行政院國家科學委員會補助專題研究計畫成果報告

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※（計畫名稱）AH26 與 AH plus 根管充填劑之溶出物分析及致突變性研究 ※
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本成果報告包括以下應繳交之附件：

□赴國外出差或研習心得報告一份

□赴大陸地區出差或研習心得報告一份

□出席國際學術會議心得報告及發表之論文各一份

Ø 國際合作研究計畫國外研究報告書一份

Ø 執行單位：

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fillings may gain access to surrounding tissues, like periodontal ligament and alveolar bone, through numerous connections, e.g., dentinal tubules, accessory and lateral canals, and apical foramina.[1.] Clinically, there are three kind of filing materials used, one is based on the zinc oxide eugenol, two is based on the calcium hydroxide, three is based on the epoxy resin.

The epoxy resin sealer AH 26 is based on bispheonl-A-diglycidylether. Additionally, the powder contains hexamethylene-tetramine. Spangberg et al. found that AH26 releases formaldehyde after mixing, with maximum release after 2 days.[2] McNamara et al, 1992, indicated that freshly polymerized compared to prepolymerized hydron (poly-2 hydroxyethyl methacrylate hydrogel) and freshly mixed AH26 compared to Tubli-Seal in L929 fibroblast cultures. During polymerization, Hydron proved to be as highly cytotoxic as Tubli Seal. Set Hydron inhibited cell functions to the same extent as AH26. All materials induced marked alterations of the cellular metabolism. AH26 has been demonstrated to be a highly cytotoxic in several cell culture systems.[3.] Formaldehyde released from AH26 mixtures is thought to be the main cause of inducing cytotoxicity.

The new root canal filling material AH plus is based upon a epoxy amine polymer. AH plus is an imprved and perfected, version of the DENTSPLY DeTrey endodontic classic AH26. According to the manufactory description, the cytotoxicity of AH plus to cells was considered to be lower than that of AH26. Schmalz et al.(1998) study the activity of the root canal sealing cement AH plus through the bacterial gene mutation assay(Ames test). The result showed that at least two different compounds of AH plus are biologically active in DMSO elutes to cause mutagenic and toxic effects in S. typhimuriam TA100 and TA98. [4]

In our previous data showed that AH26 was proved cytotoxic to oral cancer cell line(OC2) using by MTT assay.[5.] The AH26 also has mutagenicity on OC2 cell line by nick translation assay.[6] The test solution of these studies has been eluted and stored more than one year. They are still showed cytotoxic and mutagenic to OC 2 cell. It is doubted that except the formaldehyde, are there any others components can cause the cytotoxicity and mutagenicity. High performance liquid chromatography (HPLC) can used to detect the mutagenic compounds in root filling material. The chemical structure of the major mutagenic fraction of root filling material can be characterized by UV spectra and liquid chromatography-mass spectrometry (LC-MS). The first aim of this study is to determine the possible components caused the cytotoxicity that released from AH26 and AH plus sealers.[7.]

Purpose

The aim of this study are to investigate the releasing components of the filling materials AH26 and AH plus after mixing by using the HPLC and LC-MS assay, and discuss the mutagenicity of the AH 26 and AH plus on different cells by using Comet assay. It is hope that these data can provide the filling material selection in clinical.

三、結果與討論

Result:

1.After HPLC chromatogram of AH 26 and AH Plus were compared. The formaldehyde component was found in AH 26 group, but not in AH Plus group. The rest peaks of the HPLC chromptogram, can be found as the rest components of unmixed materials.

2. The comet assay of the AH 26 and AH

plus cement showed that there were existed degrees of genotoxicity. The migration ratio of the comet of the AH 26 is larger than the AH plus.

Discussion

1. The Formaldehyde can be detected in the early mixed AH 26. This is same as other author's report that the mixed time in initial 24 hours will show higher formaldehyde than other times. In this experiment, there is need further to investigate the longer time for the cement immersion liquid. Also, the small peaks of the AH Plus may be attribute to impurities or improper mixed method. The rest component of the N,N-dibenzyl-5-oxanonanediamine-1,9-TCD-diamine can be detected in sometimes. The HPLC chromatographic conditions may be another influence factor on the analysis. The mobile phase used is 80% methanol and 20% water, the flow rate is 2.0ml/min, the temperature is constant as 22℃, the detector is UV 254nm, the loop is 20 ul. The condition of the present used need to be further reevaluate. The result might be more reliable.

2. For the AH 26 is releasing the formaldehyde. As we know that the formaldehyde can caused the cytotoxicity. The genotoxicity of AH 26 also showed the tail of the nucleus of the OC2 cell is higher than that of the control group. That is AH 26 existed genotoxicity. The AH plus though no formaldehyde released, but in comet assay, they existed various degrees of toxicity as comparing to the control. The higher concentration, the tail length can be showed higher. In present study, there is need to investigate the real factor that cause the genotoxicity.

計劃成果自評

From the present findings, the toxicity of the root canal sealers can be thought as the formaldehyde from the AH 26,but can not be defined that is from the AH plus. It is thought that the finding can be a thought for the next step of investigation. The mechanism of the toxicity happened is needed to be investigation. The chemical acted on the cell, what is the signal transduction method should be analysis. So, the author is planned to continue the above works.

Reference

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Table The comet assay of resin based root canal sealer.

Material	Concentration	N	Shape Factor= Length/Diameter (Mean ± SE)	Migration Factor (:m)=Length-Diameter (Mean ± SE)
DMSO (Negative Control)		50	1.00±0	44.16±1.47
4NQO (Positive Control)		50	2.75±0.19	57.89±5.84
F value			89.23	5.20
P value			0*	0.025*
Topseal	0.1mg/ml	50	4.06 ± 0.12	82.87 ± 2.63
	0.5 mg/ml	50	3.72 ± 0.16	81.74 ± 3.36
	2.5 mg/ml	50	3.72 ± 0.13	100 ± 3.13
F value			2.03	11.22
P value			0.15	0 *
AH 26	0.1 mg/ml	50	2.62±0.17	58.26±4.69
	0.5 mg/ml	50	3.63±0.14	67.74±2.32
	2.5 mg/ml	50	3.54±0.12	89.13±3.21
F value			14.90	19.91
P value			0*	0*
AH Plus	0.1 mg/ml	50	3.89±0.27	80.20±4.90
	0.5 mg/ml	50	3.73±0.14	79.04±3.23
	2.5 mg/ml	50	4.97±0.17	110.83±3.33
F value			11.24	21.42
P value			0*	0*