Cytotoxicity of AH Plus and AH26 *in vitro* on Chinese hamster V79 fibroblasts

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Summary

The purpose of the study was to evaluate cytotoxicity of AH Plus, compared to AH26 in vitro *on Chinese hamsters V79 fibroblasts. The materials were prepared according to the manufacturer's instructions. After the initial setting period, the materials were crumbled and dissolved in dimetil-sulfoxide solution. The extracts obtained were incubated at 37C during one hour, 24 hours and 7 days. After incubation period the samples were diluted with Eagle's minimum essential medium to concentrations ranging from 1.67 µg/ml to 167 µg/ml. Each concentration was placed on a plate with 24 wells with $5x10^3$ V79 cells per 1.2 ml of medium. The number of cells was counted by electronic counter and the percentage of viable cells was determined by a light microscope. Both materials, AH26 and AH Plus were found to be cytotoxic. In both materials cytotoxic effect was related to the concentration of endodontic sealer in the extract solution. The critical concentrations, above which the sealers totally destroy the cell line are between 5.57 µg/ml and 167 µg/ml for AH Plus and 16.57 µg/ml and 167 µg/ml for AH26. The setting time did not have a statistically significant effect an cytotoxicity.*

Key words: *chinese hamster V79 fibroblasts, AH Plus, AH26, cytotoxicity.*

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Introduction

Today the market offers a variety of materials used as root canal sealers. Each of them has to fulfill certain conditions, among which biocompatibility is particularly important.

The data available from previous studies indicates that a large number of materials have high toxicity. This is particularly the case in overfilled root canals, when the filling can cause not only mechanical irritation, but can also have a cytotoxic effect on the periapical tissues (1).

A large group of root canal sealers are resin based. The most common in clinical practice are AH26 and AH Plus. AH26, epoxy-resin, has very good adherence to dentine, but high toxicity during setting time (2).

AH Plus, a new epoxy-amine resins material has been introduced which, according to the manufacturer, has better physical and clinical properties then AH26, such as faster setting time, radiopacity and easier handling (3).

Toxicity of the material has been studied *in vivo* (4,5) and on different cell cultures *in vitro* (6,7): on mouse L929 fibroblasts, human HeLa cervical cells, VERO monkey cells and NCTC2544 epithelial cells.

The purpose of this study was to determinate the cytotoxic effect of AH Plus and AH26 *in vitro* on Chinese hamster V79 fibroblasts.

Materials and methods

The tested materials were AH26 silver free (Dentsply, DeTrey, Konstanz, Germany) and AH Plus (Dentsply, DeTrey). Materials were prepared according to the manufacturer's instructions. After the initial setting period, the examined material was crumbled and dissolved in 1g/1ml dimethylsulfoxide solution. The extracts obtained were incubated at 37°C during one hour, 24 hours and 7 days. After incubation period the samples were diluted with Eagle's minimum essential medium (DMEM) to concentrations of 1.67 µg/ml, 5.57 µg/ml, 16.57 µg/ml, 55.7 µg/ml and 167 µg/ml. Each concentration was placed on a 24 well plate with $5x10^3$ V79 cells per 1.2 ml of medium. In control samples, only the growth medium (1 ml) was added. Four wells were plated for each concentration of the examined material extract. The specimens were incubated for 72 hours at 37°C. Thereafter, total cell numbers were counted by an electronic counter in 3 wells. The number of viable cells was determined under a light microscope in the last well for each concentration, using nigrosin dye which was extracted, from viable cells. For each sample, at least 100 cells were examined. The viability percentage was calculated using the following formula: % of viable cells = (A/B) x 100 where "A" was the number of viable cells in the experimental wells and "B" was the number of viable cells in the control wells. The experiment was repeated twice. The results were obtained using Student's *t*-test for independent samples.

Results

The results are shown in Figures 1 and 2. In both materials cytotoxic effect was related to the concentration of endodontic sealer in the extract solution. The critical concentrations, in which the sealers inhibit the growth of V79 cells are between 5.57 µg/ml and 167 µg/ml for AH Plus and 16.57 µg/ml and 167 µg/ml for AH26. The concentrations above these showed the percentage of viable cells identical or very near to zero. These results indicate higher cytotoxicity of AH Plus.

The percentage of viable cells in the control group was in all cases equal to 100%.

The setting time did not have a statistically significant effect ($p < 0.05$) on cytotoxicity.

Discussion

There are three levels for cytotoxicity study of material used as root canal sealers (8). Study starts in *in vitro* conditions determining the initial cytotoxicity, this is followed by an *in vivo* study on experimental animals to determine the response of tissues, and a clinical study representing the final stage.

The purpose of this study was to evaluate initial toxicity of the new AH Plus and to compare it to the AH26 whose cytotoxic effect is known. The study was conducted as part of an investigation on the biological effects of materials (9). The concentration of the sealer in the extract solution had on influence on the cytotoxic effect (higher concentration resulted in higher cytotoxic effect). The setting time, however, had no influence on the cytotoxic effect.

Greater concentrations of both sealers are extremely cytotoxic, as treatment with the extract solution in concentrations of 167 µg/ml caused total loss of viable cells. AH Plus can be considered more toxic, as its critical concentration is much lower then that of AH26.

Toxicity of AH26 is related to the release of formaldehyde in the course of material setting (10,11). AH Plus does not release formaldehyde during its setting. Its cytotoxicity, however, can be explained by its amine components, added to improve polymerization (12). These results are similar to the results of Al-Hazhan and Spangberg (13) who measured the toxicity of AH26 by the release of chrome from the membranes and determined the high toxicity of this material. On the contrary, Leyhausen et al. (14) did not find cytotoxicity of AH Plus using 3T3 cells and fibroblast cells. The difference in results could be explained by the different experimental model used. They measured cytotoxicity by DNA-synthesis, as well as by the difference in used cells.

The results of this study show the cytotoxicity of AH Plus to be higher than the cytotoxicity of AH26 on Chinese hamster V79 fibroblasts, and the cytotoxicity of both materials to be dependant on their extract concentration. Further research is necessary to determine the effect of the sealers on vital tissues.