Braz Dent J (2003) 14(1): 32-36 ISSN 0103-6440

Odontologic Use of Copper/Aluminum Alloys: Mitochondrial Respiration as Sensitive Parameter of Biocompatibility

Luiz Erlon A. RODRIGUES[1]
Antônio A.V.F. CARVALHO[1]
Antônio L.M. AZEVEDO[1]
Cecília B.B.V. CRUZ[1]
Antônio Wanderley C. MAIA[2]

[1]*Laboratory of Basic Research, EBMSP, Foundation for Science Development, Salvador, BA, Brazil*
[2]*Department of Restorative Dentistry, Faculty of Dentistry, UFBA, Salvador, BA, Brazil*

Copper/aluminum alloys are largely utilized in odontological restorations because they are less expensive than gold or platinum. However, tarnishing and important corrosion in intrabuccal prostheses made with copper/aluminum alloys after 28 days of use have been reported. Several kinds of food and beverage may attack and corrode these alloys. Copper is an essential component of several important enzymes directly involved in mitochondrial respiratory metabolism. Aluminum, in contrast, is very toxic and, when absorbed, plasma values as small as 1.65 to 21.55 µg/dl can cause severe lesions to the nervous system, kidneys, and bone marrow. Because mitochondria are extremely sensitive to minimal variation of cellular physiology, the direct relationship between the mitocondrial respiratory chain and cell lesions has been used as a sensitive parameter to evaluate cellular aggression by external agents. This work consisted in the polarographic study of mitochondrial respiratory metabolism of livers and kidneys of rabbits with femoral implants of titanium or copper/aluminum alloy screws. The experimental results obtained did not show physiological modifications of hepatic or renal mitochondria isolated from animals of the three experimental groups, which indicate good biocompatibility of copper/aluminum alloys and suggest their odontological use.

Key Words: copper, aluminum, alloy, mitochondrial respiration, mitochondria, biocompatibility.

INTRODUCTION

In order to decrease the high cost of gold or platinum alloy systems for odontological use, several other alloy systems have been used. Nevertheless, they must obey technical criteria of hardness, corrosion resistance and, mainly, biocompatibility (1). In 1988, more than 70% of the Brazilian specialized market was using these alternative alloy systems, principally copper/aluminum (2).

In 1976, Simonetti (3) reported that small pieces made of copper/aluminum alloy did not provoke any important histologic reactions when introduced in the dorsal region of adult rats and left there for 45 days. Neither copper nor aluminum ions were found in the circulatory system of these animals. However, other studies have shown tarnishing and important corrosion in intrabuccal prostheses made with copper/aluminum alloy after 28 days of use in ambulatory patients. Several copper/aluminum alloys, with or without other components such as iron, nickel and manganese, may show signs of corrosion when submitted to certain food and beverages such as fruit juices, vinegar, coffee, beer, cider and carbonated water (4).

Copper is an essential component of several important enzymes including superoxide dismutase (E.C. 1.15.1.1), a free radical scavenger and cytochrome-c oxidase (E.C. 1.9.3.1) involved in mitochondria respiratory metabolism as an electron transport protein. The essentiality of copper for humans can be demonstrated through the important role that this metal plays in infant growth, host defense mechanism, bone strength, red and white cell maturation, iron transport, cholesterol metabolism, myocardial contractility, glu-

Correspondence: Dr. Luiz Erlon A. Rodrigues, P.O. Box 4747, 40155-510 Salvador, BA, Brasil. e-mail: erlon@ufba.br

cose metabolism and brain development (5). The human normal limits of serum copper concentration are between 78 and 113 µg/dl and their urinary excretion can reach 3.95 mg/24 h, in cases of severe intoxication (6). Ingestion of more than 2 mg daily of copper induces mucous irritation, nausea, vomiting, diarrhea and, if ingested for long periods, hemolytic anemia, renal and tubular necrosis, hepatitis with hepatic failure, cirrhosis and severe cerebral lesions. The toxicity of copper at the cellular level is probably due to its capacity to inhibit sulfhydryl groups of glutathione, cysteine, homocysteine, methionine, coenzyme-A and pantetheine. Several important enzymes, i.e., glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), glyceraldehyde-3-phosphate dehydrogenase (E.C. 1.2.1.12) and glutathione oxidase (E.C. 1.8.3.3), are inhibited by copper ions (7).

