Biodegradable Poly (Lactic Acid) Microspheres for Drug Delivery Systems

Suong-Hyu Hyon

Abstract

In connection with aim of maximizing the bio-availability of conventional drugs with minimum side-effects, new drug delivery systems (DDS) continue to attracted much attention. The controlled or sustained release of drugs represents one such approach, and in this regard report upon a study of DDS using biodegradable polymers which include poly (lactic acid) (PLA), poly (glycolic acid), and their copolymers (PLGA). Much attention is being paid to the controlled release of bio-active agents from microcapsules and microspheres made of biodegradable polymers, such as lactic acid homopolymers, as well as copolymers of glycolic acid.\(^{11-21}\) Microcapsules or microspheres are injectable and able to provide pre-programmed durations of action, offering several advantages over the conventional dosage forms. This article reviews the results of a work program conducted in collaboration with a medical doctor upon DDS using biodegradable microspheres, such as PLA and PLGA.

Key Words: Controlled drug release, Biodegradable polymers

INTRODUCTION

In connection with aim of maximizing the bio-availability of conventional drugs with minimum side-effects, new drug delivery systems (DDS) continue to attracted much attention.\(^{1}\) The controlled or sustained release of drugs represents one such approach.

To develop the systems, polymeric biomaterials probably are essential as matrices, which slowly release drugs incorporated in their interiors. We have been studying DDS using such biodegradable polymers including poly (lactic acid) (PLA), poly (glycolic acid), and their copolymers (PLGA). These polymers amongst the best known biodegradable polymers, which are hydrolyzed without enzymes\(^{2,3}\) and then metabolized by the body.\(^{4}\) They have been used as surgical sutures or bone-connecting devices, and have been proven nontoxic.\(^{5}\)

Moreover, their degradation rates can be regulated by changing their molecular weights, chemical compositions, and crystallinity. Therefore, polylactides seem to be very promising DDS matrix materials. For this reason, a large number of studies which utilize these polymers have been performed in the DDS field in recent years.\(^{6-10}\) However, basic data and information upon drug release are insufficient from the viewpoint of polymer science.

In recent years, much attention has been paid to the controlled release of bioactive agents from microcapsules and microspheres made of biodegradable polymers, such as lactic acid homopolymers, and copolymers with glycolic acid.\(^{11-21}\) The microcapsules or microspheres are injectable and able to provide pre-programmed durations of action, offering several advantages over the conventional forms of dosage. This article reviews work upon biodegradable microspheres such as PLA and PLGA performed in collaboration with medical personnel on DDS.

Synthesis of poly (lactic acid) and lactic acid oligomers\(^{22}\)

Polymers from hydroxycarboxylic acids have generally been prepared in two ways. The first involves condensation polymerization of the hydroxycarboxylic acids\(^{23}\) and the other ring-opening polymerization of the monomeric, cyclic diesters of the respective hydroxycarboxylic acids. This is also the case for the polymers of glycolic and lactic acid. A schematic
presentation of the synthesis of typical polymers and oligomers is shown in Fig. 1.

The condensation polymerization method is simple but does not give high molecular weight polymers, and therefore, it is not suitable for applications requiring tough plastics and fibers. Pinkus and Subramanyam reported a method for synthesizing polyglycolic acid by condensation polymerization of bromo- and chloroacetic acid in a nitromethane solution containing triethylamine. However, the number-average molecular weights of the resultant polycondensates were around ten thousand. Fig. 2 shows the effect of reaction duration on the molecular weight of the condensation product. It is apparent that the molecular weight of the polycondensates is significantly enhanced by an increase in the polymerization duration. As is well known, this effect is characteristic of step-wise polymeric growth. On the other hand, the temperature effect on the molecular weight is not as straightforward, since high temperature often invokes depolymerization of the product. In contrast to condensation polymerization, the ring-opening polymerization of the glycolic or lactic acid cyclic dimer, that is glycolide or lactide yields polyesters with much higher molecular weights. The conventional method of preparing lactic acid monomers is based on the cyclic compound synthesis through depolymerization of the corresponding oligomers when catalyzed by inorganic metal salts. Fig. 3 illustrate the conversion and molecular weight of poly-L-lactide, respectively, as a function of reaction time for a polymerization carried out over a temperature range of 120°C to 220°C. From this figure it is evident that the molecular weight of the poly-L-lactide is increased with time during the initial stage of polymerization, and that this is followed by a second stage in which the monomer conversion and the molecular weight of polymer produced starts to decrease. This tendency becomes more pronounced.

