

EFFECT OF STORAGE TIME ON DETECTABLE FLUORIDE IN RAT NAILS

EFEITO DO TEMPO DE ESTOCAGEM NO FLÚOR DETECTÁVEL NA UNHA DE RATOS

Tatiana de Almeida FURLANI

Undergraduate student from Biological Sciences Faculty, University of São Paulo State

Gary Milton WHITFORD

Department of Oral Biology, School of Dentistry, Medical College of Georgia, Augusta, GA, USA.

José Mauro GRANJEIRO

Marília Afonso Rabelo BUZALAF

Department of Biological Sciences, Bauru Dental School, University of São Paulo, Brazil.

We are conducting studies to evaluate the suitability of nails as a biomarker for fluoride (F) exposure in rats. The aim of the present study was to determine whether the F content of rat nails remains unchanged as a function of time after they have been clipped. Weanlings male Wistar rats received deionized water and food containing 25.9 ppm F for 56 days. Blood and nails were then collected for F analysis using the electrode following HMDS-facilitated diffusion. The nails were assigned to three groups that differed with respect to the time elapsed between collection and analysis: immediate (G1); 2 months (G2) and 3 months (G3). Plasma F concentrations were similar among the groups (0.017 ± 0.004 ppm). The nail F concentrations, however, showed major differences among the groups: G1 37.40 ± 10.70 ppm; G2 2.72 ± 2.70 ppm; G3 1.38 ± 0.57 ppm. ANOVA and the Tukey's post-hoc test showed that the concentration for G1 was significantly higher than those for G2 and G3 ($p < 0.001$). The cause of the time-dependent reduction in the amount of F that was detected has not been explained. It may be the result of the formation of a F compound that is not diffusible using the HMDS method or the formation of a volatile F compound. Research is in progress to evaluate these possibilities and to more precisely determine the time course of the phenomenon.

UNITERMS: Fluoride; Dental fluorosis; Rat; Nails; Biomarkers.

INTRODUCTION

In an international workshop conducted in Bethesda, Maryland, USA, in 1999, the participants established a research agenda for fluoride (F)². One of the recommended research topics was the development of reliable biomarkers for the past intake and body burden of F, which is, for all practical purposes, equal to the amount of F in the calcified tissues of the body⁹. Thus, it was recommended that the relationships between bone F levels and F in plasma, ductal saliva, dentin and fingernails be determined².

There are many reports suggesting the use of nails as biomarkers for F exposure in humans, as nail sampling is simple and non-invasive^{3-7,11}. Our research group has been studying the possibility of

using nails as indicators of F exposure in rats¹. In one of the experiments, rat nails were analyzed three months after collection and, compared to nails that had been analyzed immediately after their collection, the fluoride concentrations were lower by an average of 85%. Thus, the aim of the present study was to determine whether the F content of rat nails remains unchanged as a function of time after they have been clipped.

MATERIALS AND METHODS

Male Wistar rats (n=24) were received from the Central Vivarium of Bauru Dental School (University of São Paulo, Bauru-SP) as weanlings and housed in plastic cages. The animals had free

access to a regular diet (Purina, 25.9 ppm total fluoride soluble in HCl M) and deionized water. Fifty-six days after, when the animals reached the age of 77 days (average body weight 260 g), they were anaesthetized with diethyl ether. A heart blood sample was collected into a lightly heparinized syringe for determination of plasma F. After blood sampling, the nails were removed and the halves nearest the growth end were analyzed for F. Nail clippings from right and left feet were analyzed separately and a mean value obtained for each animal. The nails were assigned to three groups that differed with respect to the time elapsed between collection and analysis: immediate (G1); 2 months (G2) and 3 months (G3). Prior to analysis, plasma was stored at -20°C and nails at room temperature.

