Possibility of Allergic Reaction to Dentin Primer — Application on the Skin of Guinea Pigs —

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We studied the allergic reaction of guinea pigs to glyceryl methacrylate (GM), hydroxyethyl methacrylate (HEMA) and meso-erythritol methacrylate (EM), which are used as dentin primers. On the 18th day of the application test, when macroscopic investigation revealed an inflammatory reaction, the methacrylic acid-treated group showed marked eschar formation in comparison with the control group. In each of the dentin primer groups, a slight degree of skin redness was noted, but there were no serious symptoms. On the 25th day, the applications were resumed macroscopic inspection on the 32nd day found eschar in the methacrylic acid group only. Therefore, this experiment with dentin primers suggests a delayed allergic reaction. Local irritability test showed a more severe reaction than the application test. In this test, all experimental dentin primers and methacrylic solution promptly showed inflammation, and the chemical compound, methacrylic acid was a factor in inflammation.

Key words: HEMA, Glyceryl methacrylate, Dentin primer

INTRODUCTION

Since Munksgaard and Asmussen's report on GLUMA primer, an aqueous mixture of 35% hydroxyethyl methacrylate (HEMA) and 5% glutaraldehyde[1], various dentin primers have been reported effective in promoting the efficacy of dentin bonding systems[2,3]. We modified the components of GLUMA primer by eliminating glutaraldehyde in order to avoid possible skin discoloration from the primer. The 35% HEMA solution exhibited an effect comparable to that of GLUMA primer when followed by an effective dentin bonding agent. Discoloration of soft tissue by GLUMA primer is due to protein coagulation caused by glutaraldehyde[4]. Although such discoloration is easily prevented by using the 35% HEMA solution, contact dermatitis is still experienced after frequent direct contact with the HEMA solution.

Our studies, both in vivo and in vitro, showed that HEMA primer: 1) promotes the efficacy of dentin bonding systems, 2) reduces the sensitivity of dentin[5], and 3) causes contact dermatitis in soft tissues. The third action is an undesirable side effect which should be eliminated.

In order to develop a dentin primer which has good bonding efficacy but a decreased risk of side effects, we reported on two experimental dentin primers, i.e. aqueous solutions of glyceryl methacrylate (GM) and erythritol methacrylate (EM), both of which are metha-
crylate esterified with a polyvalent alcohol\(^6\). To explore the mechanism of contact dermatitis and the possibility of side effects from GM and EM solutions, we performed skin tests with guinea pigs. Guinea pigs were selected because they are more sensitive to antigens than rats or rabbits.

**MATERIALS AND METHODS**

Three experimental dentin primers were prepared by diluting methacrylate derivatives, (HEMA*, GM**, and EM***) in distilled water at concentrations of 35, 35 and 40% by volume, respectively. As the basic chemical structure of methacrylate derivatives and a negative control, respectively, an aqueous solution of 24% methacrylic acid (MCA)**** and a normal saline solution were also prepared.

*Examination of the possibility of allergic reaction*

In order to observe the possibility of immediate allergic reaction, we examined 1) Active Systemic Anaphylaxis, 2) Passive Systemic Anaphylaxis, 3) Passive Cutaneous Anaphylaxis and 4) Reverse Passive Arthus Reaction.\(^8\) Male Hartley guinea pigs, weighing approximately 280g, were used in this experiment. Several antigen solutions were prepared by mixing experimental dentin primer and an equal volume of complete Freund's adjuvant (CFA).*****

Rabbit antisera, used for Passive Systemic Anaphylaxis and Passive Cutaneous Anaphylaxis was obtained by sensitizing several male Japanese white rabbits (body weight: 3kg) with the antigen solutions.

*Primary cutaneous irritability test*

The experimental animals were four Hartley guinea pigs, weighing approximately 280g. The possibility of delayed allergic reaction was examined as follows.

Fifty \(\mu l\) of the above-mentioned solutions were measured with a micropipet and applied to the shaved dorsal skin of the guinea pigs every eight hours for 18 days, causing obvious dermal discoloration. After a 7-day interval in which the disappearance of dermal change was confirmed macroscopically, application of the solution was resumed for 7 more days. The application of dentin primers was finished 32 days after the first application.

*Local irritability tested by intracutaneous injection*

Local irritability of the guinea pig dorsal skin was examined by an intracutaneous injection of 0.2ml of each dentin primer and methacrylic acid. After injecting the dentin primers, macroscopic observation of the dorsal skin of guinea pig was performed 2 hours and 7 days later, respectively.

The results of the application test and the local irritability test by intracutaneous injection were scored quantitatively, as follows. A score of 1 was redness, 2 was edema, 3 was redness and vesicle formation, 4 was eschar formation and 5 was ulceration.