\[ \text{Fig. 1. Reaction condition for lactide synthesis and its polymerization.} \]

\[ \text{Fig. 2. Increase in molecular weight of D, L-lactic acid polycondensates as a function of reaction time and temperature.} \]

\[ (\text{C}_{17}\text{H}_{34}\text{CO}_2\text{Sn}, \text{C}_6\text{H}_{12}\text{OH}) \quad \text{vac., 120–220°C} \]

\[ \text{Fig. 3. Conversion and molecular weight of poly-L-lactide.} \]

\[ \text{Yonsei Med J Vol. 41, No. 6, 2000} \]
at higher temperatures. The increase in molecular weight with polymerization time suggests that the ring-opening polymerization of L-lactide proceeds not only by a chain reaction mechanism but also by a step-growth reaction mechanism. This aspect has been also reported for the ring-opening polymerization of other type of cyclic esters.28

In this manner, polymers can be prepared with molecular weights of up to $6 \times 10^3$ from $1 \times 10^3$.

Lactic acid oligomer microspheres containing an anticancer agent for selective lymphatic delivery29,30

Aclarinomycin or aclacrubcin hydrochloride (ACR) is one of the most potent anticancer agents.21 However, in common with other potent anticancer agents, this drug is associated with a number of side effects including nausea, vomiting, anorexia, leukocytopenia, and thrombocytopenetic toxicities. A possible way of optimizing its pharmacological action is to target the cancer tissue sites using parenteral controlled release systems.32,33 The present work was carried out to prepare ACR encapsulated in poly (lactic acid) microspheres, which would be suitable for selective lymphatic delivery. Both the poly (lactic acid) and ACR are lipophilic and soluble in common organic solvents. This solubility characteristic makes it possible to obtain a poly (lactic acid) microspheres in which the ACR is homogeneously distributed.

Microspheres containing ACR were prepared by a solvent evaporation method.34 Briefly, a given amount of ACR and one gram of LA-oligomer were dissolved in 10 ml of methylene chloride. The resulting solution was then emulsified in 100 ml of 2 wt% poly (vinyl alcohol) (PVA) aqueous solution by sonication or agitation at room temperature. The emulsion was agitated with a magnetic stirrer, while protected from light by aluminum foil. Stirring was continued (7 h) under atmospheric pressure at room temperature until the methylene chloride solvent had completely evaporated. The microspheres were collected by centrifugation at 10,000 rpm, and 0°C for 10 min, washed three times with cold distilled water, and lyophilized.

ACR was entrapped with little loss (90 to 95% recovery) in the LA-oligomer microspheres probably

---

**Fig. 3.** Dependence of molecular weight on the reaction time and temperature for bulk polymerization of L-lactide; ○: 120°C, ●: 140°C, ◆: 160°C, □: 180°C, ●: 200°C, ●: 220°C.

**Fig. 4.** SEM photographs of different LA-oligomer microspheres containing ACR (Mw of oligomer=3,600): (A) prepared under sonication at 60 W (MS-S); (B) prepared under sonication at 15 W (MS-M); (C) prepared under stirring at 350 r.p.m. (MS-L).
because of its high lipophilicity. SEM photographs of the microspheres prepared under different conditions are shown in Fig. 4. As it can be seen, all the microspheres have a spherical shape but their sizes vary, depending on the preparation conditions. The diameter was smaller than 1 μm when the microsphere was prepared under sonication at 60 W, whereas microspheres of 1 to 5 μm were obtained by sonication at 15 W. Simple agitation with a magnetic stirrer gave microspheres as large as 10 to 100 μm. Fig. 5 shows in vitro release profiles of ACR from the LA-oligomer microspheres with different drug loadings. A nearly constant and sustained release of ACR was obtained over a period of 30 days without significant effects being observed from different initial loadings of ACR. In other words, the initial ACR amount did not affect the cumulative percentage of ACR released. Metabolism of ACR involves both glycoside- and aglycone-types. After in vivo administration to rats in this work, only glycoside-type metabolites (MA144MI and -N1) were detected in the blood and the lymph, moreover, it has been reported that there is little difference in the antitumor activities of ACR and these two metabolites. Fig. 6 indicates total ACR (ACR + two glycoside-type metabolites) levels in the plasma and lymph of the thoracic duct after administration of ACR dissolved in PBS into the peritoneal cavities of rats. The peak level of ACR was detected at 1 h (in plasma) and 3 h (in lymph), and subsequently the circulating ACR levels in both fluids decreased slowly. Little is known about the lymphatic transfer of ACR, but as can be seen in Fig. 7 the ACR level in the lymph was continuously lower than that of the plasma. The pattern of lymph ACR level was similar to that of plasma level, and ACR in both fluids diminished within 12 h of administration. This demonstrates that ACR itself administered in the peritoneal cavity has no lymphotrophy.

