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Preparation and Phase II Clinical Examination of Topical Dosage Forms for the Treatment of *Carcinoma Colli* Containing Bleomycin, Carboquone, or 5-Fluorouracil with Hydroxypropyl Cellulose[1,2]

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With the aim of developing a dosage form for the treatment of *carcinoma colli*, stick-like preparations containing bleomycin hydrochloride (BLM), carboquone (CQ), and 5-fluorouracil (5-FU) held in a mixture of hydroxypropyl cellulose (HPC) and Carbopol 934 (CP) were prepared and clinically tested in volunteers suffering from *carcinoma colli* after various *in vitro* tests. The results of preliminary tests of drug release using the agar gel bed method indicated that the addition of sodium lauryl sulfate enhanced the release of CQ, but the effect was not very great. Therefore, in order to enhance the release of CQ, the contents of HPC in the base and CQ were increased. The preparations of BLM and 5-FU of 2 mm diameter showed faster drug release than those of 4 mm diameter according to the Kerami filter method. In the preparations of 4 mm diameter, the release of CQ took place at almost the same rate as that of BLM, *i.e.*, about 40% within 24 hr, due to the modification of the formula for the preparation of CQ. In the case of the preparation of 5-FU, the release was so rapid that about 100% of the drug was released within 24 hr. The present Kerami filter method seemed suitable and convenient for measuring the drug release from the present dosage forms.

Clinical examination indicated the stick-like shape of the present dosage form to be favorable for the treatment of foci in the cervical canal. A high percentage of complete disappearance of the cancerous focus was obtained for patients of stage 0 in the cases of BLM and 5-FU, and a similar result was obtained for stage Ia in the case of CQ.

Keywords——topical dosage form; *carcinoma colli*; clinical examination; hydroxypropyl cellulose; bleomycin; carboquone; 5-fluorouracil; releasing property; local therapy; sustained release

In a previous paper,[4] the authors reported that a newly developed topical disk-like dosage form, which was prepared by direct compression of a mixture of bleomycin hydrochloride (BLM) and a combination of hydroxypropyl cellulose (HPC) and Carbopol 934 (CP), might be suitable for use as a treatment of *carcinoma colli* in the early stages.

The above disk-like dosage form, which can usually be applied to the *portio vaginalis*, seems inconvenient for treatment of the remnant foci often found inside the cervical canal because the released drug does not reach the target area effectively. Therefore, in the present study we attempted to prepare stick-like preparations containing BLM, carboquone (CQ), or 5-fluorouracil (5-FU) for treatment of the lesion in the cervical canal, on the basis of *in vitro* data concerning the effect of additives on the release of the drugs. We also carried out clinical examination in volunteers suffering from *carcinoma colli* as in the previous study.[4]

1) This paper forms Part XVII of "Pharmaceutical Interactions in Dosage Forms and Processing." Preceding paper, Part XVI: Y. Machida and T. Nagai, *Chem. Pharm. Bull.*, **28**, 1082 (1980).
2) A part of this work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April 1978.
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4) Y. Machida, H. Masuda, N. Fujiyama, S. Ito, M. Iwata, and T. Nagai, *Chem. Pharm. Bull.*, **27**, 93 (1979).

Experimental

Materials——Hydroxypropyl cellulose-H (HPC), Carbopol 934 (CP),[5] agar powder J.P.IX, and bleomycin hydrochloride J.P.IX (BLM) used were the same materials as described in the previous paper.[4] Sodium lauryl sulfate J.P.IX (SLS) and macrogol 4000 J.P.IX (MCG) were obtained commercially. Carboquone (CQ) and 5-fluorouracil (5-FU) were supplied by Sankyo Co. Ltd., and Teijin Co. Ltd., respectively.

Preparation of Dosage Form——Stick-like preparations 40 mm in length and with a diameter of 2 mm (150 mg) or 4 mm (300 mg) were made by compressing the mixed powder in a specially designed set of die and punches, shown in Fig. 1. The upper and the lower punch were of 35 mm and 10 mm, respectively, in height. The upper and lower punch contained 10 and 20 parallel grooves of semiellipsoidal section, 4 mm and 2 mm in diameter, for the preparation of 4 mm and 2 mm diameter sticks, respectively. The stick of 4 mm diameter provided sustained release of the drug as will be described later.