Fluoride analysis

The nail samples were cleaned with deionized water using a piece of cloth and then sonicated in deionized water for 10 minutes, dried at 60°C for 2 hours and weighed. The weight of the samples ranged from 4.20 to 14.8 mg. The fluoride concentrations of the nail samples were determined after overnight, HMDS-facilitated diffusion (Taves⁸) as modified by Whitford¹⁰ using the ion-specific electrode (Orion Research, Cambridge, MA, USA, model 9409) and a miniature calomel reference electrode (Accumet, #13-620-79) both coupled to a potentiometer (Orion Research, model EA 940). During the diffusion process, which was carried out at room temperature, the solutions in the non-wettable Petri dishes (Falcon, No. 1007) were gently swirled on a rotatory shaker to facilitate diffusion. Fluoride standards were prepared in triplicate and diffused in the same manner as the nail samples. In addition, nondiffused standards were prepared using the same solutions (0.05 N NaOH, 0.20 N acetic acid) that were used to prepare the diffused standards and samples. The nondiffused standards were made to have exactly the same fluoride concentrations as the diffused standards. The comparison of the millivolt readings demonstrated that the fluoride in the diffused standards had been completely trapped and analyzed. The fluoride concentration in plasma samples was analyzed in the same way.

Statistical Analysis

The data were tested for statistically significant differences by ANOVA and Tukey's post hoc test. A significance level of 0.05 was selected a priori.

RESULTS

Plasma F concentrations were similar among the groups (0.017 ± 0.004 ppm). The nail F concentrations (mean \pm SD, $n=8$, unit ppm) were 37.40 ± 10.70 ; 2.72 ± 2.70 and 1.38 ± 0.57 for G1, G2 and G3, respectively (Table I). ANOVA and the Tukey's post-hoc test showed that the concentration for G1 was significantly higher than those for G2 and G3 ($p < 0.001$). Although the G3 nail F concentration was lower than that of G2 the difference was not statistically significant. Thus after both 2 and 3 months of storage at room temperature, a large decrease in nail F concentration was observed.

DISCUSSION

Nails have been used as biomarkers for chronic exposure to fluoride both in humans^{3-7,11} and in laboratory animals¹. One of the subjects studied by Whitford et al.¹¹ approximately doubled his daily F intake by ingesting an additional 3.0 mg per day for 30 days. The additional intake was reflected in increased nail F concentrations after a lag time of 3.5 months. The authors, therefore, proposed that F enters fingernails via the matrix (growth end) and not through the nail bed. Based on this, we hypothesized that F in nail clippings could be used as an additional post-mortem test in cases of poisoning due to acute F ingestion if the growth end of the nails were analyzed. We have been conducting animal studies to investigate this possibility. However, during these experiments we observed that the storage of rat nails for 3 months prior to analysis resulted in a marked reduction in their F concentrations. Thus we decided to investigate this phenomenon.

In the present study, three groups of weanling rats were raised on the same diet for 2 months prior to the collection of blood and nails. The fact that levels of F intake did not differ significantly among the groups was confirmed by the lack of a statistically significant difference in the plasma F concentrations. Despite the similarity in F intake, the nail F concentrations of G2 and G3 were less than 10% that of G1. The only known difference among the groups was the time elapsed between collection and analysis of the nails.

The mechanism of this time-dependent reduction in the amount of detectable F has not been discovered. It may involve the formation within nails of a compound that is not diffusible using the HMDS-

facilitated method or, less likely, the formation of a volatile F compound. If the former process occurs and the F actually remains in the nails after prolonged storage, then dry ashing the nails at high temperature to destroy the organic phases should liberate the F and permit its detection. Research is in progress to evaluate this hypothesis and to more precisely determine the time course of the phenomenon. The effect of temperature during storage is also being investigated.

It is important to note that, using the HMDS-facilitated method, the amounts of F in distal nail clippings from humans¹¹ and rats (data not shown) are not affected by prolonged periods of storage. Thus, our ongoing research is needed if sections of rat nails taken from the growth end are to be analyzed for the purpose of confirming recent exposures to high amounts of F.

ACKNOWLEDGEMENTS

This work was supported by FAPESP (Grants 00/00826-6 and 01/02881-7).