We next observed ACR levels in both fluids after the intraperitoneal administration of ACR-MS suspended in PBS (Fig. 7). In the plasma, ACR levels were extremely low, barely over the detection limit (10 ng/ml), regardless of the size of ACR-MS, at any sampling time during a 2 week period, the ACR level in the erythrocytes (not shown) was almost equal to that of the plasma. On the other hand, relatively high levels of ACR were found in the lymph. In particular, during the first 10 days, almost constant levels of ACR were observed in the lymph (approximate 300–400 ng/ml for ACR-MS(S) and 400–600 ng/ml for ACR-MS(L)). There is some incompatibility between the results of the amount of ACR released from MS (Fig. 5) and these lymphatic ACR levels (Fig. 7). We speculate that there may be differences in MS lymphotrophy depending on the size of microsphere.

This is the first report in which biodegradable MS, hitherto mostly employed as a material for controlling the release of drugs, is applied as a selective lymphotrophic carrier. The results in this study offer an

---

**Fig. 5.** Release profiles of ACR from ACR/LA-oligomer microspheres (Mw=4,000): (▲) 3% loading; (○) 5% loading; (●) 10% loading.

**Fig. 6.** Lymph and plasma concentrations of total ACR after intraperitoneal administration of ACR dissolved in PBS ◆, plasma; ▲, lymph, results are expressed as the mean ± S.E. of at least 3 experiments.
interesting and potentially effective method for the prevention and treatment of lymphatic tumor metastasis in cancer chemotherapy.

Lactic acid oligomer microspheres containing hydrophilic drugs

Hydrophobic drugs such as steroids can be readily incorporated in the polymer matrix in a molecularly dispersed state. However, if the drug is too hydrophilic to be soluble in organic solvents, microcrystalline fragments of the drug will be incorporated in the final polymer microparticle in the same state as was initially suspended in the polymer solution. Moreover, the water-soluble drug fragments will diffuse out into the outer continuous aqueous phase, resulting in low drug entrapment within the microsphere and the initial rapid release of the drug. This is called the ‘burst’ effect, which has always been a problem in controlled release systems. In this study, we describe a new microsphere preparation method, applicable to hydrophilic drugs, using a solvent evaporation process. For this purpose, we use a water-miscible organic solvent in which not only the lactic acid oligomer, but also the hydrophilic drug, are soluble. In addition, the solvent chosen is easily dispersible in a nonvolatile oil containing an emulsifier. The resulting oil-in-oil (O/O) emulsion is subjected to a process which allows the evaporation of the solvent to yield lactic acid oligomer microspheres incorporating the hydrophilic drugs. Thus, our preparation method is different from the conventional method reported by Beck et al. and Benoit et al. in that hydrophilic drugs can be homogeneously incorporated into the microspheres, although the solvent evaporation technique is also

![Fig. 7. Lymph and plasma concentrations of total ACR after intraperitoneal administration of ACR-MS suspended in PBS, plasma (ACR-MS(S)); ○, plasma (ACR-MS(L)); ▲, lymph (ACR-MS(S)); ◯, lymph (ACR-MS(L)). Results are expressed as the mean ± S.E. of at least 3 experiments.](image)

![Fig. 8. Schematic illustration of the solvent evaporation methods for preparing ω-lactic acid oligomer microspheres containing a hydrophilic drug.](image)
Fig. 9. Effects of drug loading on the release profiles of ADR from ADR-loaded L-lactic acid oligomer microspheres: (A) plot based on absolute weight; (B) plot based on percentage (weight of each microsphere=50 mg; Mw of oligomer=3,400). Key for drug loading: (△) 1%; (●) 2%; (○) 3%.

employed in our method.

Doxorubicin hydrochloride (adriamycin, ADR), one of the most potent anticancer drugs, was chosen for this study because it is both slightly lipophilic, and hydrophilic. Insulin was also incorporated into the microspheres during the course of this study, as a representative example of a polypeptide drug. The injectable microspheres of PLA containing adriamycin (ADM), Sigma Chemical Co, were prepared by the solvent evaporation method. The molecular weight of PLA was 3400. Fig. 8 gives a schematic presentation of the solvent evaporation process. Briefly, 20 mg of adriamycin and 180 mg of PLA were dissolved in 0.5 ml of distilled water and 4.5 ml of acetonitrile at 40°C and the solution emulsified in 500 ml of liquid paraffin containing 2% (W/W), sorbitan mono-oleate by agitation. The oil in the emulsion was agitated for 24 hr under atmospheric pressure at room temperature until the acetonitrile solvent evaporated. Thereafter, the microspheres were collected by centrifugation, washed with hexane to remove the liquid paraffin and the emulsifier from the surface of the microspheres, and dried under vacuum until the solvent had evaporated. The microspheres were observed to be almost spherical and no drug microcrystals were detected on their surfaces. The influence of loading on the release profiles of ADR is shown in Fig. 9. It is evident that ADR is nearly constantly released over a period of >2 weeks without a significant burst effect. The amount of ADR released from the microspheres over a period of time is almost pro-

Fig. 10. Effects of drug loading on the release profiles of insulin from insulin-loaded L-lactic acid oligomer microspheres (Mw of oligomer=4,700). Key for drug loading: (○) 5%; (●) 10%; (△) 20%.
9 for ADR and insulin, respectively. As can be seen in the figures, the release rate is reduced in both cases as the molecular weight of the oligomer increases. These results are almost the same as those of the lactic acid oligomer microspheres incorporating a lipophilic anticancer drug, ACR, which were prepared by the O/W type solvent evaporation process, except that the release rate is lower than that of the ADR and insulin oligomer.

