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INFLUENCE OF AMALGAM FILLINGS ON HG LEVELS AND TOTAL ANTIOXIDANT ACTIVITY IN PLASMA OF HEALTHY DONORS

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MOTS CLES: amalgame, plasma humain, activité antioxydante totale
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ABSTRACT

In order to evaluate the influence of mercury (Hg) levels on antioxidant power in human plasma, 26 healthy people were evaluated by a dentist and their plasma analyzed for Hg content by atomic absorption and total antioxidant activity (TAA) by FRAP method. Hg plasma concentration correlated with number of amalgam restorations, suggesting that Hg released from fillings is a source of Hg in non-occupational exposed people. Fish consumption, in fact, showed no influence on Hg plasma levels, perhaps because Italian subjects examined in the present group used low quantity of fish at week or kinds of fish with light contamination. TAA negatively correlated with Hg plasma revealing a pro-oxidant role of Hg released from amalgam fillings

RESUME

Afin d'évaluer l'influence de certains facteurs spécifiques sur les niveaux de mercure (Hg) et sur le pouvoir anti-oxydant du plasma humain, 26 (vingt-six) donneurs sains ont été soumis à un contrôle dentaire et leur plasma a été analysé selon la méthode FRAP pour y déterminer le contenu de Hg par absorption atomique ainsi que l'activité anti-oxydante totale (AAT). La concentration de Hg dans le plasma est proportionnelle au nombre d'obturations réalisées avec de l'amalgame; cela confirme que le Hg émis par les obturations est la source principale de Hg chez les sujets non exposés par leur profession. La consommation de poisson, en fait, n'a eu aucune influence sur les niveaux de Hg dans le plasma, probablement parce que les sujets en question consommaient de très petites quantités de poisson par semaine. Aucune variation de Hg dans le plasma ne peut être imputée à l'âge. L'AAT était négativement corrélée avec le Hg du plasma, révélant un rôle pro-oxydant de l'Hg émis par les plombages en amalgame. Aucune influence du tabac ou de l'âge ne fut observée sur l' AAT.

INTRODUCTION

Dental amalgam is the most diffused filling material used in restorative dentistry and it contains approximately 50% of metallic mercury. It has been widely demonstrated that amalgam restorations continuously release significant amount of Hg into mouth air (Halbach S. et al. 1995) and into saliva (Lorscheider FL et al. 1995). Swallowed with saliva, released Hg enters the blood stream through the gastro-intestinal tract with a low coefficient of absorption (about 10%) while up to 80% of inhaled Hg passes the alveolar membrane and reaches plasma.

Circulating Hg accumulate in brain as demonstrated in many human autopsy studies (Eggleston DW et al. 1987; Nylander M et al. 1987), in kidney as detected in tissues from living kidney donors (Barregard L. et al. 1999), liver, jaw and kidney tissues as analyzed in animal experiments (Hahn LJ et al. 1989; Hahn LJ et al. 1990). Previous reports described a positive correlation between number of fillings and Hg vapor in intra-oral air (Vimy MJ et al. 1985; Berglund A et al. 1988), total Hg in saliva (Bjorkman L et al. 1997; Ott KH et al. 1984; Pizzichini M et al. 2000), plasma (Molin M et al. 1987; Barregard L et al. 1995) and urine (Langworth S et al. 1988, Sallsten G et al. 1996).

Fish consumption has also been extensively analysed and shown to contribute on Hg levels in different biological fluids (Berode M et al. 1976; Dennis CA et al 1975; Svensson BG et al. 1992; Suzuki T et al. 1975) and scalp hair (Pallotti G et al. 1979; Murata K et al. 1999; Renzoni A et al. 1998).

Among described harmful effects of highly toxic heavy metals, greater attention has being recently focusing on the pro-oxidant effect of mercury. In fact, a number of studies demonstrated the ability of Hg, like other metal ions, to interact with soluble and protein bound -SH groups, and to produce reactive oxygen species (ROS) like superoxide anion, hydrogen peroxide, hydroxyl-radical, able to induce oxidative injury to tissues through diverse mechanisms (e.g. lipid peroxidation, DNA damage, alterations of calcium homeostasis) (Stohs SJ et al. 1995). Living organisms are normally protected against oxidative stress by a number of enzymatic and non-enzymatic compounds endowed with antioxidant activity and the total antioxidant activity (TAA) can be measured with different analytical methods in biological fluids (Rice-Evans C et al. 1994; Cao G et al. 1993; Aejmelaeus RT et al. 1997; Kohen R et al. 1992).

