Preparation and Evaluation of New Metronidazole Gel Using Hydroxypropyl Methylcellulose

Kazuhiro WATANABE[*1], Seigo NAKAMURA[*2], Toshio SHIMAMOTO[*3], Shunji URAMATSU[*3], Kouzo KISHI[*3], Toshinobu UEMURA[*3], and Hitoshi SHINIKE[*3]

[*1] Pharmacy Practice and Research Center, Showa Pharmaceutical University, 3-3165 Higashi-Tamagawagakuen, Machida 194-8543, Japan
[*2] Breast Surgical Oncology, Breast Center, St. Luke's International Hospital, 9-1 Akashi-cho, Chuo-ku, Tokyo 104-8560, Japan
[*3] Daido Chemical Corporation, 4-4-28 Takeshima, Nishiyodogawa-ku, Osaka 555-0011, Japan

(Accepted on May 18, 2009)

Abstract: We use metronidazole carbopol gel (MTZ-Gel) to manage odor from cancerous skin ulcers in advanced breast cancer patients. However, application to affected areas has presented problems to bonding between the affected areas and gauze, which makes the gauze difficult to remove. Therefore, we developed MTZ-SW Gel with Sangelose® as a raw material, and clinically evaluated its effectiveness and applicability. Overall, our findings suggest that MTZ-SW Gel is effective and clinically useful for treating cancerous skin ulcers. We previously conducted a clinical study of MTZ-SW Gel, and reported its efficacy, safety, and applicability. In this study, to maintain the quality of this preparation, we evaluated its consistency and spreadability by pharmaceutical assessment. MTZ-SW Gel was softer than MTZ-Gel, with a greater spreadability. A stability test of MTZ-SW Gel showed that there were no differences in the MTZ content of this preparation between two conditions, 28 and 40°C, demonstrating its stability over 60 days. On a drug release test, the rates of MTZ release from MTZ-SW Gel and MTZ-Gel after 8 h were 97.6 and 89.7%, respectively, suggesting a higher drug release rate from the base of MTZ-SW Gel.

Key words: hospital preparation, metronidazole, hydroxypropyl methylcellulose, cancerous malodor, breast cancer

Introduction

Cancerous skin ulcers in breast cancer patients are accompanied by malodors caused by infections, pain due to inflammation, as well as bleeding and exudate, which decrease the quality of life (QOL) of patients markedly. Among these, malodors caused by infections (cancerous malodor) are very offensive and distressing symptoms for not only patients themselves, but also their surrounding families and health care professionals.

Contact Address: Kazuhiro Watanabe, Pharmacy Practice and Research Center, Showa Pharmaceutical University, 3-3165 Higashi-Tamagawagakuen, Machida 194-8543, Japan
E-mail: kazunabe@ac.shoyaku.ac.jp

Cancerous malodor is said to be caused mainly by infections of the dead tissues of cancerous lesions by anaerobic bacteria such as *Bacteroides* and *Peptostreptococcus* spp[1-3]. As for the therapeutic drug, external preparations containing metronidazole (MTZ), which has a wide antibacterial spectrum against anaerobic bacteria, as active ingredient are effective[1-3], and the MTZ external preparations are recommended in the guidelines of the World Health Organization[4] and the American Society of Clinical Oncology[5] for the treatment of cancerous malodors. However, since the MTZ external preparations have not been approved in our country, the MTZ external preparations are prepared by their own pharmaceutical departments in many hospitals to apply to the patients[6].