Aluminum, one of the most common metals found on earth, seems to have no role in human body physiology. In contrast, the absorption of small amounts of this element is potentially dangerous. Aluminum intoxication causes severe lesions to the nervous system, kidneys and bone marrow medulla. Plasma values between 1.65 and 21.55 µg/dl are dangerous enough to start signs of aluminum intoxication (8).

Mitochondria are intracellular organelles, existing in eukaryotic cells, responsible for production of ATP, heat generation, intracellular calcium regulation, reactive oxygen species generation and are involved in apoptosis and cell death (9). These organelles are extremely sensitive to minimal variations of cellular physiology. Small changes in pH, osmolarity, cellular oxygenation and the presence of several substances may interfere in mitochondrial respiratory metabolism. Copper is one of them.

Based on these findings, the hepatic and renal mitochondrial respiration was used as a sensitive parameter to evaluate the biocompatibility of copper/aluminum alloys implanted *in vivo* in rabbit femurs.

MATERIAL AND METHODS

Three groups of New Zealand, adult, male rabbits (weight 1.9-2.9 kg) were maintained in individual cages, *ad libitum*, at 25ºC with natural illumination. The control group (sham-operated animal) was composed of 10 rabbits that were submitted to the same conditions as the experimental groups, except the screw implant. Titanium group was composed of 6 animals, each with screws made of titanium alloy (ENGIMPLAN®, Engenharia de Implantes Ind. Com. Ltda., Rio Claro, SP, Brazil), implanted in one of their femurs, remaining for 45 days. Copper/aluminum group was also composed of 6 animals submitted to the same conditions, except the screws were made with copper/aluminum alloy (GOLDENT LA®, AJE Comércio Imp. Exp. Ltda., São Paulo, SP, Brazil).

Screw Characteristics

The screws made with the titanium alloy, measuring 3 x 9 mm, are normally used in orthopedic proceedings with confirmed biocompatibility. The chemical composition of the screw alloy in percent (w/w) was 99.79 titanium, 0.14 oxygen, 0.05 iron, 0.01 carbon, 0.007 nitrogen and 0.005 hydrogen. The copper/aluminum alloy was used to make the screws utilized in the third experimental group. They had the same characteristics as the titanium screws and their chemical composition in percent (w/w) was 76.0 copper, 12.0 zinc, 6.5 aluminum, 5.0 nickel and 0.5 manganese.

Surgical Proceedings

The surgical proceedings for each animal followed the protocol: general anesthesia (Ketamin®, Cristália Prod. Quim. Farmac. Ltda., Itapira, SP, Brazil), depilation of the internal femoral region, antisepsis with lugol solution, incision and access to the femur, bone perforation using an odontological drill (BUR® type HP703L, Beavers Dental Div. Sybron, Ontario, Canada), screw implant and finally reconstitution of tissues.

Separation of Rich Mitochondrial Fraction from Livers and Kidneys

Each animal was sacrificed by cervical traumatism, without anesthetic to avoid interference in the mitochondrial respiratory metabolism. Immediately after sacrifice, samples of the liver, kidney and of the operated femur around the screw were collected, identified and separately washed in 25 x 10^{-2} M saccharose solution and refrigerated at 2-4ºC. A small piece from each organ was removed for histological study in order to detect any tissue morphological modification.

The remaining portions of hepatic and renal tissue were reduced to small fragments, washed again in the same solution and finally suspended in a proportion of 1 g of tissue to 10 ml of a mannitol buffer solution, that can preserve mitochondria for a period of at least 6 hours. This solution consisted of 10^{-2} M phosphate buffer, 2 x 10^{-3} M potassium chloride, 2 x 10^{-3} M TRIS, 4 x 10^{-4} M EDTA, and 25 x 10^{-2} M mannitol, adjusted to pH 7.4.

The samples were then homogenized using a Potter-Elvejhem tissue homogenizer with a Teflon® pestle (Arthur Thomas Co., Philadelphia, PA, USA) at a velocity of 250 rpm for a maximum of 2 min. The homogenates were then subjected to the scheme of refrigerated differential centrifugation (Biofuge Stratos, Heraeus Inst., Osterode, Germany) shown in Figure 1, in order to isolate the subcellular fraction rich in mitochondria.

