Experimental Assessment of Biological Compatibility of Gold Alloys by Functional Indices of Salivary Glands

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The effect of implanted gold alloys on composition and rate of excretion of oral fluid was studied on rats. It was found that gold alloy 900 modifies the function of the major salivary glands.

Key Words: *salivary glands; biological compatibility; sialometry; saliva composition; gold alloys*

Gold-based alloys are widely used in orthopedic dentistry [4,5]. These alloys were certified for biological compatibility by the routine tests. However, their effects on salivary gland were not examined. Standards for biological compatibility (ISO 10993-6, 1994) [10] do not specify the tests for possible effects of dentures on the structure and function of salivary glands. There are no published data on biological compatibility in available for glandular tissues [3].

Our aim was to evaluate possible effect of gold dental alloys on the function of salivary glands in experiments on animals.

MATERIALS AND METHODS

Experiments were carried out on 50 random-bred rats weighing 250±10 g. Gold denture alloys ZlSrM-900-40, Super KM, and Super TZ were implanted subcutaneously on the back. Medical glass was used as the control. All methods complied with the requirements of ISO 10993-6, 1994, part 6 "Testing for local effect after implantation" and to Requirements of Preclinical Assessment of Safety of Pharmacologic Agents (GLP) [5,6]. Salivary function (SF) was assessed 12 weeks after implantation by collecting oral fluid after pilocarpine stimulation (5 mg/kg). Ionic composition of the saliva was determined by murexide method (total Ca, [1]), molybdenum blue (inorganic P, [9]), an ETs-59-M Ion Meter (Kvertilab, accuracy ±0.3 and ±4.0 mM for K and Na, correspondingly). Total protein, urea, and glucose were determined by biuret test [8], photometry (Lachema, [7]), and enzymatic method (Lachema, [14]), respectively. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using Lachema kits and Ritis coefficient AST/ALT was calculated [11]. Activity of α-amylase was measured as described elsewhere using Lachema kits [13]. The data were standartized (per 1 m^2 body surface area and 10 ml/min/m^2 salivation rate).

After experiments the rats were euthanized.

The data were processed statistically using Student's t test at $p<0.05$. Discriminative analysis was performed with Statgraphics Plus 5.0 software.

RESULTS

The choice of narcotizing agent is extremely important for the analysis of SF. In some experiments with routine hexenal or thiopental narcosis, salivation activator pilocarpine provoked pulmonary edema and led to fatal outcome. Ketamine (Kalipsol) used in our experiments was less "toxic" [10]. Since SF is determined by integral interaction of many factors and body sys-

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tems, individual control was used in each experimental series.

Sialometry (measurement of the rate of mixed saliva formation) showed that in female rats salivation rate did not significantly change 12 weeks after implantation of gold alloys. In males ZlSrM-900-40 significantly decreased salivation rate by 24% from 8.85±1.02 (in control rats) to 6.17±1.00 ml/h/m^2 (in experimental rats).

The aim of biochemical studies of the saliva was assessment of functional state (homeostasis) of salivary glands. Saliva secretion was evaluated by the content of total protein and α-amylase. Excretion was assessed by the concentration of urea, and recretion by the content of K^+, Na^+, and glucose. Necrosis was determined by activities of AST and ALT. These markers were detected in mixed saliva.

A pronounced and significant increase in Na^+ concentration by 438% was observed in male and female rats implanted with ZlSrM-900-400 alloy (Table 1).

Analysis of organic composition of the saliva (7 indices) was carried out by another scheme. As the first step, the mean values and standard errors were calculated by routine methods. Table 2 shows that all these indices significantly differ from the normal. The most pronounced increase in AST activity was induced by ZlSrM-900-400 (513%, $p<0.05$). In addition, this alloy increased ALT by 481%. However, the balance of activities of these enzymes was significantly and most strongly disturbed in the group implanted with Super TZ alloy (329%). All alloys potentiated synthetic function in the salivary glands (production and secretion of α-amylase). In this respect, the effects of Super KM and Super TZ were more pronounced (544%), while the effect of ZlSrM-900-400 was somewhat weaker (410%). The content of total protein (a marker of secretion) significantly increased only in the group implanted with ZlSrM-900-400 (135%). Glucose significantly decreased in the groups implanted with Super KM and ZlSrM-900-400. The smallest indices were observed in the group implanted with Super KM (31%). In all groups the concentration of urea decreased significantly and pronouncedly, the greatest drop (to 27%) was noted in the group implanted with Super TZ.