RESUMO

Estamos realizando estudos para avaliar a viabilidade de se usar unhas como indicadores da exposição ao flúor (F) em ratos. O objetivo do presente estudo foi determinar se a concentração de F em unhas de ratos permanece inalterada em função do tempo após a coleta das mesmas. Ratos Wistar machos recém-desmamados receberam água deionizada e ração contendo 25.9 ppm F por 56 dias. O sangue e as unhas foram coletados para análise de F usando o eletrodo, após difusão facilitada por HMDS. As unhas foram divididas em três grupos que diferiram em relação ao tempo entre a coleta e a análise: imediata (G1), 2 meses (G2) e 3 meses (G3). As concentrações plasmáticas de F foram similares entre os grupos ($0,017 \pm 0,004$ ppm). Entretanto, as concentrações de F nas unhas foram muito diferentes entre os grupos: G1, $37,40 \pm 10,70$ ppm; G2, $2,72 \pm 2,70$ ppm; G3, $1,38 \pm 0,57$ ppm. A ANOVA e o teste de Tukey mostraram que a concentração de F do G1 foi significativamente maior em relação ao G2 e ao G3 ($p < 0,001$). A causa na redução na quantidade de F dependente do tempo ainda não foi explicada. Pode ser resultado da formação de um composto não difusível pelo método do HMDS ou da formação de um composto volátil contendo F. Estudos estão sendo

realizados para se avaliar estas possibilidades e para se determinar mais precisamente a cronologia deste fenômeno.

UNITERMOS: Flúor; Fluorose dentária; Unha; Rato; Biomarcadores.

REFERENCES

- 1- Buzalaf, M.A.R. et al. Unha como indicador da exposição crônica ao flúor em ratos. **Pesq. Odont. bras.**, v. 15, p. 14, 2001. Supplement. /Abstract H009/
- 2- Clarkson, J. et al. International collaborative research on fluoride. **J. dent. Res.**, v. 79, p. 893-904, 2000.
- 3- Czarnowski, W.; Krechniak, J. Fluoride in the urine, hair, and nails of phosphate fertilizer workers. **Brit. J. Ind. Med.**, v. 47, p. 349-51, 1990.
- 4- Machoy, Z. Effects of environment upon fluoride content in nails of children. **Fluoride**, v. 22, p. 169-73, 1989.
- 5- Schamschula, R.G. et al. Physiological indicators of fluoride exposure and utilization: An epidemiological study. **Comm. Dent. oral Epidemiol.**, v. 13, p.104-7, 1985.
- 6- Schmidt, C.W.; Leuschke, W. Fluoride content in fingernails of individuals with and without chronic fluoride exposure. **Fluoride**, v.23, p. 79-82, 1990.
- 7- Spate, V.L. et al. Determination of fluoride in human nails via cyclic instrumental neutron activation analysis. **J. Radioanalyt. Nucl. Chem.** v. 170, p. 27-33, 1994.
- 8- Taves, D.R. Determination of submicromolar concentrations of fluoride in biological samples. **Talanta**, v. 15, p. 1015-23, 1968.
- 9- Whitford, G.M. Intake and metabolism of fluoride. **Adv. dent. Res.**, v. 8, p. 5-14, 1994.
- 10- Whitford, G.M. **Some characteristics of fluoride analysis with the electrode.** In: The metabolism and toxicity of fluoride. Monogr. Oral Sci., H.M. Myers, Ed. Karger, Basel, Switzerland, 1996, p. 303-333.
- 11- Whitford, G.M. et al. Fingernail fluoride: a method for monitoring fluoride exposure. **Caries Res.**, v. 33, p. 462-7, 1999.

Endereço para correspondência:

**Faculdade de Odontologia de Bauru
Universidade de São Paulo
Al. Dr. Octávio Pinheiro Brisolla, 9-75
Departamento de Bioquímica
Cep.: 17012.901 - Bauru - SP**