One report on the effect of Hg on TAA indicated a negative influence of Hg on the antioxidant status of saliva (Pizzichini M et al. 2000). The aim of this investigation was to relate number of amalgam restorations, Hg levels and TAA in plasma of healthy donors. The influence of fish consumption on Hg plasma levels was also evaluated.

MATERIAL AND METHODS

Twenty-six healthy donors (16 females, 10 males; mean age = 40 years; range =22-60 years) were chosen to participate in this study and all gave their informed consent. An anamnesis was performed in order to exclude both subjects with pregressive (for the last month) or actual pathologies, such as those with pregressive or actual pharmacological therapy. The number of fish meals/week has been recorded. Each participant received a visit by a dentist in order to evaluate the number of amalgam restorations.

Venous blood was collected in vacuntainer tubes tested free of mercury contamination (under detection limit) with heparin and immediately separeted by centrifugation. 0,2 ml of plasma, to which the antioxidant butyl hydroxytoluene (BHT) was added (0.1 mM final concentration), were used to determine plasmatic TAA with the FRAP assay, a simple test measuring the ferric reducing ability of plasma (Benzie FF et al. 1996). TAA values were expressed in arbitratry units. Cold vapor atomic absorption spectrometry (Perkin Elmer FIMS 400) was used to determine the total Hg. Plasma samples (1 ml) were digested with 3 ml of nitric acid (Merck Suprapur) in Teflon vessels under pressure for 8 hours at 120°C (Chien Y et al. 1996; Drexler H et al. 1998). Each series of analysis was accompanied by concurrent mineralization and identification of Standard Reference Materials (SRMs) n° 1577b "Bovine Liver" (3μg/l) from NIST (Gaithersburg, USA). Batches with accompanying SRMs outside the certified range were repeated. For quantitation, mercury standard for atomic absorption from C. Erba Reagents (1mg/ml) was used and the detection limit was of 0,1ng/ml. The reliability of Hg determination, expressed as the coefficient of variation on repeated assays of the same samples, was below 5%. Hg values are expressed in μg/l.

A simple regression analysis was used to compare the experimental results. Only p values < 0.05 were considered as significant.

RESULTS

Table 1 reported number of fillings, plasmatic Hg (P-Hg), FRAP values, number of fish meals/week, age and smoked cigarettes/day of all the examined subjects. A positive correlation was found between n° of fillings and P-Hg (r= 0.388; p< 0.05) (Fig. 1), while a significant

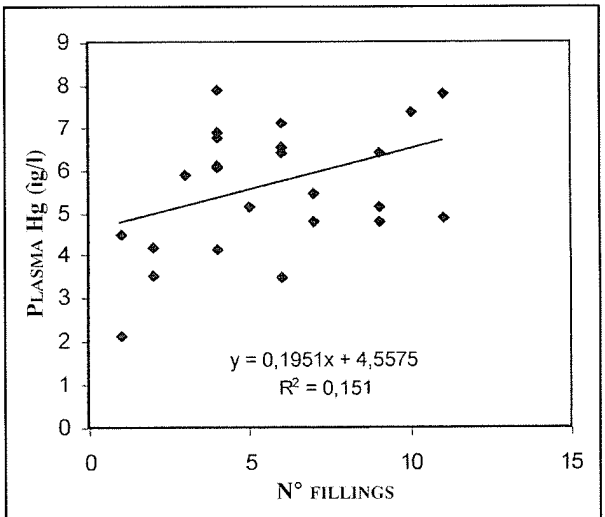

Fig. 1 - Correlation between number of amalgam fillings and plasmatic Hg for all examined subjects.

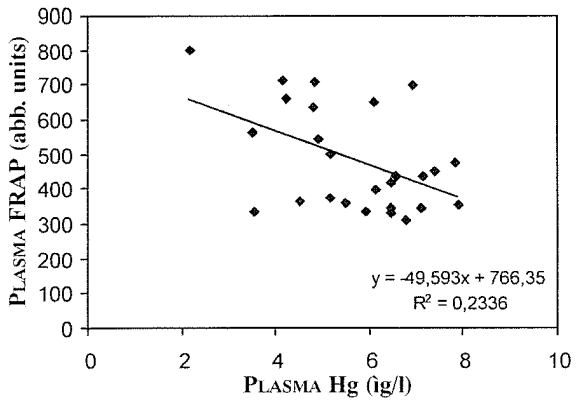

Fig. 2 - Correlation between plasmatic FRAP and Hg for all examined subjects.

n° fillings	P-Hg	P-FRAP	fish cons.
1	2,15	802	3
6	7,1	346	2
4	6,78	313	0
6	6,56	440	1
6	6,43	348	3
9	6,43	419	1
10	7,39	455	2
1	4,5	368	1
2	3,54	337	0
9	5,14	377	0
4	6,11	404	2
7	5,46	364	0
7	4,82	711	1
4	6,07	655	3
9	4,79	640	1
4	4,14	718	0
4	6,9	700	2
2	4,2	662	3
6	6,44	334	2
4	7,89	360	2
5	5,14	501	2
11	7,8	479	3
11	4,9	547	3
3	5,89	340	2
6	7,11	440	4
6	3,5	566	1

Tab. 1 - Number of amalgam fillings, plasmatic Hg (P-Hg), plasmatic FRAP (P-FRAP), fish meals/week (fish cons).

