Evaluation of Tumor Tissue Fixation Effects of Formulation Modified Mohs Pastes in Mice and Their Water-Absorbing Properties

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Mohs paste (MP) is a hospital preparation containing zinc hydrochloride and zinc oxide starch. It is a topical medication used to fixate tissues for the removal of inoperable skin tumors and the management of hemorrhage and exudates, and to prevent foul odor resulting from secondary infections. However, it has problems, such as changes in hardness and viscoelasticity with time and liquefaction by exudate. It has been reported that the modified MP with α-sorbitol (S-MP) and the modified MP using the cellulose instead of starch (C-MP) have excellent physicochemical stability and better handling than original MP (O-MP). In this study, the effect of prescription improvement of MP on the pharmacological effect was examined with reference to water absorbing property, and its tumor tissue invasion fixation depth as an indicator. In the S-MP and C-MP, the amounts of water absorption did not differ significantly from those in the O-MP. The hardness of S-MP was decreased and liquefied like O-MP after absorbing water. In contrast, C-MP retained its form even after water absorption. The subcutaneous tumors in mice treated with modified MP formulations were measured for invasion fixation depth at 6 and 24 h after application. And the tissue status was observed using computed tomography. In all MPs, invasion fixation depth increased depending on application time. S-MP and O-MP depths did not differ significantly. The invasion depths of the C-MP significantly increased compared with those in the O-MP. These results suggest that C-MP had a high tissue fixation rate.

Key words  Mohs paste; modified prescription; tissue invasion depth; water absorbing property; subcutaneous tumor

Mohs paste (MP) is a topical medication composed of zinc chloride and zinc oxide starch as the main ingredients and used for the palliative treatment of patients with surgically untreated malignant tumors. In terminally ill patients with skin cancer, the tumor surface gradually becomes ulcerative, hemorrhagic, and malodorous, developing into disintegrated gigantic cancer tumors. This worsens the patients’ QOL. MP is a histopathological fixative based on zinc chloride; it was developed for conducting chemosurgery of malignant skin tumors. It is also used to fix tissues for the removal of inoperable superficial tumors. MP also helps manage hemorrhage and reduce exudate fluid derived from self-destructive wounds by fixing the organization surface. Furthermore, it prevents the foul odor resulting from secondary infections caused by fixing the organization surface. The application time of MP varies depending on the tumor condition and treatment purpose. The application time of MP for skin tumor chemosurgery is approximately 6–24 h, whereas that for managing bleeding and reducing exudate fluid is approximately 15–180 min.

Examples of using MP in medical settings in Japan have been scattered to improve the QOL of patients receiving palliative care. MP is usually prepared in hospitals as it is not available commercially. Hospital-prepared MP (Original prescription of MP: O-MP) is difficult to apply to affected areas because its physicochemical properties, such as hardness, viscoelasticity, and malleability, dramatically change over time after preparation. Addition of 4% α-sorbitol reportedly makes MP easier to apply as it prevents changes in the physical properties of MP immediately after preparation. Kikuchi et al. reported that compared with O-MP, it is easier to apply MP with sorbitol (S-MP) to affected areas; they also reported that S-MP required a comparatively shorter application time. In a clinical case study on breast cancer skin metastasis, Hashiguchi et al. reported that S-MP and MP were equally effective on hemostasis. However, S-MP develops higher viscosity several weeks later. We have developed a starch-free MP (C-MP) that solves the problems of dramatic physical property changes; this formulation remains stable even after storage for more than a month, as confirmed by an acceleration test.

Here, we examined the effect of prescription improvement of MPs on pharmacological effects. MP controls bleeding and effusion by fixing the tissue while absorbing and maintaining the liquid component. Neoplastic cell or tumor vessel proteins are denatured owing to the chemical solidification ability of the zinc ions in MP. The disintegration wound surface simultaneously dries because of the water absorptivity of MP, thereby decreasing bleeding and effusion. Therefore, here, we aimed to evaluate the absorbing water properties of prescription improvement of MPs.