The morphological changes of the insulin microspheres accompanying in vitro hydrolytic degradation are shown in Fig. 11 (A-E). The microspheres before hydrolysis appear spherical in shape, but have some cracks and pores on their surfaces (Fig. 11A). After immersion in an aqueous medium for 1 day, many more surface pores and channels are generated (Fig. 11B). This is probably due to vacuolization by diffusion or dissolution of the drug existing near the surface area into the surrounding medium. The microspheres undergo noticeable degradation after day 5, which is accompanied by shape changes (Fig. 11C-E). At 30 days, the microspheres still remaining have become extremely porous and are no longer spherical (Fig. 11E). A similar morphological change was observed for microspheres with other molecular weights and for the ADR microspheres, but such changes appeared delayed in the higher molecular weight micro-spheres. A new method was developed preparing biodegradable lactic acid oligomer microspheres containing hydrophilic drugs. The microspheres were obtained by removing solvent from an O/O (oil-in-oil) emulsion by evaporation. The solvent used for the dispersed phase solution was an acetonitrile:water mixture, while the continuous phase medium was cottonseed oil. Doxorubicin hydrochloride (ADR) and insulin were successfully entrapped in the microspheres with high trapping efficiencies of 80 to 90%, and their release profiles were not accompanied by a significant burst effect. The release rates of the drugs from the microspheres were greatly affected by the initial drug loadings and the molecular weights of the lactic acid oligomer.

Biodegradation and antitumour effect of adriamycin-containing poly (L-Lactic acid) microspheres

Anticancer agents generally harm not only tumour cells but also normal cells, causing unavoidable side effects. Therefore, reduction of the systemic drug concentration is required to obtain the maximum pharmacological activity with minimal systemic side effects. Many attempts have been made to deliver antitumour drugs selectively to neoplasias. We also

![Fig. 11. The SEM photographs of insulin-loaded L-lactic acid oligomer microspheres subjected to hydrolytic degradation in vitro for different durations.](image-url)
prepared adriamycin (ADR)-containing poly (L-lactic acid) microspheres (ADR-MS) and obtained a preliminary result that demonstrated they had slow-release and long-acting anticancer effects. Poly (L-lactic acid) has the advantages of a suitable biodegradation rate in vitro and that it can be administered in various forms. It is also interesting that the polymer is absorbed without scar formation at the implanted site during absorption. In the present paper, we describe tissue reactions due to ADR release from ADR-MS and the antitumour effects of ADR-MS for tumour-bearing mice.

The ADR-MS employed during our present study were almost spherical and homogeneous in size with an average diameter of 50 μm and contained 6.1 μg/mg of ADR which represented a loading of 10 vol% of ADR. The average molecular weight of the poly (L-lactic acid) used for the microsphere preparation was 3400. ADR-MS suspended in tris buffer showed a nearly constant release of ADR from the microspheres without any remarkable burst effect and we tried to prepare the ADR-MS to release 100% of ADR at 20 d. Retention of the pharmacological activity was confirmed by bioassay using B (data not shown). SEM observation revealed that the ADR-MS was hydrolyzed in tris buffer to yield many small perforations.

Injection of the ADR-MS suspension into the marginal ear vein of rabbits caused death, indicating that pulmonary embolism had occurred. ADR was not detected (the detection limit (d.l.)=0.073 μg/ml) for up to 14 d in the serum of the rabbits that survived. The hemorrhage in the autopsied lung observed 14 d after injection also suggested pulmonary embolism and optimal microscopic examination revealed that much of the ADR-MS was trapped in the capillaries of the lung parenchyma and biodegraded.

ADR was not detected in the serum for up to 14 d, when the ADR-MS suspension was injected into the femoral muscles of rabbits (d.l.=0.029 μg/ml). However, ADR remained in the muscles until day 10, but was not detected on day 14 (d.l.=0.098 μg/ml). Macroscopically, an orange-colored elastic hard mass was recognized at the injection site for up to 10 d and white scar tissues appeared 14 d after the injection. Histological examination, as shown in Fig. 12, revealed that ADR-MS was biodegraded in the muscles, but little necrotic tissue was observed. Figure 13 shows muscle tissues 2 wk after the injection, scar tissues in evidence after the disappearance of the ADR-MS. However, when the suspension of PLA-MS was injected into the muscle, the injection site could not be located macroscopically or microscopically, indicating that the injection site had healed without scar formation. As a control, when 500 μg of F-ADR in solution was injected into rabbit femoral muscles. ADR was not detected in serum for up to 3 d (d.l.=0.015 fig/ml), but 46.069 μg of ADR re-
mained in the muscles on day 3 (d.l. = 0.069 pg/ml).

