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# BLEOMYCIN-LIPIODOL SUSPENSION FOR INTRALYMPHATIC ADMINISTRATION IN ESOPHAGEAL CANCER

(bleomycin suspension / releasability / cancer chemotherapy)

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Bleomycin (BLM)-Lipiodol suspension was prepared for intralymphatic chemotherapy of esophageal cancer. The suspension was prepared by dispersing BLM directly into lipid contrast medium, Lipodol. One half of the suspension was then ultrasonicated. The dispersibility, physico-chemical stability and releasability of BLM in sonicated suspension were compared in vitro with those of non-sonicated suspension. The clinical availability of sonicated suspension was also evaluated. BLM in sonicated suspension was kept dispersed more uniformly as smaller particles than that in non-sonicated suspension for at least 3 days after the preparation. BLM in these suspensions was fairly stable under storage at room temperature for 3 days, showing the residual intact drug contents at higher than 96%. The in vitro release of BLM was so slow as to show only about 15 and 20% released from both suspensions in 60 min at 50 and 100 rpm, respectively. Under any rotatory condition, the release of BLM was or tended to be rather sustained from the sonicated suspension. After intralymphatic injection of the sonicated suspension, BLM was transported into regional lymph nodes and was detected at a considerable level ranging from 0.1 to 23.0 μg/g. BLM levels in thoracic and abdominal lymph nodes tended to be higher than those in cervical lymph nodes. These findings suggest that BLM-Lipiodol suspension may be a useful preparation for the targeting chemotherapy to esophageal cancer.

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In surgical treatment for esophageal cancer, adequate and complete dissection of metastatic lymph nodes in the upper mediastinum is generally difficult. Therefore, chemotherapeutic agents have been used as adjuvant therapy to surgery for the

prevention or delay of further recurrence. To enhance the efficiency in cancer chemotherapy, it is important to deliver anticancer agents in sufficiently high concentration to both primary growth and metastasis with minimal side effects.

Takahashi et al. (1-3) have reported that anticancer agents, when adiministered topically in the form of fat emulsion prepared with sesame oil, are distributed in regional lymph nodes and are retained there in high concentration over a relatively extended period. It was experimentally demonstrated that the topical injection of a drug in emulsion into lymphatic tissue enhanced drug accumulation and prolonged its retention within drainage lymph nodes (4-6). Natsuda (7) detected bleomycin (BLM) in lymph nodes dissected from the esophageal cancer patients who were intraoperatively given its emulsion, which was prepared with sesame oil, into the tracheal bifurcation lymph nodes. Although the utilization of fat emulsions would be promising to facilitate drug transportation into lymph, physical instability of the preparation is often disadvatageous to a wide clinical application. We have reported recently that adriamycin suspension perpared with lipid lymphographic agent, Lipiodol, has an excellent physico-chemical stability in vitro and retain adriamycin in the tumor tissue for a prolonged period after intraarterial administration to the patients with hepatocellular carcinoma (8).

In the present work, we prepared the suspension of BLM in Lipiodol, investigated the physico-chemical stability and drug releasability of the suspension, and carried out clinical application of this preparation to patients with esophageal cancer.

## MATERIALS AND METHODS

### Materials

Lipiodol Ultra-Fluide (Lipiodol), an ethyl ester of the fatty acid of poppyseed oil containing 38% iodine by weight, was purchased from Kodama Co., Ltd., Tokyo, Japan. BLM hydrochloride was purchased from Nippon Kayaku Co., Ltd., Tokyo, Japan.

### Preparation of Suspension

Suspension was prepared by pulverizing BLM (150 mg as potency) and by directly mixing with Lipiodol (10 ml) in mortar

and pestle. The mixture was either ultrasonicated at 20 KHz for 3 min by Sonifier (Branson Co., USA) in an ice bath (sonicated suspension) or not (non-sonicated suspension). Each preparation was distributed in ampoules and sealed. All of these procedures were carried out on the clean bench (class 100) in biological clean room.

Examination of physical stability

Green diameter of BLM particles in each suspension was microscopically determined. One ml of each freshly prepared suspension was loaded into glass cylinder (2.55 × 300 mm) which was commonly used for erythrocyte sedimentation test, and was allowed to stand for 3 days. Dispersibility of the suspension was evaluated by measuring periodically the thickness of precipitated BLM particles in this glass cylinder.

Determination of BLM content

Sonicated and non-sonicated suspension were stored at room temperature (26 - 28°C) for 3 days. The content of BLM during the storage was determind by bioassay after extraction with distilled water from the suspension.

