**APPROACH TO NEW FORMULATIONS OF BIOACTIVE PEPTIDES**

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**Introduction**

Administration of drugs in conventional dosage forms often results in seesaw fluctuations of drug concentrations in systemic circulation and tissue compartments. The "peak and valley" pattern is more striking for drugs with a short biological half-life.

Drug delivery systems are expected to make a significant contribution not only in improving the delivery of already existing drugs but also in future drug development. Since many new bioactive substances under development such as peptides and proteins are rapidly destroyed by the body, it will be critical to develop effective systems for them.

In order to maintain a steady drug concentration in blood circulation and increase the efficacy of drugs and minimize the incidence and severity of adverse side effects, we studied sustained-release drug delivery systems of bioactive peptides and developed new formulations which are applicable to various kinds of drugs.

This report describes the application of these formulations to interferon as an example of protein drugs.

Clinical studies on various interferons have shown that it is important to enhance the clinical effectiveness by applying appropriate administration methods and dosage forms (1,2). The *in vitro* cytocidal activity of interferons is time-dependent, suggesting that the anti-tumor activities in clinical use are enhanced by sustaining an effective concentration of interferon (3).

Collagen, a major protein of connective tissue of animals, is a biocompatible and biodegradable material with wide utility for many applications, including implantation for cosmetic surgery to smooth acne scars (4,5). Two types of sustained-release preparations of interferon, oil-suspension and mini-pellet, using collagen as a carrier material have been developed (figure 1) and *in vivo* studies were performed (6,7).

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**Figure 1** Schematic diagram of collagen matrix preparations
Experimental

Materials
Atelocollagen (Koken Co., Ltd., Japan) was used as the drug carrier material. Interferon was human lymphoblastoid interferon (HLBI) prepared from Sendai virus-induced human Namalwa cells (a product of Sumitomo Pharmaceuticals Co., Ltd., Japan).

Oil-suspension preparation and evaluation
An aqueous solution of interferon was mixed with collagen solution homogeneously. The mixture was lyophilized and pulverized at -30 °C using liquid nitrogen. Drug-loaded collagen powder (particle size: 100-150 μm) was suspended in oil. The suspension was administered subcutaneously to beagle dogs (3x10^6 U/kg). In comparison, solutions of interferon were injected into beagle dogs. Blood samples were collected and serum was separated and analyzed for interferon concentration by radio immunoassay (RIA), using an Interferon-α kit (Dainabot Radioisotope Lab. Ltd., Japan). Body temperature was recorded at specific times after dosing.

Mini-pellet preparation and evaluation
A gel mixture of interferon and collagen was subjected to molding and then dried to give a cylindrical mini-pellet (1 mm in diameter and 8 mm in length). In vivo studies were carried out in mice and beagle dogs. Mini-pellets containing 5x10^5 U of interferon were injected subcutaneously in mice. A control group was injected with solutions of interferon. Similar experiments were done in beagle dogs at a dose of 4x10^6 U/kg.

![Figure 2](image_url)

Figure 2 Mean serum interferon concentrations in beagle dogs after subcutaneous administration at a dose of 3x10^6 U/kg as a oil-suspension (●) or an aqueous solution (○).
Results and discussion

It was apparent that the interferon serum concentration profiles varied with the formulations as shown in figure 2. Aqueous injection resulted in earlier (3 vs 7 hr) and much higher Cmax (2900 vs 380 units/ml) than those of oil-suspension injection. After aqueous injection, serum concentrations declined quickly and no interferon was measured after 48 hours. In the case of oil-suspension, the significantly prolonged activity of interferon was observed and detectable levels were noted for 72 hours. There also was distinct difference in the body temperatures for the formulations. An elevated body temperature was observed after aqueous injection, suggesting that the temperature was related to the magnitude of the serum concentration. No such side effect was noted with oil-suspension.

The serum interferon concentrations in mice after administration of mini-pellets is shown in figure 3. The mini-pellet exhibited the desired sustained-release for 7 days.

The serum peak levels of interferon in beagle dogs given with mini-pellets are significantly reduced and interferon concentrations were continuously maintained for extended periods. An elevated body temperature was not observed. The host response to the mini-pellet exhibited no observable inflammatory characteristics.

These results demonstrate that interferon distributed through a collagen is released slowly to give a constant serum level.

Figure 3 Mean serum interferon concentrations in mice after subcutaneous administration of mini-pellet, 5x10^5 U/mouse (●) or aqueous solution, 1x10^5 U/mouse (○).
Conclusion

The present study was done to develop new formulations and administration methods of interferons. It is recognized that oil-suspension exhibits the prolonged serum levels of interferon for several days by conventional subcutaneous injection. The trans-catheter embolization also would be an effective modality for cancer treatments. Mini-pellet can be used as a long-term sustained-release preparation by subcutaneous injection or surgical treatment. Moreover, these preparations can be administered into the tumor sites using a fiberscope to maintain high interferon levels locally for a long period of time. In addition, both formulations offer a significant advantage in interferon therapy because peak serum concentrations are reduced, allowing increased dosage and reducing dose-related side effects (8).

The advantages of these systems are: 1) carrier material is a biodegradable natural protein; 2) they are manufactured under mild conditions without any organic solvent or heating process; 3) they are easily administered in the same way as conventional injections. Therefore, they are applicable to various kinds of drugs which are effective in small amounts and whose activities are enhanced by maintaining blood levels over a long period of time.

References