Injection of the ADR-MS suspension into the peritoneal cavity of mice also resulted in the absence of ADR in serum for up to 7 d (d.l. = 0.073 µg/ml). On the other hand, when F-ADR was injected at a dose 13.7 mg/kg into the peritoneal cavity, 0.050 µg/ml of ADR was detected in serum on day 1, but ADR was not detected in serum thereafter (d.l. = 0.015 µg/ml). In addition, injection of the ADR-MS suspension at doses of 27.4 (E) and 41.1 mg/kg of ADR (F) produced death in all mice between days 21 and 27, and from day 10 to day 15, respectively. And the suspension of PLA-MS in saline (H) produced no deaths (Fig. 14). These results were statistically significant (P < 0.01) with respect to comparisons between B and C, B and F, C and E, C and F, and E and F (see Fig. 14).

ADR-MS formulated in our laboratory showed an almost constant release of ADR to tris buffer and disappeared within 20 d. ADR-MS, administered in muscles of rabbits were biodegraded, releasing all of the ADR in less than 2 wk with the formation of scar tissues. Local administration of ADR-MS led to a high concentration of released ADR at the administration site, which could be related to a strong anticancer effect and a very low concentration of ADR in serum, conducive with low systemic side effects. When ADR-MS was administered at a suitable dose, it proved more effective than F-ADR against tumour cells.

Evaluation of a bilayer artificial skin capable of sustained antibiotic release

The most frequent complication of artificial skin is infection, which often occurs about 1 wk after application. The infection is generally not serious because it subsides after partially removing the silicone sheet over the infected area and using a conservative wound dressing. However, to use artificial skin safely even infected wounds, an artificial skin resistant to infection is necessary.

For this purpose, an artificial skin capable of the sustained release of an antibiotic was developed. Microspheres containing an antibiotic were incorporated underneath the silicone sheet of the artificial skin. This chapter describes the effect of the new type of artificial skin as evaluated by in vitro studies. PLA weighing 0.75 g was dissolved in 10 ml of acetonitrile mixed with water at 10 vol%. Both hydrophobic PLA and hydrophilic Tobramycin (TOB) are soluble in this mixed solvent system. TOB (0.25 g) was added to this solution, followed by the dropwise addition of water to effect its complete dissolution. This solution of PLA and TOB was added in a drop-wise manner under agitation to cottonseed oil containing an emulsifier. The temperature was raised to 40–45°C to evaporate the solvent from this oil-in-oil type emulsion. The microspheres were hardened by this process, and then filtered using sieves to obtain a fraction of microspheres containing TOB (TOB-MS) ranging from 50 to 100 µm in diameter. Excess emulsifier and cottonseed oil on the surface of TOB-MS were then removed by washing with petroleum ether. The TOB-MS was dried under reduced pressure.

![Fig. 14. Survival of mice after intraperitoneal injection of a solution of free Adriamycin (F-ADR) and a suspension of Adriamycin-containing poly(l-lactic acid) microspheres (ADR-MS). Statistically significant (P = 0.01) by generalized Wilcoxon test as compared with B and C, B and F, C and E, C and F, and E and F. Dosage form and dose: (A) F-ADR, 13.7 mg/kg; (B) F-ADR, 27.4 mg/kg; (C) F-ADR, 41.1 mg/kg; (D) ADR-MS, 13.7 mg/kg of ADR; (E) ADR = MS, 27.4 mg/kg of ADR; (F) ADR-MS, 41.1 mg/kg of ADR; (G) saline; (H) microspheres without Adriamycin (ADR).](image)

![Fig. 15. Structure of a new type of artificial skin with sustained release of Tobramycin (TOB).](image)
and sterilized with EOG gas.