The penetration depth of MP chemical fixation depends on its time of application to an affected region. However, in clinical settings, it is difficult to compare the penetration rates...
for tissue fixation of MPs because the penetration rate depends on the condition of the skin structures. Therefore, here, we used sarcoma-180 bearing mice to compare the fixation effects of S-MP and C-MP on subcutaneous tumor tissues.

MATERIALS AND METHODS

Preparation of MP

Materials

Zinc chloride (Kishida Chemical, Osaka, Japan), Japanese Pharmacopoeia Zinc oxide starch powder (Kenei Pharmaceutical, Osaka, Japan), Zinc Oxide (Nacalai Tesque, Kyoto, Japan), D-Sorbitol (Kishida Chemical), Microcrystalline Cellulose (CEOLUS® PH-101, Asahi Kasei, Tokyo, Japan), and Glycerin (Nacalai Tesque) were the chemical reagents used in this study. All chemicals used were of special grade. The compositions of formulas are presented under the materials.

Preparation of O-MP

An aqueous solution of zinc chloride was prepared by dissolving 50 g of zinc chloride in 25 mL of purified water and cooling at room temperature. To prepare O-MP, 25 g of zinc oxide starch powder were gradually added to 25 mL of zinc chloride solution and stirred in a pestle and mixed.

Preparation of S-MP

To prepare an aqueous solution of zinc chloride, 50 g of zinc chloride was dissolved in 25 mL of purified water and cooled. To prepare S-MP, 4 g of sorbitol was added to 25 mL of zinc chloride solution and was completely dissolved; next, 25 g of zinc oxide starch powder was gradually added with stirring in a pestle and mixed.

Preparation of C-MP

To prepare an aqueous solution of zinc chloride, 50 g of zinc chloride was dissolved in 25 mL of purified water and cooled. To prepare C-MP, 12.5 g of zinc oxide and 3.125 g of microcrystalline cellulose were mixed and 8.5 g of macrogol ointment and 2 mL of glycerin were added and kneaded well with a muddler. Then, 50 mL of zinc chloride solution was gradually until a uniform mixture was obtained.

Evaluation of the Water Absorption Properties

MP samples prepared 1 h before use were evaluated. Each MP sample weighed approximately 10 g and was molded onto a 50×50 cm filter paper. Each filter paper was accurately weighed and placed into a petri dish on a Kimwipe soaked in 25 mL of purified water and incubated for 3 h at room temperature for the absorption of purified water.

Test for Water Absorption Properties

After absorbing the water, each MP sample was weighed

Table 1. Formula of Mohs Paste

<table>
<thead>
<tr>
<th></th>
<th>O-MP</th>
<th>S-MP</th>
<th>C-MP</th>
</tr>
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<tbody>
<tr>
<td>Zinc chloride (g)</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Purified water (mL)</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>D-Sorbitol (g)</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zinc oxide starch powder (g)</td>
<td>25</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Zinc oxide (g)</td>
<td></td>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>Microcrystalline cellulose (g)</td>
<td>3.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Macrogol ointment (g)</td>
<td>8.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycerin (mL)</td>
<td>2</td>
<td></td>
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</tr>
</tbody>
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and dried for 120 h under constant temperature (DRYING OVEN® MOV-112, SANYO Electric, Osaka, Japan) set at 80°C. Each MP sample was weighed again after drying, and the amount of water absorbed per gram of each sample was calculated; the water absorption property of each MP sample was then evaluated. For controls, each MP sample without filter paper or purified water absorption was weighed after drying.

Morphology of Each MP Sample after Water Absorption

The morphology of each MP sample at 3 h after water absorption was observed.

Measurement of Hardness of Each MP Sample

The hardness of each MP sample before and after water absorption was evaluated using the press mode of a rheometer (COMPAC-100II® Sun Scientific, Tokyo, Japan). A container with an internal diameter of 28 mm and depth of 12 mm was filled with each MP sample without introducing air bubbles. The hardness of each sample was tested by raising the platform carrying the container filled with each sample at a speed of 1 mm/min and measuring the force detected by the pressure-sensitive shaft when it was pushed 1 mm into the sample.