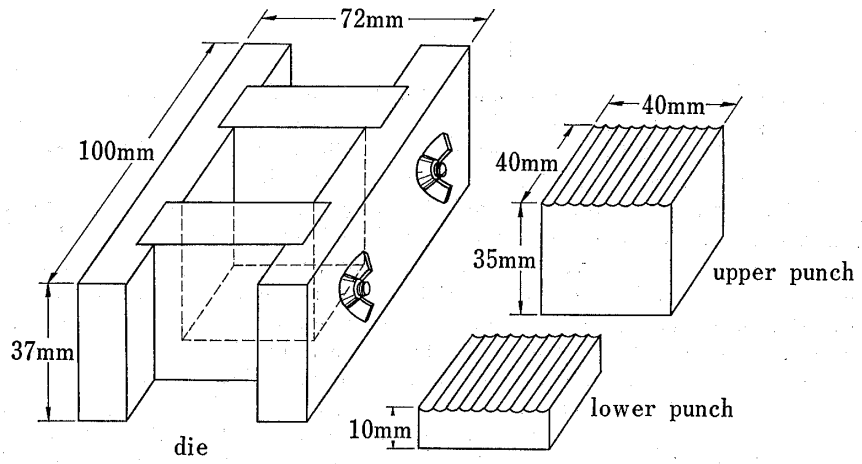

Fig. 1. Apparatus for making Stick Preparations

Evaluation of *in Vitro* Drug Release from Preparations——a) Agar Gel Bed Method: The stick preparation was inserted into the center of a 20 ml gel bed of 1% agar in saline in a test tube of 15 mm diameter. The top of the stick was located 10 mm below the surface of the agar bed. The test tube was kept in an incubator at 37±1°, and the stick was removed after 6, 12, 24, 48, and 72 hr. The agar gel bed was then homogenized for 5 min in 40 ml of acetone–$CHCl_3$ (2:1) using a Nihon Seiki homogenizer. The homogenized mixture was centrifuged in a glass-stoppered centrifuge tube for 10 min at 3000 rpm. An aliquot (2 ml) of the acetone–$CHCl_3$ layer was transferred into a 10 ml volumetric flask and the solvent was evaporated off at 40° under a nitrogen stream. The residue was dissolved in methanol (up to 10 ml). The concentration of CQ in methanol was determined spectrophotometrically at 330 nm using a Hitachi 124 spectrophotometer by reference to a calibration curve.

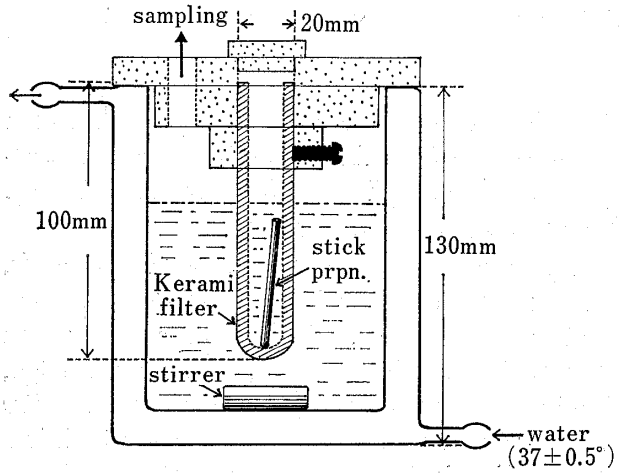

Fig. 2. Apparatus used in the Drug Release Test by the Kerami Filter Method

b) Kerami Filter Method:[6] The apparatus shown in Fig. 2 was used following the report of Sakurai *et al.*[7] The stick was put in a Kerami filter set in 400 ml of saline stirred with a magnetic stirrer at 37±0.5°. Aliquots (5 ml) of the saline were pipetted out at intervals and the concentration of drug was determined spectrophotometrically using a Hitachi 323 spectro-

5) Carboxypolymethylene, product of B.F. Goodrich Chemical Co.
6) Product of Koshin Rikagaku Co.; porous cylindrical filter made mainly of Al_2O_3, 100 mm in length, inner and outer diameters of 12 mm and 20 mm, respectively.
7) S. Sakurai, N. Nambu, and T. Nagai, at the 98th Annual Meeting of the Pharmaceutical Society of Japan, Okayama, April 1978.

Table I. Stick Preparations used in Clinical Examination

Preparation	(diameter)	Drug content (mg)	Total weight (mg)	Vehicle (HPC : CP)
BLM	(2 mm)	25	150	3 : 1
	(4 mm)	50	300	3 : 1
CQ	(2 mm)	6	150	5 : 1
	(4 mm)	12	300	5 : 1
5-FU	(2 mm)	75	225	3 : 1
	(4 mm)	150	450	3 : 1

photometer by reference to a calibration curve at 290 nm, 335 nm and 265 nm for BLM, CQ, and 5-FU, respectively.

