± 0,50	6,85 ± 0,42[a]
		OMTM n = 5	19,72 ± 1,14	5,93 ± 0,38[a]
5	EG + PI+ echinacea compositum			
	C + Teraflex	without OMTM n = 10	15,84 ± 0,91	7,56 ± 0,42[c]
		OMTM n = 5	17,52 ± 0,86[d]	7,23 ± 0,30[d]

*a - statistically significant differences from values in an intact group; b - statistically significant differences from indexes in rats with OMTM; c - statistically significant differences from indexes in rats with a goiter without OMTM; d - statistically significant differences from indexes in rats with a combination of a goiter and OMTM. (Values are Mean ±SE, p.asymp.sig t-Student and p.asymp.sig. U Mann-Whitney <0,05)

complex of drugs to experimental animals. So, in the 5th group AP activity in rats with a goiter and on the background of treatment by a complex of drugs has authentically reduced (P_2<0,02) to a level in intact animals (P>0,5). Values of AP activity in the gingival homogenates of rats with a goiter, orthodontic intervention and animals receiving a recommended complex also corresponded to a level in healthy animals (P>0,1 and P_3<0,001). As it is specified in the table 2, application of «Echinacea compositum C» in rats of the 3rd group has not rendered the expressed influence on activity of other marker of inflammation of elastase in a gingival of animals (P_2 and P_3>0,3). Injection of Teraflex to rats of the 4th group has led to authentic reduction of elastase activity in a gingiva only in animals whom replicated orthodontic intervention (P_3<0,05) that testifies about certain anti-inflammatory influence of these drugs introduced separately. After application of the full schema of treatment for animals of the 5th group elastase activity in a gingiva of rats has reduced to a normal level (P>0,2-0,3; P_2<0,002 and P_3<0,02).

Activity of Indexes of Antioxidative-prooxidative System in Gingival Tissues of Rats: In the Table 3 the data of research of a level of malonic dialdehyde (MDA) - a finished product of POL and activity of one of the basic antioxidative ferments of catalase in a gingiva of rats on the background of modeling of a goiter pathology, orthodontic teeth movement and prophylaxis by drugs of an offered complex are given. The introduced results testify that fixation of an orthodontic spring in healthy rats has led to lifting of MDA maintenance in 1,53 times (P<0,002) and has not influence on activity of catalase (P>0,4) in a gingiva of animals. Modeling of a goiter by means of sodium perchlorate on the contrary has not rendered essential influence on MDA level, but has led to authentic reduction of catalase activity (P<0,002) in the gingival homogenates. Associative influence (orthodontic

intervention on the background of an experimental goiter) has led to authentic change of both indexes of antioxidative-prooxydative system in a gingiva of rats: MDA level was enlarged (P<0,01) and catalase activity has reduced (P<0,001) on the average in 1,44 times. Injection of «Echinacea compositum C» or Teraflex as monotherapy to rats has had certain a positive effect on MDA in a gingiva: the maintenance of this product despite doubtful reduction (P_2>0,1-0,2 and P >0,1-0,2) corresponded to a level in healthy animals (P>0,1-0,4). On the background of «Echinacea compositum C» application has led to normalization of activity of catalase in a gingiva of rats of the 3rd group both with an experimental goiter and in rats after orthodontic intervention on the background of a goiter (P>0,4-0,6; P_2<0,05 and P_3<0,02). Injection of Teraflex to rats of the 4th group has not affected activity of catalase in a gingiva, conserved on the same low level, as well as in rats of the 2nd group unlike echinatseya. In a gingiva of rats of the 5th group, which were introduced drugs of all complex stage by stage, maintenance of MDA and activity of catalase corresponded to a level in healthy animals, despite on modeling of a goiter and orthodontic intervention (P>0,2-0,6; P_2<0,02 and P_3<0,01-002).

DISCUSSION

Increase of inflammation markers level (AP and elastase) in gingival homogenates testifies about aggravation of inflammatory processes in gingival tissues after modeling of orthodontic teeth movement in rats with an experimental goiter. It is compounded with researches which have shown that under the influence of the mechanical load induced by orthodontic forces, there is a change of microcirculation in periodontal tissues [12]. At the first stages of tooth movement metabolic changes occur in periodontal ligament, as a result of hypoxia and trophic disturbances in a pressure region first of all. In the conditions of hypoxia in cells the anaerobic glycolysis is observed [13]. At destruction of cells which could not adapt to ischemic conditions, a considerable quantity biologically active agents, potentiated inflammatory reaction is released [14]. Local synthesis and release of cytokines, the colony stimulating factor, growth factors and metabolites of arachidonic acid is observed. The started cascade of cellular signal reactions stimulates metabolism in PDL, a transitive aseptic inflammation and further local resorption and opposition of bone tissue [12]. Thus it is necessary to consider that an unfavorable background is the expressed disturbances have been revealed by scientists in gingival tissues at dysfunction of a thyroid gland [7-9]. It demands carrying out of the conforming correction. AP activity in rats with a goiter and OMTM at application of drugs complex has authentically reduced to a level in intact animals, activity of elastase - to normal values. The resulted findings of investigation of inflammation markers in a gingiva of experimental animals convincingly prove anti-inflammatory character of action of a complex «Echinacea compositum C», Teraflex and potassium iodide applied at stages of orthodontic intervention at an experimental goiter. One of the important systems of tissues and organs providing their resistance to influence of unfavorable factors is antioxidative-prooxidative system. Correction of its main parts allows essentially increase nonspecific resistance and quality of dentist treatment. Summing up to the biochemical researches carried out in a gingiva of rats with an experimental goiter andOMTM, it is possible to conclude that dysfunction of a thyroid gland and the orthodontic intervention replicated on-separateness make negative influence on antioxidative-prooxidative system in gingival tissue of animals. It proves to be true works of explorers which have shown that disturbances of function of a thyroid gland cause oxidative stress in an organism [1, 15]. At fixation of a spring on the background of a goiter antioxidative protection is exhausted in a greater degree on the background of more expressed outbreak of peroxide oxidation of lipids. «Echinacea compositum C» in a combination with potassium iodide displays the expressed antioxidative action, stimulating activity of catalase and reducing MDA level in a gingiva of rats with a diffuse goiter and orthodontic intervention. Teraflex with P² on the background of reproduction of investigated influences is capable to reduce only maintenance of MDA in a gingiva. Application of an offered complex for rats completely prevents disturbances in the antioxidative-prooxidative system caused by modeling of a goiter and fixation of an orthodontic spring.

CONCLUSIONS

The developed complex of drugs in rats with a goiter and orthodontic intervention allows essentially reducing intensity of inflammation, a level of peroxide oxidation of lipids and to increase antioxidative protection in gingival tissues. The mentioned research can serve as substantiation for application of an offered complex of drugs for children with a diffusive nontoxic goiter at treatment of maxillodental anomalies.

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