At the second stage, the experimental data including seven indices (Table 1) and salivation rate were subjected to multivariate analysis, which assessed all

TABLE 1. Ionic Composition in Stimulated Mixed Saliva of Female Rats 12 Weeks After Implantation of Gold Alloys (*M*±*SE*)

Substance	Control	Alloy		
		super TZ	ZlSrM-900-40	super KM
P	1.63±0.06	0.72±0.23*	1.75±0.59	1.31±0.26
Ca	1.69±0.11	1.69±0.26	0.95±0.34	1.41±0.23
Ca/P	1.03±0.08	2.60±1.03	0.66±0.33	1.12±0.15
Chlorides	26.17±2.69	21.17±1.92	39.17±6.45	24.50±4.07
K	36.02±0.55	34.60±0.68	35.98±0.89	32.80±2.35
Na	5.53±0.26	6.28±0.66	24.22±2.15**	6.43±0.46
Na/K	0.150±0.007	0.18±0.02	0.67±0.06**	0.20±0.03*

Note. *$p<0.01$, **$p<0.001$ compared to the control.

TABLE 2. Organic Composition in Stimulated Mixed Saliva of Female Rats 12 Weeks After Subcutaneous Implantation of Gold Alloys (*M*±*SE*)

Substance	Control	Alloy		
		super TZ	ZlSrM-900-40	super KM
Total protein, g%	1.50±0.07	1.66±0.08*	2.02±0.09*	1.98±0.12*
ALT, U/liter	2.770±0.017	4.48±0.82*	10.93±0.65*	4.58±0.96*
AST, U/liter	2.51±0.21	11.4±1.3*	12.88±0.74*	7.33±0.88*
AST/ALT	1.11±0.04	3.29±0.84	1.22±0.14	1.92±0.37
Urea, mM	2.23±0.14	0.74±0.09*	1.04±0.06	1.06±0.17*
α-Amylase mmcat/liter	1656±94	9019±278*	6798±196*	9090±220*
Glucose, mM	0.54±0.07	0.63±0.09*	0.32±0.05*	0.17±0.02*

Note. *$p<0.05$ compared to the control.

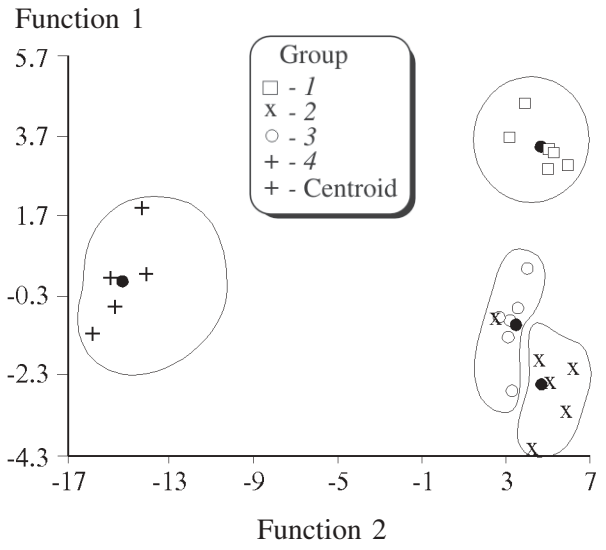

Fig. 1. Distribution of alloys on the coordinate plane of the first and second discriminative functions. 1) Super TZ; 2) ZlSrM-900-40; 3) super KM; 4) control. Bold dots mark the centers in the space of canonic discriminative functions (centroid). Ordinates: functions 1 and 2 of the classifying discriminative functions.

parameters together (Fig. 1). The dispersion plot clearly revealed four independent classes. The groups 1 (Super TZ) and 4 (control) belong to clear-cut independent classes, which did not overlap with other groups. By contrast, the groups 2 (ZlSrM-900-400) and 3 (Super KM) overlapped in the discriminative functional space, albeit they clearly differed from the control group.

Integrating these data one can conclude that subcutaneous implantation of some gold alloys used in dentistry modifies the work of salivary glands: it changes the composition of electrolytes (Na, K) that strongly depend on activity of ionic pumps. In addition, it modifies activity of all examined enzymes and the content of various organic metabolites produced by acinar cells. Activation of aminotransferases activity indicate necrotic processes in the glandular tissue. Clinical data revealed decrease in activity of some enzymes such as alkaline phosphatase, AST, and ALT in patients with gold dentures.

Thus, it is established that gold dentistry alloys, which contain some other elements in addition to gold, affect synthetic, barrier, and salivation functions of the salivary glands. This fact was not previously studied in view of biological compatibility. Further studies should clarify whether these functional changes are adaptive or pathological.

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