Drug release

Drug release from the suspension was investigated by the rotating basket method of dissolution test based on JP XI. The dissolution test apparatus used was Toyama NTR-5S, Osaka, Japan. One ml of the freshly prepared suspension was gently introduced into the basket (36 mesh), which was agitated at 50, 100 or 200 rpm in 500 ml of isotonic phosphate buffer solution (pH 7.4, 37 ± 0.5°C). Four ml of the buffer solution was withdrawn at appropriate time intervals and equal volume of the fresh buffer solution was added to maintain the initial volume. Concentrations of BLM released in the buffer solution (4 ml) which were centrifuged for 10 min at 3000 rpm were determined spectrophotometrically at 290 nm. The amount of drug released was expressed as a percentage of total absorbance corresponding to whole drug in the sample. There was no degradation of BLM during this test.

Clinical application

Sonicated suspension was used for the chemotherapy in two

patients (61 and 74 years old, male) with resectable esophageal cancer, who gave their informed consent to receive surgical operation. One ml of the suspension (15 mg BLM as potency) was intraoperatively administered into the bifurcation lymph nodes immediately after thoracotomy, and then esophagectomy and lymph node dissection were performed within 2 h after intralymphatic injection. The content of BLM in the dissected specimen was determined by bioassay method after extraction with 7.5% trichloroacetic acid solution from the homogenized tissue.

Assay methods of BLM

An antimicrobiological activity assay was used for the determination of BLM in the suspension and the lymph nodes. The paper disc method using *Bacillus subtilis* PCI-219 as test microorganism was employed for BLM assay (9).

## RESULTS

Physico-chemical properties of the suspension

Figure 1 shows photomicrographs of sonicated and non-sonicated suspension immediately after the preparation. In the sonicated suspension, the size of BLM particles was mostly smaller than 9 μm and almost uniform dispersion of BLM was obtained in Lipiodol. In contrast, non-sonicated suspension consisted of relatively large particles together with the particles smaller than 9 μm and involved some coagulation among particles. Figure 2 compares particle size distribution of BLM between sonicated and non-sonicated suspensions. In the sonicated suspension, almost 95% of all particles consisted of those smaller than 9 μm. On the other hand, the non-sonicated suspension showed rather wide size distribution such as 68.5, 23.5 and 8.0% for the particles smaller than 9 μm, ranging from 10 to 29 μm, and larger than 30 μm, respectively. Thickness of the precipitated particle layer was measured in order to evaluate dispersibility of BLM in the suspension more quantitatively, as summarized in Table 1. The sedimentation of BLM particles in sonicated suspension was almost negligible after 1 to 3 days, showing only about 1 mm thickness of the sedimentary layer 3 days after the preparation. The sonicated preparation seemed to keep relatively good suspensibility in Lipiodol even after 3 days. On the other hand, non-sonicated suspension caused particle

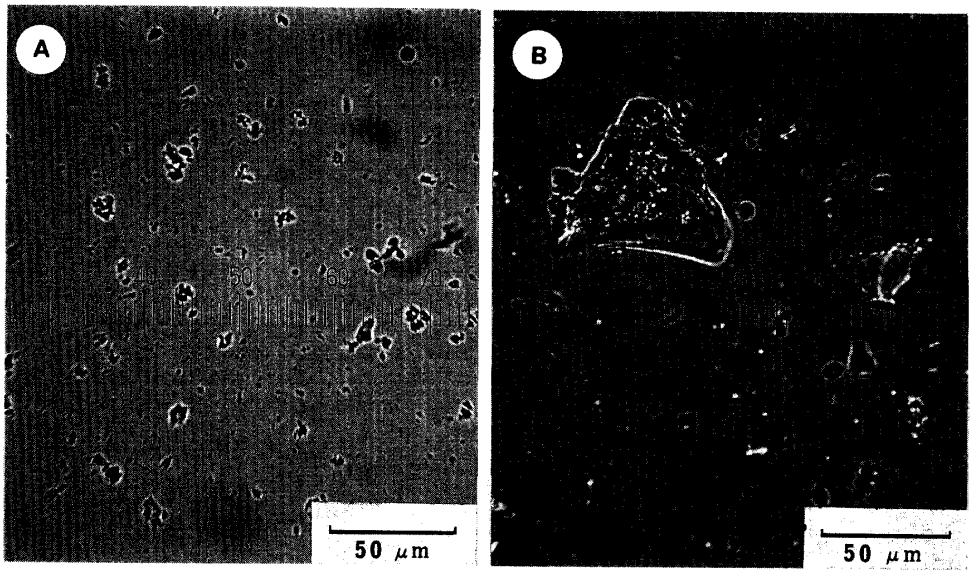


Fig.1. Microphotographs of freshly prepared BLM-Lipiodol suspension with (A) and without (B) sonication