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* (2-hydroxyethyl)-methacrylate; E. Merck, Darmstadt, Germany
** glyceryl methacrylate; Nippon Oil and Fat Co., Tokyo, Japan
*** erythritol; Tokyo Kasei Kougyou Co. LTD., Tokyo, Japan
**** methyl methacrylate; Wako Pure Chemical Industries, LTD., Tokyo, Japan
***** complete Freund's adjuvant; Difco Laboratories, Detroit, MI, USA
RESULTS

The possibility of allergic reaction

Active systemic anaphylaxis

None of the administered test solutions induced symptoms typical of anaphylaxis, i.e. piloerection, sneezing, clonic spasms, tachypnea, etc. With MCA administration, some animals showed slight piloerection and emitted crying sounds, while some animals jumped a number of times immediately after the administrations of GM and EM. These reactions were not judged to be anaphylactic symptoms.

Passive systemic anaphylaxis

No signs of anaphylaxis were observed in the guinea pigs passively sensitized with 2ml each of the rabbit antisera prepared against each of the test solutions. No abnormalities were noted even when the administered dose of rabbit antisera was increased (to 4ml).

Passive cutaneous anaphylaxis (PCA)

None of the experimental animals showed any changes such as edema, redness, hemorrhage, etc., at the site of the antisera infection. It was thus judged that there were no symptoms indicative of anaphylaxis.

Reverse passive arthus reaction

In the reverse passive Arthus reaction test, as well, there were no findings of blue dye leakage at the site of injection at any concentration of antisera. The PCA reaction was thus judged to be negative.

Primary cutaneous irritability test

Sensitivity tests were carried out by applying 50μl of each test article solution to the shaved skin of guinea pigs, three times per day (at 8hour intervals) on Days 1~18 and Days 25~32. On the 18th day of the application test, when an inflammatory reaction was first recognizable on macroscopic inspection, the methacrylic acid-treated group showed marked eschar formation in comparison with the control group. In each of the dentin primer groups, a slight degree of redness of the skin was noted, but there were no serious symptoms (Fig. 1). On the 25th day, the applications were resumed. Macroscopic inspection on the 32nd day found eschar in the methacrylic acid group only (Fig. 2, 3). In histopathological studies, on both the 18th and 32nd days, the skin of the animals in the methacrylic acid group showed acceleration of severe keratinization, enlargement of sebaceous glands and infiltration by inflammatory cells. Comparing the time required for manifestation of gross inflammatory changes observed after the methacrylic acid application, less time was required after the second application series than after the first application series (Table 1).

Local irritability tested by intracutaneous injection

The results of local irritability test after 2 hours were as follows. The injection of methacrylic acid solution caused severe eschar compared to the control, and was given a score of 4 by macroscopic examination. Vesicle formation was observed after injecting GM solution, and given a score of 3. The HEMA and EM solutions both formed redness and vesicles; these were also given scores of 3. After 7 days, methacrylic acid, HEMA, GM and EM solutions all formed eschars. These were scored at 4. Therefore, all of the experimental solutions and the dentin primers in this study proved strongly irritating. (Table 2, Fig 4, 5).
Fig. 1 Guinea pig skin observed macroscopically after application of saline, 24% methacrylic acid. 35% HEMA, 35% GM and 40% EM solution (after application for 18 days).

Fig. 2 Guinea pig skin observed macroscopically after application of saline, 24% methacrylic acid. 35% HEMA, 35% GM and 40% EM solution (after application for 25 days).

Fig. 3 Guinea pig skin observed macroscopically after application of saline, 24% methacrylic acid. 35% HEMA, 35% GM and 40% EM solution (after application for 32 days).

Fig. 4 Guinea pig skin observed macroscopically after intracutaneous injection of saline, 24% methacrylic acid. 35% HEMA, 35% GM and 40% EM solutions (immediately after 2 hours).

Fig. 5 Guinea pig skin observed macroscopically after intracutaneous injection of saline, 24% methacrylic acid. 35% HEMA, 35% GM and 40% EM solutions (immediately after 7 days).
Table 1. Degree of cutaneous irritability to dentin primer applied guinea pigs

<table>
<thead>
<tr>
<th></th>
<th>18 Days</th>
<th>32 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MCA</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>HEMA</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>GM</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>EM</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

0: non irritably 1: redness 2: edema 3: redness and vesicle 4: eschar 5: ulcer

Table 2. Degree of cutaneous irritability to dentin primer in guinea pigs after of intracutaneous injection

<table>
<thead>
<tr>
<th></th>
<th>2 Hours</th>
<th>7 Days</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MCA</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>HEMA</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>GM</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>EM</td>
<td>3</td>
<td>5</td>
</tr>
</tbody>
</table>

See explanation in Table 1.