At the St. Luke's International Hospital, MTZ-carbopol 934-P Gel (MTZ-Gel) contained in the 5th edition of Byoin Yakkyoku Seizai[7] has been prepared, and the clinical efficacy, safety and benefit etc. have been reported[8, 9]. However, when dressing the affected area, it was found that to remove the gauze was difficult due to the bouning between the protective gauze coated with MTZ-gel and the affected area. To solve this problem, we developed MTZ-Sangelose Gel (MTZ-SW Gel) using hydrophobized hydroxypropyl methylcellulose (Sangelose®)[10, 11] which was applied as the vehicle of gel for the cosmetics and the quasi-drugs in recent years. The clinical evaluation has been already conducted using MTZ-SW Gel. As the results, the malodor improvement effect of MTZ-SW Gel was equivalent to MTZ-Gel, and it was suggested that MTZ-SW Gel has the possibility of decreasing the irritating sensation which is the adverse event compared to MTZ-Gel. In addition, from the results of evaluation for the impression of use, MTZ-SW Gel was assessed as a "water-retentive" and "easy peeling" preparation, and it was suggested that MTZ-SW Gel is a clinically useful preparation which overcomes the problem of MTZ-Gel that to remove the gauze was difficult due to the bounding between the affected area and the protective gauze.[12]

This time, we conducted the pharmaceutical evaluation on the physical properties of the preparation and the stability of active ingredient to ensure the quality of MTZ-SW Gel preparation as the hospital preparation.

Materials and Methods

1. Raw Materials

For the preparation of MTZ-SW Gel and MTZ-Gel, following raw materials were used: Metronidazole (2-methyl-5-nitroimidazol-1-ethanol, MTZ) (Tokyo Chemical Industry Co., Ltd.); Sangelose® 60L and Sangelose® 90L (Daido Chemical Corporation) as the vehicle of gel; propylene glycol (Maruishi Pharmaceutical Co., Ltd.) and sodium

hydroxide (Koso Chemical) as the solubilizing agent. For Water for injection, the medicinal product contained in the Japanese pharmacopoeia was used. All other reagents used in each test were the guaranteed grade or analytical grade reagents.

2. Preparation of MTZ-SW Gel

MTZ-SW Gel was prepared as follows: 0.8g of MTZ were weighed and were dispersed well with 10 mL of propylene glycol in a mortar. Separately, to 90 mL of water for injection, previously warmed at 70°C, 0.25 g of Sangelose® 60L and 0.75 g of Sangelose® 90L were added slowly and mixed. This solution was cooled to 40°C, and then previously prepared MTZ suspension was added, and mixed thoroughly. MTZ-Gel, which was used as the control preparation in each pharmaceutical test, was prepared according to the method described in the 5th edition of Byoin Yakkyoku Seizai[7] (Table 1).

Table 1 Formula of MTZ-SW · Gel

Rp. 0.8% MTZ- SW · Gel	
2- Methyl -5- nitroimidazol -1- ethanol	0.8 g
Propylene glycol (JP)	10 ml
Sangelose® 60L	0.25 g
Sangelose® 90L	0.75 g
Water for injection	90 ml
Rp. 0.8% MTZ · Gel	
2- Methyl -5- nitroimidazol -1- ethanol	0.8 g
Propylene glycol (JP)	10 ml
Carbopol® 934-P	0.88 g
Water for injection (JP)	90 ml
10% Sodium hydroxide solution	2 ml

3. Experimental Methods

The pharmaceutical evaluation was conducted by performing the consistency and spreadability test, the stability test of the preparation, and the release test of MTZ from the preparation. Thirty grams each of prepared MTZ-SW Gel and MTZ-Gel were filled in plastic containers, stored for 60 days, and used as the sample.

3-1 Consistency

For the measurement of consistency, the penetrometer (Rigo Co., Ltd., JIS) was used.[13-15] The consistency was obtained from the average value of 10 times measurements in which the distance at 5 seconds after the penetration of the penetrometer needle was measured. The consistencies of MTZ-SW Gel and MTZ-Gel were measured immediately after preparation (Day 0).

3-2 Spreadability

For the measurement of spreadability, a spreadmeter (Rigo Co., Ltd., JIS) was used.[13, 15, 16] The spreadability was obtained from the average value of 5 times measurements in which the diameters of spread were measured after 10, 50, 150, 200, 300, 400, 500, 600, 700, 800 and 900 seconds at room temperature. The spreadabilities of MTZ-SW Gel and MTZ-Gel were measured immediately after preparation (Day 0).