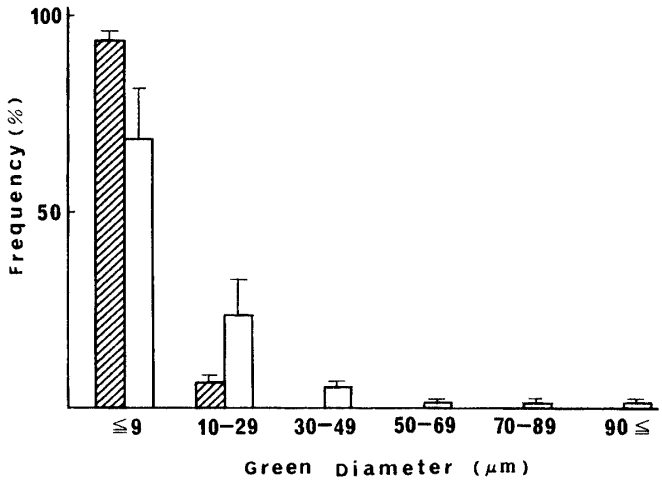


Fig.2. Particle size distribution of BLM in the sonicated (▨) and non-sonicated suspension (□)

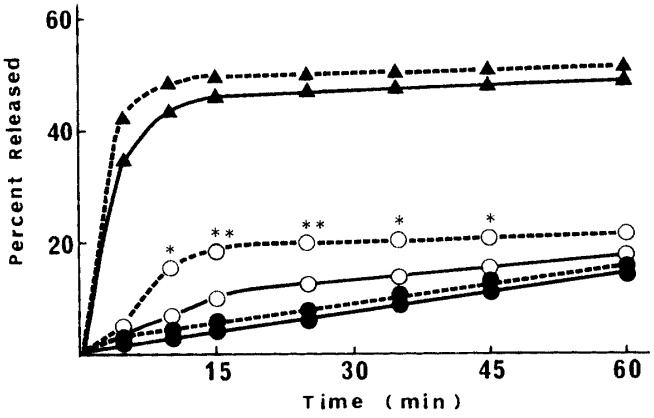


Fig.3. Release profile of BLM from the sonicated (——) and non-sonicated suspension (----) at 50 (●), 100(○) and 200 rpm (▲)
Each point represents the mean of 5 experiments. There were significant differences at 100 rpm between the sonicated and non-sonicated suspension by $t$-test (*$P$<0.05,**$P$<0.01).

**Table 1.** Thickness of sedimentary layer for BLM in the suspension

| Suspension | Thickness (mm) | | |
|---|---|---|---|
| | 1 day | 2 days | 3 days |
| Sonicated | 1.1 ±0.2 | 1.2 ±0.2 | 1.2 ±0.2 |
| Non-sonicated | 12.8 ±3.3 | 15.1 ±3.5 | 15.5 ±3.4 |

Each value represents the mean ± S.D. of 5 experiments.

**Table 2.** Stability of BLM in the suspension at room temperature

| Suspension | Residual percent of BLM | | |
|---|---|---|---|
| | 1 day | 2 days | 3 days |
| Sonicated | 104.5 ±6.6 | 101.1 ±5.1 | 100.8 ±3.0 |
| Non-sonicated | 99.4 ±8.5 | 95.6 ±5.5 | 97.3 ±6.8 |

Each value represents the mean ± S.D. of 5 experiments.

**Table 3.** BLM level in dissected lymph nodes after intralymphatic injection of the sonicated suspension

| Location of lymph node | No. of lymph node | Mean ( Range ) μg/g |
|---|---|---|
| Cervical | 9 | 0.45 (0.10 - 1.05) |
| Thoracic | 23 | 4.50 (0.13 - 23.00) |
| Abdominal | 5 | 6.51 (0.12 - 12.44) |

Lymph nodes were dissected within 2 h after intralymphatic injection.

sedimentation so rapidly and extensively that it resulted in remarkable phase-separation after 3 days. The thickness of sedimentary layer was 12.8 mm after 1 day and 15.5 mm after 3 days.

The stability of BLM in the suspension during storage is shown as percentage of the initial potency in Table 2. There was no significant decrease of the drug potency either in sonicated suspension or in non-sonicated suspension, which retained more than 96% of the initial potency at room temperature after 3 days.

Figure 3 shows the release profiles of BLM from the suspension at 3 different rotation speeds in the basket. The release of BLM from sonicated or non-sonicated suspension seemed to be relatively sustained. Both suspensions showed only about 15% release in 60 min at 50 rpm. At 100 rpm, the release of BLM was more sustained from the sonicated suspension than from non-sonicated preparation and the release from each suspension was about 20% or less in 60 min. In contrast, at 200 rpm, about 50% of the drug was released from both suspensions within 60 min, and the release from sonicated preparation tended to be rather sustained.