Clinical Application——The stick preparations shown in Table I were tested clinically by insertion into the cervical canal of voluntary patients, prior to surgical operations. As clinical circumstances required, two sticks or doubled sticks were used. Figure 3 shows the sticks inserted into the cervical canal of a patient (originally two doubled sticks). The effect of the treatment was evaluated as described in the previous paper.[4]

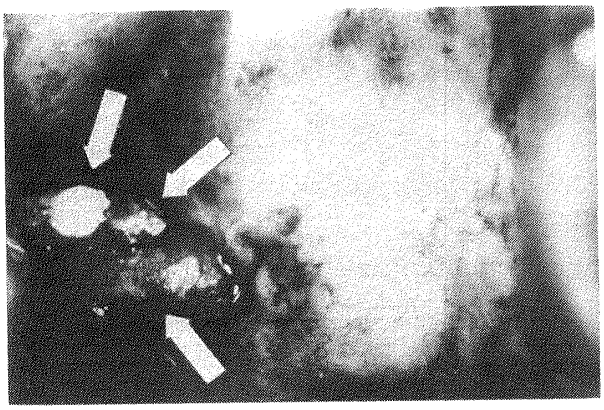

Fig. 3. Sticks inserted into the Cervical Canal of a Patient

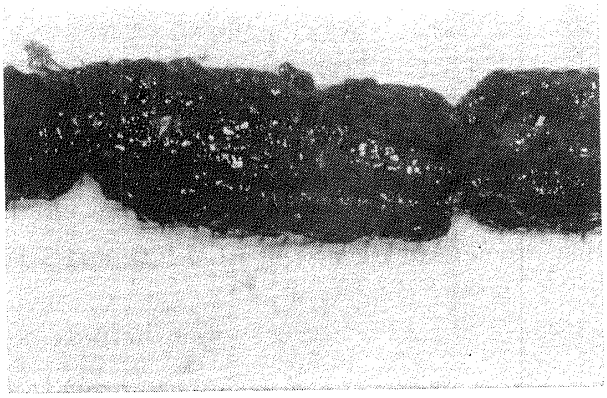

Fig. 4. Swollen Stick removed from a Patient after 4 Days

Results and Discussion

Effect of Water-Soluble Additives on the Release of CQ, a Slightly Soluble Drug

BLM, which was chosen in the previous work as a suitable active ingredient,[4] is a water-soluble antibiotic and specifically attacks the cell at the G_2 and M stages of the cell cycle, while CQ is almost insoluble in water and acts as an alkylating agent. It shows cell killing activity regardless of the stage of the cell cycle; the activity is dependent on the concentration of the drug and the period of contact with cancer cells, as in the case of BLM.

Figure 4 shows a stick of 3 mg of CQ in 147 mg of a mixture of HPC and CP (3: 1),[8] which was removed from a patient after 4 days. The stick was swollen with secreted fluid but the shape of the preparation was fairly well maintained. The dull violet color of the swollen preparation indicated that some CQ remained.

Although the sustained release property is an advantage of the present dosage form, excessively slow and incomplete release of the drug may result in a decrease of therapeutic effect. Therefore, it is necessary to investigate how to enhance the release of a slightly soluble drug such as CQ. For this purpose, the use of an additive seemed effective. For example,

8) An increase in the amount of HPC enhances the release of the drug[4] and improves the molding properties of the thin stick preparation.

MCG, which is readily soluble in water compared with HPC and CP, is considered to form pathways in the swollen preparation to accelerate the release of CQ. Apparently, however, the addition of MCG did not enhance the release of CQ at the concentration examined. This might be because the pathways formed by the dissolution of MCG become filled by the gel-like HPC formed, thus being ineffective for the release of CQ.

Table II. Release of CQ from Stick Preparations containing SLS as measured by the Agar Gel Bed Method

Concentration of SLS (%)	CQ released (mg)				
	6 hr	12 hr	24 hr	48 hr	72 hr
0	0.40	0.54	0.55	0.62	0.72
1	0.42	0.56	0.62	0.64	0.75
2	0.46	0.61	0.69	0.70	0.80

On the other hand, SLS, a surface-active agent, enhanced the release of CQ, as shown in Table II. The enhancement of release by the addition of SLS might be due to the solubilizing effect of SLS. However, the effect was not marked at the concentration of SLS examined. The addition of a large amount of SLS seemed undesirable for clinical use because the evaluation of the antitumor agent for local therapy might be complicated by the effect of SLS on the diseased part. Finally, therefore, in order to enhance the release of CQ for clinical use, the composition of the vehicle was changed to 5: 1 (HPC: CP)[8] and the content of CQ was increased to 6 mg per stick, as shown in Table I, without using SLS.