3-3 Stability Test

The stability of MTZ-SW Gel was evaluated by observing the changes of appearance immediately after preparation (Day 0) and after 14, 30 and 60 days, and also determining the concentration of MTZ in the MTZ external preparation by using the High Performance Liquid Chromatography (HPLC).[17-20] The preparation procedure of the sample for measurement was as follows: Thirty milligrams each of MTZ-SW Gel were weighed exactly, and a mixture of methanol and water (1:1) containing 200 μg of ranitidine (SIGMA-ALDRICH, USA) as the internal standard was added to make 10 mL. These solutions were sonicated for 5 minutes to make homogeneous. The obtained solutions were filtered with DISMIC®-3JP (pore size: 0.5 μm; Advantec Toyo Kaisha, Ltd.), and 20 μL each of these filtrates were injected into HPLC. The concentration of MTZ was calculated from the average of 5 times results using the calibration curve prepared from the ratio of peak area between MTZ and the internal standard.

HPLC conditions: Pump: HITACHI L-7100 (Hitachi, Ltd.); Detector: HITACHI L-7420 (UV wavelength: 324 nm; Hitachi, Ltd.); Column oven: Shimadzu CTO-10AS (Column temperature: 30°C; Shimadzu Corporation); Column: TSK-GEL ODS-80TM (250 mm x 4.6 mm i.d., 5 μm; Tosoh Corporation); Injection syringe: Hamilton Syringe MICROLITER #705 (Hamilton Compnay, USA); Mobile phase: A mixture of acetonitrile and 0.1 mol/L potassium dihydrogen phosphate (KH_2PO_4) buffer solution, pH 7.4 (1:9); Flow rate: 1.0 mL/min.

3.4 Release Study

The release of MTZ from MTZ-SW Gel and MTZ-Gel was determined using Franz type diffusion cells.[13, 21, 22] A cellulose membrane (cellulose tube size 30/32, Sanko-Jyunyaku Co., Ltd.) was placed in a Franz type diffusion cell, LG-1084-MPC (Laboratory Glass Apparatus, USA) and 0.31 g of newly prepared MTZ-SW Gel or MTZ-Gel was coated onto the membrane in the donor cell. The receptor cell was filled with Ringer's solution (JP) as the solvent, and the cumulative permeated amount of MTZ from the donor

cell to the receptor cell at a temperature of 37°C was determined by HPLC. The samples were collected after 0.5, 1, 1.5, 2, 3, 4 and 8 hours.

Results

1. Consistency

The measurement results of the consistency of MTZ-SW Gel and MTZ-Gel immediately after preparation (Day 0, 28°C) were more than 400 x 10^{-1}mm for MTZ-SW Gel and 330 x 10^{-1}mm for MTZ-Gel, respectively.

2. Spreadability

The measurement results of the spreadability of MTZ-SW Gel and MTZ-Gel immediately after preparation (Day 0, 28°C) were as follows: Y-intercept: 3.08 cm for MTZ-SW Gel and 3.03 cm for MTZ-Gel; slope: 0.58 for MTZ-SW Gel and 0.24 for MTZ-Gel (Fig. 1).