Clinical application of the sonicated suspension

In 2 patients with esophageal cancer, 15 mg of BLM as the sonicated suspension was intraoperatively administered into the tracheal bifurcation lymph node. BLM was detected in 37 of 41 (90.2%) dissected lymph nodes within 2 h after the administration. BLM level was below the detection limit only in each two cervical and abdominal lymph nodes. Table 3 shows the mean BLM level and its range in the dissected lymph nodes. Nine cervical lymph nodes showed the mean BLM level of only 0.45 μg/g, whereas 23 thoracic lymph nodes showed 4.50 μg/g and 5 abdominal lymph nodes 6.51 μg/g. Neither remarkable complication nor side effects was encountered in any case.

## DISCUSSION

In surgical operation of esophageal cancer, complete resection of metastatic lymph nodes was difficult due to anatomical restrictions or operative injury. Particularly the upper mediastinum is not usually resectable. Therefore anticancer agents have been often administered systemically to compensate the limitation of the surgical operation. However, the systemic dosage was unfavorable due to relatively low level and short residence of the drug at tumor site as well as high incidence of toxic reactions. To improve these points, local (or topical) injection to target the chemotherapeutic agents as some sustained-release forms has been favored recently. The lipid lymphographic agent, Lipiodol, had been found to remain selectively in tumor tissue for a prolonged period (10-12). On the basis of this finding, anticancer agents emulsified in

Lipiodol have been applied to clinical trials and proved to be effective in the targeting of chemotherapy for cancer of the digestive organs (13,14). With adriamycin, the suspension was found to be much better than the emulsion for the adequate physico-chemical properties and clinical efficacy as well (8).

In the present study, we aimed to prepare better BLM-Lipiodol suspension applicable to the intralymphatic injection by prolonging the efficacy and minimizing its toxicity. Particularly, the effect of ultrasonication was examined on the physico-chemical properties of the suspension. Sonicated suspension consisted of much more micronized drug particles and gave better and more uniform dispersibility than non-sonicated preparation, so that only slight sedimentation occurred after standing for 3 days. In practice, we could redisperse the slightly precipitated BLM in the sonicated suspension readily by an instant shaking. Hence it appears that ultrasonication plays a role not only to micronize the drug particle but also to prevent the coagulation among particles. Severe sedimentation of the non-sonicated drug particles was thought to be inadequate for the intralymphatic application. From these results, it was suggested that the sonicated suspension might be a favorable preparation for clinical use.

There were no reports on the stability of BLM in Lipiodol. BLM was stable in both suspensions at least 3 days after the preparation and there was no effect of sonication on the stability. It has been reported that adriamycin-Lipiodol suspension is completely stable during a long-term storage (8). BLM suspended in Lipiodol may also have a satisfactory stability for clinical use after the hospital manufacturing.

Antitumor effect could be enhanced by targeting anticancer agents to the tumor cells at higher concentrations for longer period. The prolongation of the clinical effect can be achieved in part by an adequate sustained-releasability of the drug at tumor site. Uniformly dispersed BLM in the sonicated suspension exhibited favorable sustained-releasability in vitro at any rotation condition.

Topical administration of BLM has been employed for the treatment of metastatic lymph nodes which could not be resected completely (7,15,16). Tanabe (16) has reported that BLM level in dissected lymph nodes is relatively low (mean, 0.82 μg/g) after the injection of BLM-Lipiodol emulsion to esophageal wall in

esophageal cancer patients. In contrast, BLM level is considerably high in the regional lymph nodes when emulsified BLM was injected directly into the bifurcation lymph node (7,15). Natsuda (7) has detected BLM in only 41.3% of the dissected thoracic lymph nodes and scarcely detected in either cervical or abdominal lymph nodes. In the present clinical study, BLM was detectable in almost all (90.2%) of the dissected lymph nodes, which were not only located in thoracic region but also in cervical and abdominal regions even within 2 h after injection of the sonicated suspension. It was considered that BLM was transported into the regional lymph nodes and retained there to a certain extent. These results suggest that Lipiodol plays a role as an adequate carrier of BLM, which may thereby be delivered to undissectable lymph nodes and may prevent the metastasis of the related lymph nodes.

In conclusion, the present sonicated suspension possessed an excellent dispersibility, sufficient stability and sustained release characteristics. Topical administration of sonicated suspension at bifurcation lymph node was fully available and proved to be effective as an intraoperative, supplemental treatment of esophageal cancer.

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