Measurement of Release of BLM, CQ, and 5-FU by the Kerami Filter Method

In addition to BLM and CQ, 5-FU was used as an active ingredient; it is a metabolic inhibitor of type II whose cell killing activity depends on the period of contact with cancer cells[9] and which specifically attacks the cell at the S stage of the cell cycle.

Measuring the release of drug by the agar gel bed method required not only a number of agar beds but also a long period for measurement. The U.S.P. rotating basket method also

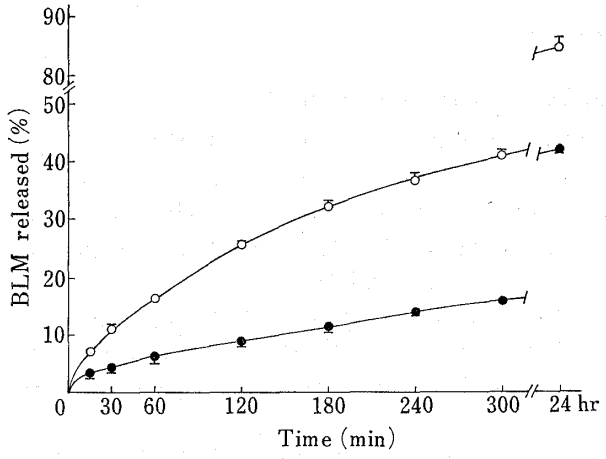

Fig. 5. Release Pattern of BLM from Preparations of 2 mm (○) and 4 mm (●) Diameter as determined by the Kerami Filter Method

Each point represents the mean ± S.D. of 5 determinations.

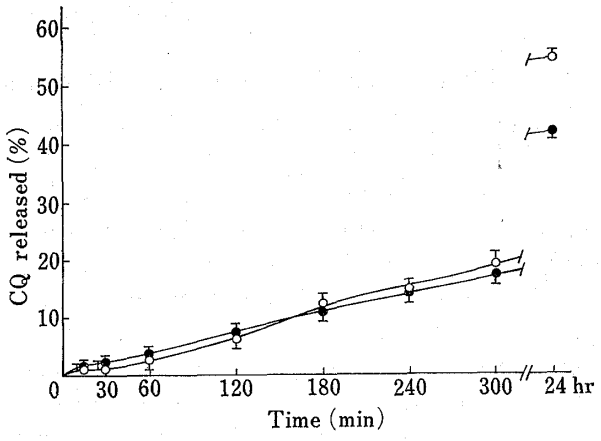

Fig. 6. Release Pattern of CQ from Preparations of 2 mm (○) and 4 mm (●) Diameter as determined by the Kerami Filter Method

Each point represents the mean ± S.D. of 5 determinations.

9) *a*) M. Shimoyama, *Saishin Igaku*, **28**, 850 (1973); *b*) M. Shimoyama and K. Kimura, *Saishin Igaku*, **28**, 1024 (1973); *c*) M. Shimoyama, *Gan to Kagakuryoho*, **3**, 1103 (1976).

seemed unsuitable for the present stick preparation because the swollen stick spread in the basket, modifying the stirring conditions.

On the other hand, the Kerami filter method seemed to avoid these problems, giving good reproducibility of data in a comparatively short period. Figures 5, 6, and 7 show the releasing patterns from 2 mm and 4 mm sticks of BLM, CQ, and 5-FU, respectively. In the cases of BLM and 5-FU, the amount of drug released was larger from the 2 mm diameter preparation than from the 4 mm diameter one. However, in the case of CQ, there was no marked difference between the preparations, except at 24 hr. This result was considered to be due to the difference in water solubility, *i. e.*, the former two drugs are soluble and the latter is only slightly soluble. The small diameter preparation was considered to take up water rapidly, resulting in a rapid release of such water-soluble drugs as BLM and 5-FU.

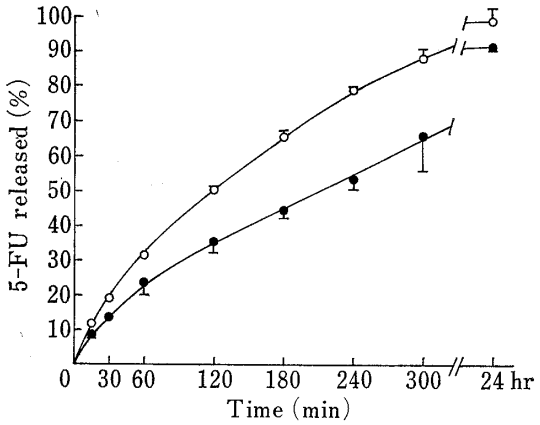

Fig. 7. Release Pattern of 5-FU from Preparations of 2 mm (○) and 4 mm (●) Diameter as determined by the Kerami Filter Method

Each point represents the mean±S.D. of 5 determinations.