TOB-MS (630 mg) was added to 30 g of a solution containing 0.3% of arelocollagen (pH 3, type I collagen, donated by Nitta-Gelatin Co., Japan) and the mixture was stirred immediately. The stirred collagen solution was poured into a mould (10 × 7 cm), frozen rapidly at −20°C and stored overnight. The frozen contents were then directly freeze-dried for 48 h to yield a collagen layer that contained TOB-MS at a loading of 9 mg/cm² (TOB 2.25 mg/cm²), and which served as a reservoir of TOB-MS, however, the collagen sponge sheet did not contain TOB-MS (Fig. 15). The collagen layer containing TOB-MS was attached to a silicone layer of 25 μm to fabricate the combined sheet (Fig. 15). The silicone doped film was cross-linked at room temperature overnight. The combined sheet, which permitted the sustained release of TOB, constituted the upper layer of the new bilayer artificial skin (Fig. 15). Fig. 16 shows the SEM structure of the layered sheet. In Fig. 17 burst release is observed initially for both the TOB-MS and the treated sheet. The amount of TOB released from the treated sheet on the first day was controlled, compared with that from TOB-MS. However, sustained release of TOB to saline from both the TOB-MS and the treated sheet continued for at least 13 d from day 3.

The “artificial skin” without antibiotic was previously used for 13 regions in 12 patients but infection occurred rather frequently, in five of the 13 regions. Bacteria found on these infected wounds included, Pseudomonas aeruginosa in two cases, and Staphylococcus aureus, Staphylococcus epidermidis and Acromobacter xylosidans in every case. These microbes all fell within the antibacterial spectrum of tobramycin, which was, therefore, selected as the “artificial skin” antibiotic. Since the introduction of the new “artificial skin” with the drug release system, no infection has occurred in any of the six cases in which it was used (Table 1). Some problems may develop, such as, the emergence of antibiotic-resistant bacteria, and an “artificial skin” capable of releasing other antibiotics must be developed in the future in order to deal with such bacteria.

Intraperitoneal administration of 5-fluorouracil microspheres against peritoneal carcinomatosis in rodents

Intraperitoneally administered 5-fluorouracil in the form of an aqueous solution is one of the most common treatments for peritoneal carcinomatosis induced by gastrointestinal cancers. However, this cannot always control the disease, because small water-soluble molecules, such as, 5-fluorouracil (5-

---

**Table 1. Clinical Uses of the Artificial Skin with Sustained Release of Tobramycin**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Site</th>
<th>Cause</th>
<th>Extent of area grafted (cm)</th>
<th>Donor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>M</td>
<td>Chest</td>
<td>Burn</td>
<td>10 × 20</td>
<td>Scalp</td>
</tr>
<tr>
<td>2</td>
<td>23</td>
<td>F</td>
<td>Thigh</td>
<td>Naevus</td>
<td>20 × 30</td>
<td>Thigh</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>M</td>
<td>Abdomen</td>
<td>Burn</td>
<td>5 × 7</td>
<td>Inguinal</td>
</tr>
<tr>
<td>4</td>
<td>18</td>
<td>F</td>
<td>Lower limb</td>
<td>Naevus</td>
<td>25 × 15</td>
<td>Thigh, buttok</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>F</td>
<td>Upper limb</td>
<td>Naevus</td>
<td>18 × 15</td>
<td>Back</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>M</td>
<td>Lower limb</td>
<td>Burn</td>
<td>7 × 10</td>
<td>Back</td>
</tr>
</tbody>
</table>

The take of secondary skin graft in all cases was perfect.
FU) in aqueous solution are rapidly absorbed through the blood capillaries into the systemic circulation, therefore, the concentration of 5-FU is not maintained at high enough levels for sufficient periods of time in the intraperitoneal area. In contrast, corpuscular particles such as microspheres are retained in the peritoneal cavity for long periods of time and are absorbed gradually.

Based on the above-mentioned differences in the absorption of aqueous solutions and corpuscular particles, we developed a new formulation of 5-FU in the form of 5-FU-MS. This following section reports upon the of 5-FU-MS distribution and its therapeutic efficacy in rodents.

5-FU-MS (Fig. 18), which consists of 5-FU incorporated into microspheres of poly (glycolide-co-lactide), was prepared as follows: −100 mg/ml of 5-FU and 900 mg/ml of poly (glycolide-co-lactide) were dissolved in 97% acetic acid. The resulting solution was emulsified in 10 volumes of liquid paraffin by stirring at 250 cycles per minute at 30°C for 48 hours, and then evaporated to form microspheres containing 5-FU.

The concentrations of 5-FU in the omentum and mesentery are shown in Fig. 19 and 20 respectively, which are considered to represent the 5-FU concentrations in the intraperitoneal tissues. In the 5-FU-MS group, the 5-FU concentration in the omentum was maintained at a high level, and was much higher than in the 5-FU solution group throughout the entire observation period of 16 days after the administration. The 5-FU concentration of the two formulations was significantly (p < 0.01 to 0.05) different at 1 hr, 6 hr and at 4 days after drug administration. In the mesentery, the 5-FU concentration in the 5-FU-MS group was 12.7 μg/g, which was lower than the 30.3 μg/g of the 5-FU solution group, 1 hr after administration. However, the concentration in the 5-FU-MS group increased to 261.5 μg/g at 6 hr, and was maintained at a high level for 16 days. In contrast, in the 5-FU solution group, the 5-FU concentration in the mesentery was 30.3 μg/g at 1 hr, and decreased to an undetectable level 4 days after injection. The 5-FU concentration in the mesentery was significantly different (p < 0.025 to 0.05) at 1 hr, 6 hr, 24 hr and 4 days.