Mice and Tumor Cell Engraftment

This study was conducted in compliance with the protocol which was reviewed by the Animal Care and Use Committee for School of Pharmacy, Aichi Gakuin University. All the animal experiments were conducted in accordance with the Regulation on Animal Experimentation at School of Pharmacy, Aichi Gakuin University.

Female ICR mice were purchased from Charles River Laboratories Japan (Yokohama, Japan). S180 cells (TKG0173) were purchased from Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer Tohoku University (Sendai, Japan). S180 cells expanded in vitro via intraperitoneal injection into ICR mice. The S180 cells (5×10⁶ cells) were implanted under the back skin of 5-week-old ICR mice; the mice were used for the experiment 3 weeks after implantation. All mice were maintained under a constant 12 h light/dark cycle (light from 8:00 a.m. to 8:00 p.m.), Groups of three mice per cage were given free access to water and solid food.

Evaluation of MP for Tissue Fixation

The mice were shaved at the tumor site using an electric shaver designed for small animals, and 0.5 g of each sample was placed in a ring-shaped container (diameter, 1 cm; height, 1 mm). The skin surrounding the container was protected by applying petroleum jelly and covering with gauze. Isoflurane (for animal use; Mylan, Tokyo, Japan) was used as general anesthesia for the mice. Tumor tissues were imaged 6 and 24 h after sample application using a 3D micro X-ray computed tomography scanner (Cosmo Scan AX®, Rigaku Corporation, Tokyo, Japan) designed for small or medium-sized experimental animals. The depths of tissue fixation and protein denaturation were measured using ImageJ (ver. 1.51) image processing software.

Statistical Analysis

Results are expressed as mean ± standard deviation (S.D.). In a study on the method of preparation, Welch’s t-test was used for comparing continuous variables. Differences between groups were evaluated using non-repeated measure ANOVA followed by Dunnett’s test. Statistical significance was defined as p<0.05. All statistical analyses were carried out using JMP® ver.11.20 (SAS Institute Inc., Cary, NC, U.S.A.).
RESULTS

The amount of water absorbed per gram of MP sample after 3 h of water absorption is shown in Fig. 1. Sample O-MP retained approximately 0.4 g water per 1 g of the sample. Compared with O-MP, samples S-MP and C-MP did not show a significant difference in water absorption. Photographs of each MP sample after water absorption are shown in Fig. 2. Samples O-MP and S-MP were liquefied, which caused the samples to flow from the filter paper. Sample C-MP retained its form even after water absorption. Next, the hardness of each MP sample before and after water absorption was measured (Fig. 3). The hardness of sample O-MP immediately after preparation was 12.6 ± 3.4 N but significantly decreased to 0.01 ± 0.006 N after water absorption. The hardness of sample S-MP before water absorption was 0.5 ± 0.7 N but significantly decreased to 0.01 ± 0.006 N after water absorption. The hardness of sample C-MP before water absorption was 1.8 ± 0.4 N and remained nearly unaffected (1.6 ± 0.3 N) after water absorption.

Each sample was applied to the tumor site of skin cancer-bearing mice, and the tissue status was observed using computed tomography (CT). The image obtained after 6 h of application is shown in Fig. 4(A). In all groups, a high absorption area was observed from the application sites on the tumor surface toward the inner side, and the area of protein denaturation was also confirmed. The image obtained after 24 h of application is shown in Fig. 4(B). Compared to after 6 h of application, degeneration proceeded and the deep region was fixed after 24 h of application in all groups. Next, the tissue invasion depth was measured (Fig. 5). The invasion depths of the O-MP group were 1.27 ± 0.24 mm after 6 h of application and 2.75 ± 0.35 mm after 24 h of application. The invasion depths of the S-MP group were 1.17 ± 0.15 mm after 6 h of application and 2.36 ± 0.37 mm after 24 h of application, and these did not differ significantly from those in the O-MP group (p = 0.95 and 0.17, respectively). The invasion depths of the C-MP group were 1.82 ± 0.48 mm after 6 h of application and 3.52 ± 0.41 mm after 24 h of application. In the C-MP group, the invasion depths after 6 and 24 h of application significantly increased compared with those in the O-MP group (p = 0.011 and 0.005, respectively).