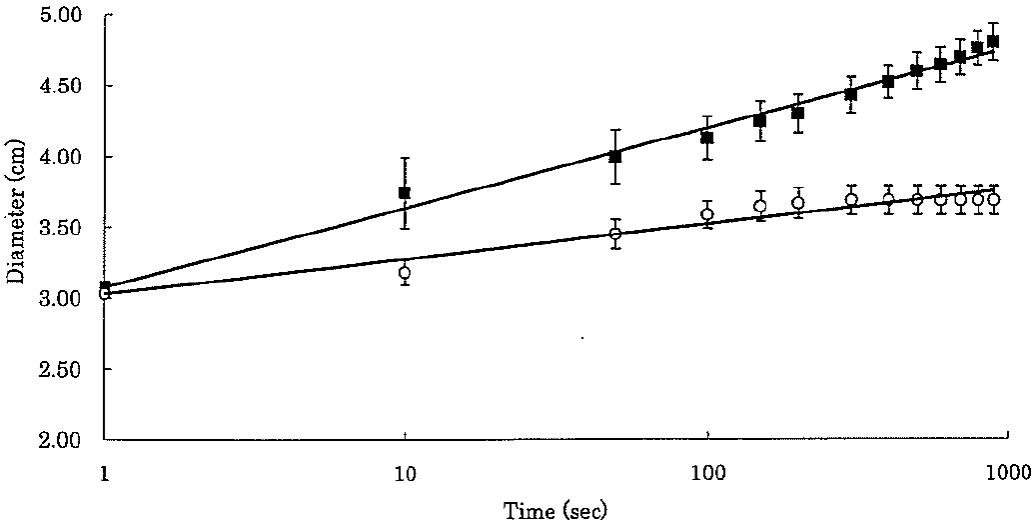

Fig. 1 Spreadability of MTZ-SW · Gel and MTZ · Gel with Spread meter for 0 day at 28℃. The diameter (Y, cm) of the spread was measured (X, s) from 10 s to 900 s using a spread meter. Each point represents mean ± *S.D.* ($n = 5$). ■—■ MTZ-SW · Gel $y = 0.58$ Log $(x) + 3.08$ $r = 0.99$. ○—○ MTZ · Gel $y = 0.24$ Log $(x) + 3.03$ $r = 0.97$.

3. Stability Test

As for the appearance of MTZ-SW Gel, although no change was observed for 60 days when stored at 28°C and 40°C, the crystallization of white needle shapsed crystal was observed after 1 day similar to MTZ-Gel when stored at 4°C. In the stability test of MTZ-SW Gel, the concentration of MTZ in MTZ-SW Gel was determined by HPLC, and

the time-course changes were observed. Almost no change of the concentration of MTZ was observed up to 60 days from preparation in MTZ-SW Gel stored at both 28°C and 40°C (Fig. 2).

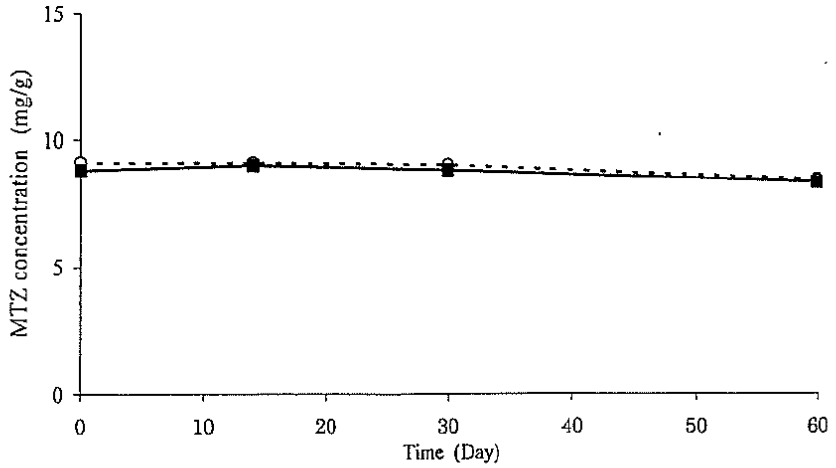

Fig. 2 Stability of MTZ in MTZ-SW · Gel Stored at 28℃ and 40℃. Each point represents mean ± *S.D.* ($n = 5$). ○—○ MTZ-SW · Gel 28℃ ■—■ MTZ-SW · Gel 40℃.

4. Release Study

The cumulative permeation ratio of MTZ at 0.5, 1, 1.5, 2, 3, 4 and 8 hours after were 25.9, 42.2, 56.3, 66.5, 76.1, 82.8, 88.8 and 89.7% for MTZ-Gel, 42.0, 70.4, 82.6, 91.6, 95.4, 97.0, 97.4 and 97.6% for MTZ-SW Gel, respectively. At each measurement time up to 8 hours after preparation, MTZ-SW Gel showed higher value than MTZ-Gel (Fig. 3).