The volume of surrounding fluid in the Kerami filter method is quite large compared with the *in vivo* condition, and the viscosity in the former case is low compared with that of the fluid secreted from the glands in cervical canal. Moreover, the preparation may be subject to some disturbance in the cervical canal. In fact, however, it was possible to remove the preparation from the patient after 4 or 5 days in a swollen state, enlarged to about 2-fold in diameter, but retaining its shape, as shown in Fig. 4. Thus, the release of drug as measured by the Kerami filter method might be comparable to that *in vivo*.

Concerning the preparations of 4 mm diameter, the release of CQ took place at almost the same rate as that of BLM, due to the modification of the formula for the preparations of CQ mentioned above. In the case of the preparation of 5-FU, the release was so rapid that about 100% of drug was released within 24 hr. If such rapid release of 5-FU is undesirable for clinical use, it may be possible to adjust the release rate by lowering the content of 5-FU or by increasing the amount of CP.

Practically, the thick preparation of 4 mm diameter usually seemed preferable to the thin one of 2 mm diameter as regards sustained release, strength, and easiness of preparation. However, the thin stick was also used when clinically desirable.

It was concluded that the present Kerami filter method was suitable and convenient for measuring the release of drugs from the present dosage form, because the data could be obtained in a short time and showed good reproducibility.

Effect of Unevenness of Compressional Force on Drug Release

The stick preparations were made by compressing the mixed powder in a die 4 cm×4 cm square, resulting in some unevenness of density in the preparation. This unevenness was detected as a lack of uniformity in color in the case of the preparations of CQ, which is red, and the apparent unevenness could be represented by the ratio of the dark regions of the stick. Regarding the correlation between the ratio and the amount of drug released for the initial 60 min, the latter decreased with increase of the former; this was statistically significant at the 5% level by the t-test ($r=0.588$). However, the absolute decrease of the release was only 1.4—2.6%, and this seems negligible in view of the conditions of application of the present preparation.

Table III. Results of Clinical Application of Preparations of BLM, CQ, and 5-FU to Voluntary Patients with *Carcinoma Colli* at Stages 0 and Ia

Stage	Drug	No. of patients	Dose in total (mg)	Disappearance[a] of focus	% disappearance
0	BLM	8	25— 275	5	63
	CQ	4	18— 30	1	25
	5-FU	4	300—1350	4	100
Ia	BLM	10	50— 325	2	20
	CQ	3	18— 42	2	67
	5-FU	3	1200—1500	1	33

a) Complete disappearance of the cancerous focus in the *cervix uteri* extirpated from patients after local therapy using a stick preparation.

Clinical Evaluation of Effectiveness of Preparations

The results of clinical application of the preparations of BLM, CQ, and 5-FU are shown in Table III; only the total doses are given without mentioning whether the thin preparation or the thick one was used, because the clinical treatment varied with circumstances. In the cases of BLM and 5-FU, a high percentage of complete disappearance of the cancerous focus was obtained for patients of stage 0. In the case of CQ, a high percentage of disappearance was obtained for stage Ia. The results that BLM and 5-FU were effective at stage 0 while CQ was effective at stage Ia may be attributable to a difference in tissue affinity or in the mechanism of cell killing action between the drugs, as the former two are water-soluble and the latter is only slightly soluble.

The application of the preparation of 5-FU gave 100% disappearance of the focus for patients of stage 0. The cell killing action of 5-FU is known to manifest itself on contact with cancer cells for a long period at a comparatively low concentration.[9] Although the present preparation of 5-FU showed very rapid release (Fig. 7), a continuous release of a small amount of 5-FU may take place after that rapid release, resulting in a high percentage disappearance of the cancerous focus. Further investigations are required to confirm this and to improve the formula of the preparation.

The percentage disappearance of the cancerous focus upon application of suppositories of BLM with Witepsol into the cervical canal was reported as 54% for stage 0 and 6% for stage Ia.[10] Thus, the percentage of complete disappearance was apparently improved by use of the present dosage form of BLM. Moreover, the appearance of side effects was greatly decreased in the present case compared with the above report, as vaginal erosion was observed in 7 of 42 cases in the previous work, but in only 2 of 32 in the present study.

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10) H. Masuda, Y. Sumiyoshi, and Y. Shiojima, at the 14th Congress of the Japan Society for Cancer Therapy, Sendai, Oct. 1976.