DISCUSSION

The water absorption properties of the two types of formulation-improved MP were evaluated to study their ability to maintain the shape after absorption of the water. In the S-MP and C-MP, the amount of water absorbed did not differ significantly from those in the O-MP immediately after preparation. After absorbing water, the hardness of S-MP decreased and it liquefied like O-MP. In contrast, C-MP retained its form even after water absorption.

Zinc oxide starch powder, a raw material of O-MP, is composed of a mixture of zinc oxide and potato starch at a ratio of 1:1; O-MP contains a starch component of 12.5%. The addition of zinc chloride solution into the zinc oxide starch powder causes it to gelatinize when the starch particles become swollen and disrupted from the influx of moisture. Cloudy gelatinized substances appeared between the particles after 24 h. 17) It is presumed that the water absorption property of O-MP can be attributed to starch and large molecules, including amylose and amylopectin, which were released after the particles were disrupted; however, no gelatinized substances were observed after adding zinc chloride solution after mixing D-sorbitol with zinc oxide starch powder, although there was a slight swelling of starch particles. 17) The water absorption property of S-MP was believed to be derived from the properties of the highly water-soluble compound D-sorbitol, which retains moisture between the starch particles.

C-MP is a preparation in which the starch is replaced by microcrystalline cellulose (MMC) and macrogol ointment. 21) MMC (CEOLUS, PH-101), a polymerized α-cellulose, is an insoluble fiber. The average particle diameter of PH-101 is 50 µm. Therefore, because of its large size, the possibility to spread among the organizations is remarkably low even if MMC is applied to an affected part. Moreover, even if a part of MMC permeates, MMC is retained in a fixed tissue and is removed by excision of tissue, so it is unlikely that it will directly affect the living body.

Macrogol ointment is a water-soluble base with water ab-
sorption properties; therefore, the water absorption property of C-MP may have been greatly influenced by the ointment base. In general, when selecting a drug formulation, those which possess water-soluble bases with high water absorption properties are selected when a large amount of effusion is produced; however, MP bases that dissolve as a result of water absorption cause damage to surrounding tissues when used in clinical treatment. It was presumed that C-MP retained its shape without becoming liquefied even after water absorption because of a cellulose component. On the basis of these findings, the risk of liquefaction-associated damage of the surrounding skin decreased on using C-MP in addition to enabling the preparation in advance.

Regarding the pharmacological action of MP, it has been previously indicated that zinc chloride is ionized because of the presence of moisture and that the tumor tissue, blood vessels, and bacterial cell membranes that cause secondary infection are hardened by the protein agglutination of the zinc ions.24) It has been reported that MP fixes the tissue depending on the application time, and the invasion depth reaches 5 mm from the surface at 24–48 h of application and 10 mm at 72 h of application when the application was made at a thickness of approximately 1 mm on the human tumor surface.1,24) Similarly, in the present study, the depth of the fixed tissue in the mouse tumor in the O-MP group was 1.3 mm at 6 h of application and 2.8 mm at 24 h of application. In the present study, the effect of prescription improvement of O-MP on the pharmacological effect was examined with reference to its tumor tissue invasion depth as an indicator. From the results of the S-MP group, the addition of 4% D-sorbitol to MP did not show any significant effect on the tumor tissue fixation effect.