5-FU-MS increased survival with increasing 5-FU doses (Table 2); the median survival period was 28.5 days (T/C% of 124%) at a 5-FU dose of 100 mg/kg, and more than 150 days (T/C% of over 652%) at a dose of 400 mg/kg. On the other hand, the 5-FU solution was not as efficacious when the 5-FU dose was increased; the median survival was 28 days (T/C% of 122%) at a 5-FU dose of 100 mg/kg, and only 29.5 days (T/C% of 128%) at a dose of 200 mg/kg. The survival curves for the 5-FU-MS group and the 5-FU-solution group were compared at the same 5-FU dosage. A 5-FU dose of 200 mg/kg in

![Fig. 17. Release of Tobramycin (TOB) from the microspheres containing TOB and the combined sheet to day 13. (●-●) Microsphere (×-×).](image1)

![Fig. 18. Scanning electron microscopic view of 5-FU microspheres shows that the mean size of the microspheres is 20 μm in diameter.](image2)
Table 2. Therapeutic Effects on Peritoneal Carcinomatosis in Mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Median survival days (range)</th>
<th>T/C%</th>
<th>No. of survivor</th>
<th>No. of toxic death</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-FU-MS 400 mg/kg</td>
<td>&gt;150 (16 to &gt;150)</td>
<td>&gt;652</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>5-FU-MS 300 mg/kg</td>
<td>36 (33 to &gt;150)</td>
<td>157</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>5-FU-MS 200 mg/kg</td>
<td>32 (27 to &gt;150)</td>
<td>139</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>5-FU-MS 150 mg/kg</td>
<td>30 (25 to 41)</td>
<td>130</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-FU-MS 100 mg/kg</td>
<td>28.5 (25 to 34)</td>
<td>124</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-FU solution 200 mg/kg</td>
<td>29.5 (11 to 37)</td>
<td>128</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>5-FU solution 150 mg/kg</td>
<td>29 (26 to 33)</td>
<td>126</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5-FU solution 100 mg/kg</td>
<td>28 (24 to 32)</td>
<td>122</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Empty-MS + 5-FU solution 200 mg/kg</td>
<td>29 (11 to 35)</td>
<td>126</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Empty-MS</td>
<td>24 (20 to 27)</td>
<td>104</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Non-treatment</td>
<td>23 (20 to 26)</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

T/C%: Median survival day in the treatment group/Median survival day in the non-treatment group. No. of toxic deaths: Number of mice dead from drug toxicity, as determined by autopsy. 5FU-MS: 5-fluorouracil incorporated in microspheres.

Fig. 19. 5-FU concentration in omentum. In the omentum 5-FU concentration was higher at 1, 6 hr and 4 days after administration in rats given 5-FU-MS than in rats given 5-FU solution.

Fig. 20. 5-FU concentration in mesentery. In the mesentery 5-FU concentration was higher at 1, 6, 24 hr and 4 days after administration in rats given 5-FU-MS than in rats given 5-FU solution.

the 5-FU-MS formulation prolonged the survival time significantly (p < 0.05) as compared with the same dose of aqueous 5-FU. There were no significant differences between the 5-FU-MS group and the 5-FU-solution group in terms of the survival curves of mice treated with 150 mg/kg or 100 mg/kg of 5-FU.
No differences were found in therapeutic effect between mice given the 5-FU solution and those given the 5-FU solution plus empty-microspheres.
The empty-microspheres induce neither toxic death nor therapeutic effect on the survival of the mice. Mice that survived for 150 days were cancer-free, as determined by autopsy. The intraperitoneal administration of 5-FU-MS, which is a relatively convenient method, distributes a high concentration of 5-FU selectively into the intraperitoneal tissues for a long period of time, as was shown in the present experiments. Since the anticancer activity of 5-FU depends on the length of time it is present as well as on its concentration, the therapeutic effects against peritoneal carcinomatosis should be enhanced by the 5-FU-MS formulation. Drug distribution experiments showed that the plasma 5-FU concentrations were lower in rats given 5-FU-MS than in those given the 5-FU solution. This result suggests that 5-FU-MS causes less systemic toxicity than the same dose of
aqueous 5-FU. These results also suggest that intraperitoneal 5-FU-MS may be more efficacious in the treatment of peritoneal carcinomatosis because it yielded enhanced therapeutic effects in these animal studies.