The pellet containing chiefly the mitochondria was suspended to the same volume of original homogenate, using the buffer solution. The respiratory metabolism of mitochondria isolated from hepatic or renal tissues was measured polarographically with a Clark-type oxygen electrode (YSI-53, Yellow Spring, OH, USA) at 37°C, in 3.0 ml reaction mixtures (2.8 ml of mitochondrial suspension plus 0.2 ml of sodium succinate, 3.3 x 10^{-3} M) (10) and the amount of mitochondrial proteins of each sample was determined by the Folin-phenol technique (11).

The data were analyzed statistically by the unpaired (one-tailed) Student t-test and the results were reported as mean ± SD.

RESULTS

Because mitochondria are related to cellular energetic metabolism, the study of their respiratory activity has been used as a sensitive parameter to evaluate the physiological conditions of the cells, tissues and even, the organs. Table 1 shows the endogenous respiratory activities of mitochondria isolated from livers and kidneys of rabbits implanted or not with screws. There were no statistical differences among the experimental groups. The endogenous mitochondrial respiration was practically the same for the control animals and for those implanted with titanium or copper/aluminum alloys. The existing differences between the specific activities of livers and kidneys, almost three times more for kidneys, are histologically explained. The kidney is an organ much richer in mitochondria than the liver.

As can be seen in Table 2, the utilization of sodium succinate as substrate for liver and kidney

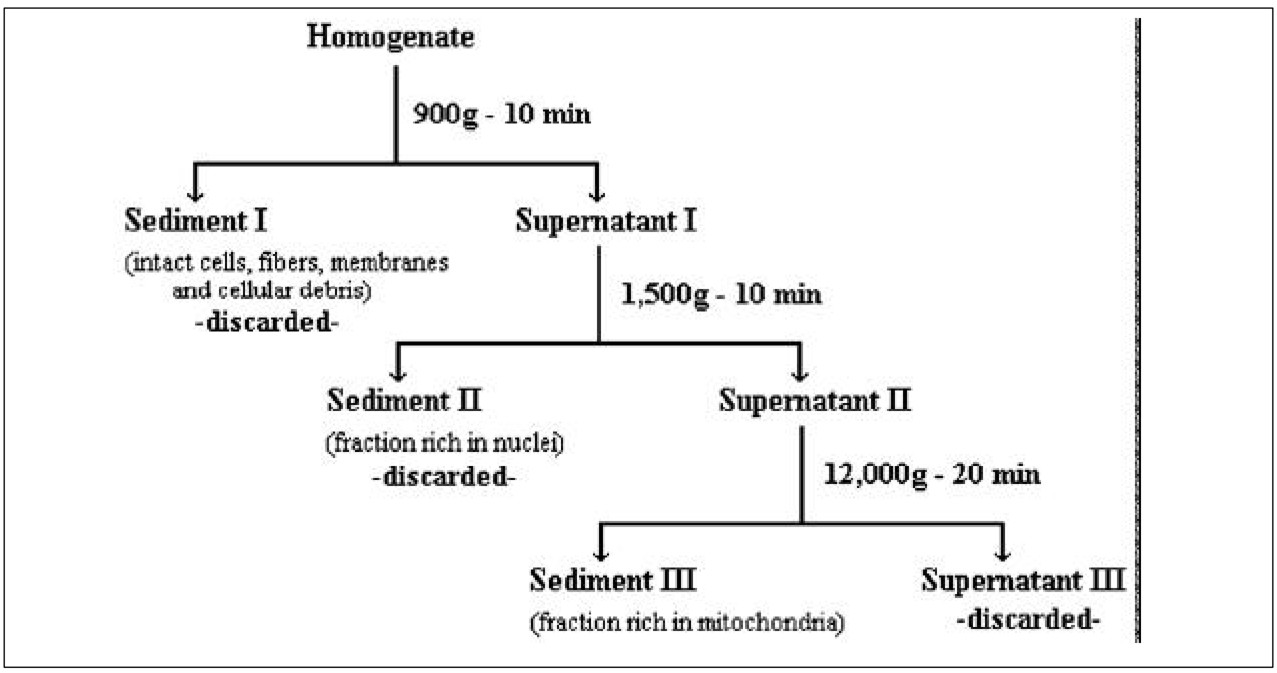

Figure 1. Scheme of the refrigerated centrifugation employed for isolation of the subcellular fraction rich in mitochondria.

mitochondrial respiration increased oxygen consumption, almost at the same rate for the three experimental groups.

DISCUSSION

The direct relationship between mitochondrial respiratory metabolism and cellular physiology has been largely employed in studies of cellular lesions provoked by toxic agents. The respiratory chain has been the target of a number of toxins developed as pesticides (12).