	Plasmatic Hg
Fish meals *per* week	0.247

Tab. 2 - Correlation coefficient (r) obtained from simple regression analysis.

inverse correlation emerges between P-Hg and P-FRAP ($r= 0.483$; $p< 0.02$) (Fig. 2). No influence of fish consumption on P-Hg was evident (Tab. 2).

DISCUSSION

Amalgam fillings constitute a well documented source of exposure to Hg° vapors. Extra-cellular fluids, SNC, liver and kidney tissues of human amalgam bearers display detectable amounts of Hg which positively correlate with the amalgam load of the subject. Many factors such as low oral hygiene, dental brushing, salivar composition, chewing use, pH and termical variations, can influence the corrosion of amalgam, so determining enhanced release of Hg (Brune D et al. 1985). In agreement with many other reports (Barregard L. et al. 1999 ; Molin M et al. 1987), our results confirm that higher Hg plasma levels are present in people bearing amalgam restorations.

The fish consumption represents another important source of Hg (WHO 1990) and many authors indicated that very high fish consumption correlated with Hg levels in blood cells (Svensson BG et al. 1992; Birke G et al. 1972), plasma (Berode M et al. 1976; Dennis CA et al 1975; Bregdahl IA et al. 1998) urine (Svensson BG et al. 1992) and hair (Murata K et al. 1999; Renzoni A et al. 1998). The Swedish National Food Administration recommended that certain fresh-water fish (i.e., species pike, perch, pike-perch and burbot) not be consumed during pregnancy and lactational period (Oskarsson A et al. 1996). Many of these studies have been based on populations with very high intake of contaminated fish such as fishermen and their families, but studies of the general population are rare. In Italy, fishermen and their families from Tyrrhenian area eating at least four seafood meals a week showed higher Hg concentration in the scalp hair (Renzoni A et al. 1998). A correlation was found between Hg blood levels and the number of fish meals, but not for hair in subjects from Province of Rome (Pallotti G et al. 1979), and no influence of fish

consumption, smoke or age on urinary Hg excretion was observed in people from Province of Bari (Soleo L et al. 1998). Our results showing no correlation between Hg plasma and fish meals are easy explained considering that subjects of the present group used different species of fish or poor quantity ingested at week.

Many studies were undertaken to demonstrate the pro-oxidant role of Hg linked to ROS production in vitro (Lund BO et al. 1991; Kappus H et al. 1994; Olivieri G et al. 2000) and in vivo (Lund BO et al. 1993) and some variations of antioxidant compounds were obtained in rat organs after exposure to Hg organic (Woods JS et al. 1995; Hussain S et al. 1997) or in blood of people occupationally exposed (Queiroz ML et al. 1998). Barregard et al. (1990) found no differences between exposed workers in a chloralkali plant and a control group with respect to the antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase(GSH-Px). Bjorkman et al. (1993) found similar activities of catalase and GSH-Px in erythrocytes of individuals occupationally exposed to Hg and in subjects with amalgam fillings, but not occupationally exposed.

It is now generally accepted that the measurements of any individual antioxidants is less representative of the total antioxidant activity. Despite several procedures available to determine TAA, this more global appraisal has never been used to investigate the relationship between Hg from dental amalgam and antioxidant status. In the past, only Pizzichini et al. (2000) demonstrated a significant positive correlation between salivary mercury and number or extension of amalgam fillings, while TAA (measured by the FRAP method) negatively correlated with salivary Hg in female subjects. The present data indicated, for the first time, that Hg influenced plasma antioxidant status in a group of healthy people of different age.

In conclusion, our results confirm that Hg released from amalgam fillings is the main source of the Hg present in plasma, without influence of fish consumption, and that total antioxidant plasma activity negatively correlated with plasmatic Hg in the examined group.

The antioxidant defence can be defined as an integrated and effective mechanism which protect biological systems against oxidative stress through a variety of enzymatic and non enzymatic compounds. Then, any factor or substance which is able to interfere with antioxidant defence should be carefully deepened and analyze.

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