Cisplatin-releasing bio-resorbable microspheres for the treatment of malignant pleural effusions

In the treatment of malignant pleural effusions, it is important to remove the effusion and to prevent its re-accumulation to improve the patient’s quality of life. However, the management of malignant pleural effusions is sometimes difficult. Treatment involves anticancer agents being instilled into the thorax, and occasionally systemic side effects occur due to the absorption of drugs into the serum. To obtain maximal anticancer effect at the site of tumor cells, with minimal systemic side effects, sustained release of the anticancer drug is effective. We have previously reported upon the effectiveness of treatment with adriamycin-containing bio-resorbable microspheres for patients with pleuritis carcinomatosa. Cisplatin (CDDP) is one of the most powerful antineoplastic agents but has significant toxicity. However, it has been difficult to achieve sustained release. We developed slow-releasing cisplatin-containing bioresorbable microspheres, and treated patients with the microspheres as a local chemotherapy.

Microspheres were prepared using CDDP and a polymer consisting of glycolic acid-L-lactic acid copolymer (PGLA) with a molecular weight of 27,000 by the oil in oil emulsion method. The average diameter of the CDDP-containing PGLA microspheres (CDDP-MS) was about 100 μm. The CDDP-MS contained 5% of CDDP and in vitro the CDDP was released almost constantly over a period of about 3 weeks. In animal studies, CDDP-MS was safely administered and exerted a stronger anticancer effect with less systemic side effects than the CDDP solution.

CDDP-MS was administered to seven patients with malignant pleural effusions, which were diagnosed cytologically by effusions as having adenocarcinoma of the lung. They were undergoing their first treatment for their pleuritis carcinomatosa. As a control, we also treated patients utilizing a CDDP solution instead of CDDP-MS. Chest tube drainage was performed and the effusion was removed completely. CDDP-MS, equivalent to 100 mg of CDDP, was administered through a tube with 100 ml of physiologic saline, followed by clamping of the drain. The clamp was removed after 24 h. All the pleural effusion was recovered thereafter. Hydration was not performed after the CDDP-MS administration.

After the CDDP-MS or CDDP solution had been administered, concentrations of platinum (Pt) in serum and in effusion were assayed periodically. Blood or effusion was collected in a tube with heparin sodium and the tube was immediately centrifuged at 3,000 rpm for 10 min in order to separate serum for the assay of the protein-bound Pt (total Pt=T-Pt). One ml of the same serum or centrifuged effusion was ultrafiltered at 2,000 rpm for 15 min using an Amicon centrifree® micropartition system-3 for the assay of non-protein-bound Pt.

Ed-the highlight above-Amicon?: Pt (free Pt=F-Pt). After centrifugation, these samples were immediately frozen at a temperature of -40°C. T-Pt and F-Pt were assayed by atomic absorption (Zeeman, Hitachi Co., LTD, Tokyo, Japan). The limit of detection was 0.02 g/ml.

CDDP-MS was administered as a local chemotherapy for malignant pleural effusions. CDDP-MS was made using PGLA, which has no cytotoxicity and undergoes biodegradation to bicarbonate and water. After instillation of the CDDP-MS, we closed the tube for 24 h. During this period the CDDP-MS seemed to be caught and embedded in fibrin in the pleural space. Therefore, little CDDP-MS was lost from the pleural cavity after the drain was evacuated. In some patients, two weeks after CDDP-MS administration, T-Pt was detected in the serum, indicating sustained release of CDDP from the CDDP-MS in the thorax. Furthermore, the total amount of drained T-Pt was less than 1% of the total, suggesting that almost all of the CDDP-MS.

Ed-the highlight above-confirm: Remained at the administration site. Sustained release of CDDP from the CDDP-MS would contribute to decreasing the serum concentration of T-Pt. In our cases, after the administration of CDDP-MS, the maximal concentrations of the T-Pt in serum was always lower than that after treatment with CDDP solution. We believe that low T-Pt concentrations in the serum contributed to the reduction of the systemic side effects. Therefore, even though no hydration occurred, neither gastrointestinal symptoms nor myelo-suppression were observed after treatment with CDDP-MS. In addition, since CDDP-MS released F-Pt, it
would be expected to produce a prolonged antitumor effect at the site of tumor cells. After the administration of CDDP-MS, the effusions decreased to less than 20 ml/day on days 3.5 ± 1.64 and exubations were performed on 5.0 ± 1.52 days. They were shorter than those treated with CDDP solutions. In terms of the control of pleural effusions, CDDP-MS proved superior to a CDDP solution. CDDP-MS is, therefore, considered to be useful for the treatment of malignant pleural effusions and to contribute to the improvement of the patient’s quality of life.

REFERENCES


28. Gilding DK, Reed AM. Biodegradable polymers for use in surgery-polyglycolic/poly (lactic acid) homo- and co-
polymers; 1.". Polymer 1979;20:1459-64.