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Effects of salivary acetylcholinesterase on the cytotoxicity of acrylic reline resins

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ABSTRACT

Aim: To evaluate the effect of acetylcholinesterase on the cytotoxicity of three autopolymerizing acrylic reline resins through the effect of the materials' eluates, liquids and respective pure compounds on the cellular viability of primary dermal fibroblasts cultures.
Methods: Disk shaped specimens of two direct Acrylic Reline Reins (ARR), Kooliner and Ufi Gel Hard, and one indirect ARR, Probase Cold, were studied. Cytotoxicity was studied through spectrophotometric determination of tetrazolium reduction (MTT assay) and lactate dehydrogenase activity (LDH assay). Moreover, at least 7 concentrations of each liquid and compound were prepared to determine the IC50 parameter. All data were evaluated using Kruskall–Wallis or Mann–Whitney test, after verification with Kolmogorov–Smirnov test.
Results: The fibroblasts exposed to the direct ARR eluates resulted in inhibition of the mitochondrial activity. Probase Cold eluates did not diminish cellular viability. LDH remained unaltered when fibroblasts were exposed to the eluates. Acetylcholinesterase groups of direct reline resins showed to be less cytotoxic when compared with control groups without changing their cytotoxic potential. The non-cytotoxic effect of Probase Cold did not change. The cytotoxicity of the pure compounds increased in the following order: Methacrylic Acid (MA), Isobutyl Methacrylate (IBMA) and Hexanediol Dimethacrylate (HDMA). Methyl Methacrylate (MMA) showed no cytotoxicity at the concentrations used. The direct reline resins liquids and respective pure compounds exhibited similar behavior.
Conclusions: Acetylcholinesterase did not change the cytotoxic potential of the reline resins studied. HDMA and IBMA revealed higher levels of cytotoxicity than MA, and their behavior was similar to the respective liquids.

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Efeito da enzima salivar acetilcolinesterase na citotoxicidade de resinas acrílicas de rebasamento

Palavras chave:
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Acetilcolinesterase
Fibroblastos
Concentração inibitória 50

RESUMO

Objetivos: Avaliar o efeito da acetilcolinesterase na citotoxicidade de três resinas acrílicas de rebasamento autopolimerizáveis (RRA), através do efeito dos extractos totais dos materiais, dos líquidos e dos respetivos monómeros puros na viabilidade de culturas primárias de fibroblastos.

Métodos: Foram avaliadas duas RRA diretas, Kooliner e Ufi Gel Hard, e uma resina de rebasamento indirecto, Probase Cold. A citotoxicidade foi determinada através de ensaios espectrofotométricos da redução do brometo de tetrazólio (MTT) e da atividade da enzima lactato desidrogenase (LDH), em culturas primárias de fibroblastos. Adicionalmente, foram preparadas, pelo menos, 7 concentrações de cada monómero e líquido, para determinar o parâmetro IC50. Os dados foram analisados por meio do teste Kruskall–Wallis ou Mann–Whitney, após verificação com teste de Kolmogorov–Smirnov.

Resultados: A exposição dos fibroblastos aos extratos das RRA diretas resultou na inibição da atividade mitocondrial, enquanto o Probase Cold não provocou diminuição da viabilidade celular. A atividade da LDH não sofreu alterações quando exposta aos extratos. Os grupos com acetilcolinesterase das RRA directas revelaram-se menos tóxicos, quando comparados com os grupos controlo, sem alterar o seu potencial citotóxico. A citotoxicidade dos monómeros puros aumentou na seguinte ordem: ácido metacrílico (MA), isobutilmetacrilato (IBMA) e hexanodioldimetacrilato (HDMA). Os líquidos das resinas de rebasamento direto demonstraram uma curva de citotoxicidade semelhante aos respetivos monómeros.

Conclusões: A enzima acetilcolinesterase não alterou o potencial citotóxico dos materiais estudados. O HDMA e IBMA demonstraram maiores níveis de citotoxicidade que o ácido Metacrílico, e o seu comportamento foi semelhante ao líquido das respetivas resinas.

Introduction

The use of autopolymerizing acrylic reline resins (ARR) has recently gained popularity in dentures readjustment to the continuous reabsorbed underlying tissues, providing better retention and stability for complete removable prostheses.[1,2]

However, these materials have been associated with *in vitro* toxicity and also, *in vivo* manifestations such as chemical irritation, allergic reactions,[1,3] erythema, erosion of oral mucosa and burning mouth sensation.[4] These adverse reactions caused by denture base polymers have been attributed to substances leached from these materials, especially unreacted residual monomers (RM), that remained in the resin net after polymerization.[5–7]

Given the generally reliable manufacturers intended lifetime of polymeric devices,[8] several studies have shown that polymers may be subject to numerous biodegradation processes in the oral cavity,[9] due to the important role that esterases plays in the enzymatic activity. Acetylcholinesterase (AChE) catalytic activity has recently been shown to be detectable in saliva where its catalytic activity is stable.[10] However, other study findings indicated that the intra-individual coefficient of variance of saliva AChE was 35%,[11] showing that levels of this enzyme are highly variable.

Although well demonstrated in composite resins,[12–14] the role of esterases on the biodegradation of ARR needs further investigation.

The main purpose of the present study was to investigate the influence of acetylcholinesterase on the level of cytotoxicity of three widely used autopolymerizing ARR. In addition, the purpose was to assess the level of cytotoxicity of three specific pure compounds, that are known to be present in the eluates, and the cytotoxicity of the resin liquids through the determination of the half maximal inhibitory concentration (IC50).

Materials and methods

This study enrolled two direct ARR, Kooliner (GC America Inc., Alsio, IL, USA), Ufi Gel Hard (VocoGmbH, Cuxhaven, Germany), and one indirect ARR, Probase Cold (Ivoclar Vivadent AG, Schaan, Liechtenstein), in powder liquid form (Table 1). Disk shaped specimens were prepared from three separate mixtures in stainless steel molds, with an average diameter of 50 ± 0.1 mm and an average thickness of 2 ± 0.01 mm, according to ISO recommendation for biological evaluation of biomaterials.[15,16]

Direct ARR were set at $37 \pm 2\,^{\circ}C$ for the recommended polymerization time (Table 1) in order to simulate the intra-oral polymerization of the material. Polymerization of indirect ARR was carried out in a Ivomat pressure device (Ivoclar Vivadent, Lichenstein) for the recommended time, temperature and pressure (Table 1).

After UV sterilization,[17–19] specimens of each material ($n = 6$) were randomly divided into two groups: experimental,