Succinate dehydrogenase (succinate:ubiquinone oxidoreductase, E.C. 1.3.5.1), a flavoprotein (FAD) containing iron-sulfur centers, is a mitochondrial enzyme bound to the internal membrane and represents one of the most active steps of oxygen consumption in respiratory metabolism. Because it is linked to the mitochondrial membrane system, it can be used to detect minimal physicochemical alterations of cellular physiology.

Table 1. Endogenous respiratory activities of mitochondria isolated from livers and kidneys of rabbits.

	Endogenous activities (nlO_2/ml/min)	Total proteins (mg/ml)	Specific activities (nlO_2/mg/min)
Liver			
Control	54.0 ± 6.7	3.6 ± 1.2	15.0 ± 3.9
Titanium	53.3 ± 6.5	3.8 ± 2.5	14.0 ± 4.5
Copper/aluminum	55.3 ± 8.0	3.2 ± 0.8	17.3 ± 4.4
Kidney			
Control	136.1 ± 10.5	3.2 ± 1.3	42.5 ± 5.7
Titanium	139.4 ± 8.5	3.4 ± 1.3	41.0 ± 4.9
Copper/aluminum	135.1 ± 7.8	3.3 ± 0.3	40.9 ± 4.0

There were no statistical differences among groups (p>0.05).

Table 2. Specific respiratory activities of mitochondria isolated from livers and kidneys of rabbits stimulated by sodium succinate (nlO_2/mg/min).

	Specific activities without stimulus	Specific activities with stimulus	Activation in number of times
Liver			
Control	15.0 ± 3.9	25.9 ± 5.6	1.73
Titanium	14.0 ± 4.5	24.2 ± 3.4	1.73
Copper/aluminum	17.3 ± 4.4	28.3 ± 3.8	1.64
Kidney			
Control	42.5 ± 5.7	112.6 ± 11.5	2.65
Titanium	41.0 ± 4.9	110.0 ± 9.5	2.68
Copper/aluminum	40.9 ± 4.0	115.5 ± 5.2	2.73

There were no statistical differences among groups (p>0.05).

Mitochondrial dysfunction has been implicated in a number of degenerative diseases including ischemia. Preservation of mitochondrial membrane physiology is considered to be an essential process in rescuing cells from energy-depletion in anoxic situations and inhibition of release of pro-apoptotic components from the mitochondrial intermembrane space (13).

As a by-product of electron transport, a small amount of molecular oxygen undergoes nonelectronic reduction at intermediate steps of the mitochondrial respiratory chain resulting in the generation of superoxide radical (9). Normally, respiratory-chain generated reactive oxygen species are detoxified by an efficient mitochondrial antioxidant system, represented by superoxide dismutase (superoxide:superoxide oxidoreductase, E.C.1.15.1.1), where copper ions play an important role.

Cupric ions are rapidly absorbed from the stomach and intestine and serum copper levels increase rapidly. This element is bound to albumin and ceruloplasmin, and is taken by the liver, kidneys, lungs and red blood cells. The primary route of copper excretion is through bile and feces (14).

Aluminum is one of the most common metals found on earth, but seems to have no role in the body's physiology. In contrast, absorption of small amounts of this element can be potentially dangerous to the nervous system, kidney and bone medulla. Plasma values between 1.65 and 21.55 µg/dl are sufficient to cause severe cellular damage (8). In healthy individuals, the kidneys serve as a means of collecting and disposing of excess aluminum ingested naturally through food and drinks.

Metallic implants release finite amounts of metal surrounding tissue by various mechanisms, including corrosion, wear, and mechanically accelerated electrochemical processes such as fretting corrosion, stress corrosion and corrosion fatigue. This release of metal has been associated with osteolysis, cutaneous allergic reactions and remote sites of accumulation (15). However, the implantation of components made with alloys containing 4% aluminum and 6% titanium into the femurs of 23 patients and observed during 36 months of treatment did not show statistical differences in terms of amount of circulating aluminum and urinary excretion when compared with a group of non-operated patients (16). The experimental results found in the present work also demonstrated that femoral screw implants made of titanium and copper/aluminum alloys did not modify the physiology of hepatic and renal mitochondria. These findings indicate biocompatibility of copper/aluminum alloy and suggest their odontological use.