DISCUSSION

Although dermatitis caused by skin contact with HEMA solution has been frequently experienced in the laboratory, the mechanism and the risk have not been completely elucidated.

The development of GM and EM solutions were based on the speculation that methacrylate esterified with a polyvalent alcohol would be effective in promoting the bonding efficacy of dentin bonding systems.

However, the possibility of contact dermatitis caused by GM solution was not significantly less than with HEMA solution. Our previous report, in which the histological change in rat skin was examined after repeated application of dentin primers, demonstrated this fact.

Such an insignificant difference in skin irritation can be explained because clearly severe inflammation was observed after methacrylic acid application. It was confirmed that the guinea pig or rabbit did not form antibodies, because neither the experimental dentin primer nor methacrylic acid solution combined with protein in the living body. Furthermore, delayed allergic reaction was suggested by the results of the primary cutaneous irritability test. The dorsal skin of guinea pigs exposed to repeated applications experimental dentin primers recovered more quickly than that exposed to applications of methacrylic acid solution. Therefore, local irritability was caused mainly by methacrylic acid.

The methacrylate group is employed in most resin materials because it is effective for polymerizing the monomer. The undesirable side effect of skin irritation may be prevented by developing an esterified methacrylate with a polyvalent alcohol having more hydrogen groups since xylitol or sorbitol are assumed to be safe and permitted in foods as sweeteners.

From the clinical point of view, contact dermatitis resulting from dentin primer application is easily avoided by manipulating the materials carefully or using some protection to prevent direct contact with the monomer. However, it is impossible to secure the soft tissue completely because most dentin primers are dried with a strong blast of air after application. This results in excess primer being scattered around the oral mucosa or soft tissue of the patient. Therefore, more effort is required to develop a primer which exhibits high bonding efficacy, but causes fewer side effects.
CONCLUSION

In comparison with the application test, direct intracutaneous administration resulted in a more marked inflammatory reaction. Accordingly, these tests raise concern regarding irritation of the oral mucosa by the tested dentin primers and the aqueous solution of methacrylic acid.

Based on the results of these experiments, the possibility of an immediate-type allergic reaction to the dentin primers was ruled out. However, in the application tests, repeated application of the dentin primers clearly showed a shortening of the time required for manifestation of inflammatory changes. This finding raises concerns of a possible delayed-type allergic reaction.

REFERENCES

レーザーラマン光分析を応用した接着界面の研究
――4-META/MMA-TBB レジンと牛及び、ヒト象牙質界面の分析――

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前報で4-META/MMA-TBB レジンとハイドロキシアパタイト、牛歯エナメル質との接着界面の研究を行ったが、今回は統報として4-MET と牛及び、ヒト象牙質間の化学結合の可能性を、ラマンスペクトル測定により検討した。市販モノマー液中から体積量 2/3 の MMA を揮発して得た濃縮モノマー液を 10-3 処理した牛及び、ヒトの象牙質に塗布したこと、両者共に塗の形成が認められ、この塗は、前回我々が報告したハイドロキシアパタイト及び、牛歯エナメル質表面で、4-MET が形成した塗と同様の過程で形成されたものと考えられる。一方、象牙質中の有機成分で 4-MET の間で化学変化が起こったことを示唆するバンドは認められなかった。

モルモットに対するデンチングライナーによるアレルギー反応の検討

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象牙質の接着に不可欠な試作デンチングライナーである GM，HEMA，EM，を用いてモルモットに対する免疫学的アレルギー反応を検討した。その結果、即時型アレルギー反応はいずれのプライマーおよびこれらの基本的化学構造物であるメタクリル酸においても観察されなかった。また、デンチングライナーとメタクリル酸を用いモルモットの背部皮膚に長期間の反復塗布を行った。メタクリル酸では、塗布開始後 18 日で強い発赤，浮腫が認められた。GM，HEMA，EM では，軽度の発赤を認めた。炎症が完全に消失した 25 日後からの再塗布試験においては 7 日間で 18 日後と同様の炎症が認められた。このことからメタクリル酸、GM，HEMA，EM の遅延型アレルギー発現の可能性が示唆された。皮内反応による局所刺激では、塗布試験より過激な条件であることから，2 時間後には水泡，7 日後で真皮を形成した。今回の実験結果により，試作デンチングライナーの遅延型アレルギー反応（接触性皮膚炎）発現の可能性が示唆された。

顎関節鏡下手術のための手術器具の開発

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顎関節鏡下手術は，従来の種々の画像診断法で観察できなかった観察内病態を直接鏡視下にて診断が可能なこと，診断直後に手術が行える理由により普及しつつある。