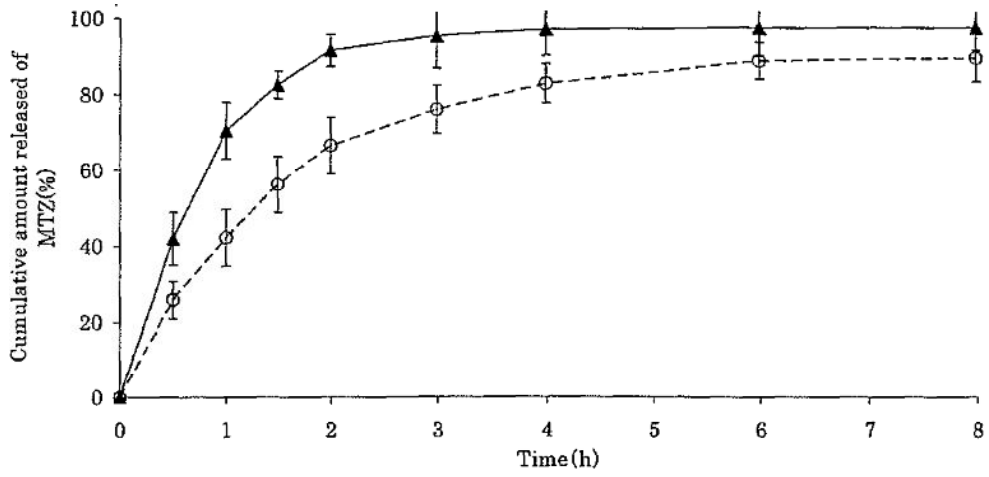

Fig. 3 Permeation of MTZ from MTZ preparation with the static diffusion Franz cell for 0 day. Each point represents mean ± *S.D.* ($n = 5$). ▲—▲ MTZ-SW · Gel ○—○ MTZ · Gel.

Discussion

Hydrophobized hydroxypropyl methylcellulose (Sangelose®), a cellulose derivative, is a gel vehicle which obtained hydrophilic property by introducing a small quantity of long-chain alkyl group (hydrophobic group) to hydroxypropyl methylcellulose. Sangelose® has two types of gel vehicle: Sangelose® 60L which has a fluidity and Sangelose® 90L which has a thickening property. It is able to adjust the hardness, viscosity and spreadability of gel by using the products selectively or together. In addition, they are gel vehicles which have high water retentivity and shape retentivity, and have good compatibility with skin and good impression of use, and they therefore have been applied for cosmetics and quasi-drugs.[10, 11, 23, 24] So we prepared new MTZ gel preparation, MTZ-SW Gel, by using this Sangelose® as the gel vehicle to perform the pharmaceutical evaluation. In addition, for the decision of MTZ-SW Gel formulation, we prepared various gels of which the mixing ratio and the concentration of two types of Sangelose® 60L and 90L were adjusted in order to improve the impression of use of MTZ-Gel which had been so far used until now, and the nurses who had been actually involved caring the cancerous skin ulcer were asked to confirm the impression of use for the gels.

In the results of physical property test, it was found that MTZ-SW Gel is softer and more spreadable than MTZ-Gel. In the results of stability test, no changes were observed up to 60 days under the storage conditions of 28°C and 40°C, and it was confirmed that MTZ-SW Gel is stable same as MTZ-Gel. From the results of the release study, MTZ-SW Gel showed greater value when compared with MTZ-Gel, suggesting that the releasability of MTZ from the vehicle is high. In the future, we would like to clarify the reason why MTZ-SW Gel could overcome the problem of MTZ-Gel that to remove the gauze was difficult due to the bouning between the affected area and the protective gauze from the view point of the water retentivity and shape retentibity of the gel in the fundamental experiments using the models, etc.

In addition, although the results are not shown in this report, it is noted as the characteristic of MTZ-SW Gel that the preparation of gel is easy. The time required to prepare MTZ-SW Gel is about 30 minutes, and it can be shortened to 1/4 when compared with about 120 minutes required for MTZ-Gel which have been prepared conventionally, and we consider that it leads to efficiency improvement of businesses for the pharmaceutical department preparing the hospital preparations.

From the above, we consider that MTZ-SW Gel is assured the same pharmaceutical

quality as MTZ-Gel in the pharmaceutical evaluation. We would like to increase the number of cases in the clinical evaluation in the future to study the evaluation of usefulness including the QOL of the patients.