RESUMO

Ligas do sistema cobre/alumínio são, atualmente, muito usadas em odontologia restauradora, principalmente devido aos seus baixos custos. Porém sofrem manchamento e corrosão quando utilizadas por mais de 28 dias, de modo intrabucal, em humanos, além do que são atacadas por vários tipos de alimentos e de bebidas. O cobre é essencial para a atividade de várias enzimas importantes, diretamente envolvidas com o metabolismo mitocondrial. O alumínio, ao contrário, é muito tóxico. Pequenas quantidades plasmáticas são capazes de causar graves lesões no sistema nervoso, rins e medula óssea. As mitocôndrias são extremamente sensíveis às mínimas variações da fisiologia celular. As relações diretas existentes entre o metabolismo respiratório mitocondrial e as lesões celulares, têm sido usadas como um parâmetro muito sensível para a avaliação das agressões às células, decorrentes de agentes externos. Este trabalho consistiu nos estudos polarográficos do metabolismo respiratório mitocondrial, a partir de fígados e rins de coelhos, em cujos fêmures foram implantados parafusos confeccionados com ligas de titânio e de cobre/alumínio. Os resultados encontrados não mostraram modificações fisiológicas nas mitocôndrias hepáticas ou renais isoladas dos animais componentes dos diferentes grupos experimentais. Eles indicam uma boa compatibilidade para as ligas de cobre/alumínio e sugerem suas utilizações em odontologia restauradora.

References

1. Wataba JC. Biocompatibility of dental casting alloy: A review. J Prosthet Dent 2000;83:223-234.
2. da Silva Filho FPM, de Sá DN, Rettondini WC. Ligas do sistema cobre-alumínio. Estudo da contração de fundição e dureza Vickers. Efeito de técnicas de fusão e tratamento térmico. Odontol Cli 1988;2:15-19.
3. Simonetti EL. Dentística Restauradora: Ligas do sistema cobre-alumínio. I. Compatibilidade biológica. Rev Fac Odontol S Paulo 1976;14:201-206.
4. Benatti OF, Miranda Jr WG, Muench A. In vitro and in vivo corrosion evaluation of nickel-chromium and copper-aluminum-based alloys. J Prosthet Dent 2000;84:360-363.
5. Cox DW. Disorders of copper transport. Brit Med Bull 1999;55:544-555.
6. Takeda T, Yukioka T, Shimazaki S. Cupric sulfate intoxication with rhabdomyolysis, treated with chelating agents and blood purification. Internal Med 2000;39:253-255.
7. Olivares M, Uauy R. Copper as an essential nutrient. Am J Clin Nutr 1996;63:791S-796S.
8. Yokel RA, McNamara PJ. Aluminum toxicokinetics: an updated minireview. Pharmacol Toxicol 2001;88:159-167.
9. Kowaltowski AJ. Alternative mitochondrial functions in cell physiopathology: Beyond ATP production. Braz J Med Biol Res 2000;33:241-250.
10. Chen J, Li Y, Lavigne JA, Trush MA, Yager JD. Increased mitochondrial superoxide production in rat liver mitochondria, rat hepatocytes and HepG2 cells following ethinylestradiol treatment. Toxicol Sc 1999;51:224-235.
11. Komsa-Penkova R, Spirova R, Bechev B. Modification of Lowry's method for collagen concentration measurement. J Biochem Biophys Methods 1996;32:33-43.
12. Schapira AHV. Research into mitochondrial function and dysfunction - Entering the powerhouse. Odyssey 1996;2:8-13.
13. Nuydens R, Novalbos J, Dispersyn G, Weber C, Borgers M, Geerts H. A rapid method for evaluation of compounds with mitochondrial-protective properties. J Nerosci Meth 1999;92:153-159.
14. James LP, Stowe CD, Argao E. Gastric injury following copper sulfate ingestion. Ped Emerg Care 2000;15:429-431.
15. Klein D, Lichtmannegger J, Heizmann U, Summer KH. Dissolution of copper-rich granules in hepatic lysosomes by D-penicillamine prevents the development of fulminant hepatitis in Long-Evans cinnamon rats. J Hepatol 2000;32:193-201.
16. Jacobs JJ, Silverton C, Hallab NJ, Skipor AK, Patterson L, Black J, Galante JO. Metal release and excretion from cementless titanium alloy total knee replacements. Clin Orthop Rel Res 1999;358:173-180.

Accepted July 29, 2002