Kikuchi et al. reported that fibrosis of cancer cells was confirmed by attaching MP at the burst area of breast cancer for 24 h. O-MP was used where the burst area was solidified to a depth of >10 mm from the skin surface, while S-MP was used where the burst area was solidified to the depth of 15 mm.18) Hashiguchi et al. reported that the effect of S-MP was equivalent to that of O-MP based on the fact that the hemostasis effect was obtained at 30 min of application and that the tissue was fixed to a 13 mm depth at 24 h of application in the case in which S-MP was applied to breast cancer disintegrated wound of solid-tubular carcinoma.19) S-MP also showed the same zinc release behavior as O-MP in the in vitro release test.20) Based on the experimental results for tumor in the present study, it was suggested that S-MP has an equivalent tissue fixation effect to that of O-MP.

In contrast, the tissue invasion depth of C-MP was 1.5-times greater after 6 h of application and 1.3-times greater after 24 h of application in comparison with O-MP. In C-MP formulation, starch of O-MP is replaced with microcrystalline cellulose and macrogol ointment to avoid the change of physical properties.21) Then the prescription change level of C-MP is greater than that of S-MP. In the in vitro release test, the zinc release amount of C-MP was 1.3-times greater at 2 h and

Fig. 3. Hardness of Mohs Paste before and after Water Absorption

Each point represents the mean±S.D. of 3 trials. Welch’s t test compared with before absorbing water. *p<0.05, **p<0.01, □: Before absorbing water. ■: After absorbing water.

Fig. 4. CT Images of the Mouse Skin Tumor Tissue after Mohs Paste Application

Cross-sectional view. (A): 6 h after MP application, (B): 24 h after MP application. The white bar indicates 2000 µm.
The invasion depths of tissue fixation at 6 h and 24 h of each sample application were measured. Each column represented mean±S.D. (n=6), Dunnett’s test, *p<0.05, **p<0.01 compared with original formulation Mohs paste.

The above findings suggest that the tissue fixation effect of C-MP was boosted by increase of zinc release rate with change in formulation.

In recent years, the use of MP has been reported in the field of veterinary medicine.\textsuperscript{23,24} Fukuyama \textit{et al.} performed CT scan and confirmed that fixation could occur at up to 10 mm depth after 1 h. of treatment in a canine case of osteosarcoma.\textsuperscript{23} In addition, Sato \textit{et al.} investigated the fixation effect in chicken breast and reported a mean invasion depth of 3.8 mm at 3 h of application.\textsuperscript{27} The speed of Zine invasion and fixation by MP was inferred to be faster in the osteosarcoma tissues with disintegrated wound having a larger quantity of exudate than in the S180 subcutaneous tumor tissue in mice and chicken breast. In general, tumors have weaker cell-binding force than healthy tissues and therefore show characteristically active angiogenesis and large amount of blood flow. It appears that the speed of tissue invasion and fixation varies depending on the cancer type, skin surface condition, exudate leakage, and bleeding situation. Shimizu reported a case of canine breast tumor disintegration in which MP was applied for 48 h for tumor volumetric reduction; when MP flowed out from the applied area to the adhering surrounding tissues, the area where erythema-induced dermatitis was observed was fixed, but the healthy tissue area was not.\textsuperscript{26} Therefore, it was inferred that compared with the disintegrated wound abundant with moisture such as exudate, the tissue fixation effect on the tumor tissues with epidermis and healthy tissues without ulcer or inflammation may be weak.

Based on the abovementioned findings, it was inferred that C-MP has a high tissue fixation effect and its treatment time can be expected to be shorter than that of O-MP. However, the fixed depth per unit time varies according to the state of tumor. Therefore, based on the value obtained in the present experiment, when used in a clinical setting, C-MP application time should be decided with reference to the evaluation of O-MP under the close tumor condition. Moreover, application time is shortened because C-MP is physically stable and clinically easy to handle. Shortening of the total treatment time is expected to reduce the patient’s position restraint time as well as the burden on medical personnel.

**Conflict of Interest**
The authors declare no conflict of interest.

\textbf{REFERENCES}


