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Sustained release of buserelin acetate, a luteinizing hormone-releasing hormone agonist, from an injectable oily preparation utilizing ethylated β-cyclodextrin

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Abstract—The possible use of heptakis (2,6-di-*O*-ethyl)-β-cyclodextrin (DE-β-CyD) as a parenteral sustained-release carrier for buserelin acetate, a luteinizing hormone-releasing hormone superagonist, has been examined. The in-vitro release of buserelin from the oily suspension was significantly retarded by the complexation with DE-β-CyD, mainly due to the poor water solubility of the complex. A single subcutaneous injection of the suspension containing the buserelin-DE-β-CyD complex to rats provided an effective continuous plasma level of buserelin lasting for at least one month, indicating a potential therapeutic efficacy for the treatment of the endocrine-dependent diseases. These results suggest that DE-β-CyD serves as an injectable sustained-release drug carrier suitable for chronic treatment with buserelin acetate.

Buserelin acetate, a highly potent analogue of a luteinizing hormone-releasing hormone (LHRH), is known to be effective in the treatment of endocrine-dependent metastatic prostate carcinoma or endometriosis (Sandow et al 1987). However, in clinical practice, chronic treatment with LHRH analogues including buserelin acetate has often been associated with disadvantages; the short biological half-lives of the drugs require long-term daily injection or frequent nasal application to maintain therapeutic concentration of the drugs. Therefore, attention has been directed toward the development of drug delivery systems with controlled-release features to realize the potential and efficacy of LHRH analogues. Several approaches have been proposed including the use of implants or injectable microcapsules of biodegradable copolymers or gel-forming agents (Banga & Chien 1988).

Molecular encapsulation of drugs with cyclodextrins (CyDs) and their derivatives has received much attention (Szejtli 1985; Uekama & Otagiri 1987; Pitha et al 1988). Among these derivatives, alkylated CyDs recently gained acceptance in pharmaceutical applications (Uekama & Irie 1987) and one of them, heptakis (2,6-di-O-ethyl)-β-CyD (DE-β-CyD), which is slightly soluble in water, was found to serve as a sustained-release type carrier for water-soluble drugs with short biological half-lives (Uekama et al 1987; Hirayama et al 1988). Thus, in a preliminary study, we investigated the complexation of buserelin acetate with DE-β-CyD as an alternative to devices for controlled-drug release as described by Sanders et al (1986) and Okada et al (1988). This paper deals with the in-vitro release of buserelin from the oily suspension containing the drug or its DE-β-CyD complex and the plasma drug levels when injected subcutaneously in rats.

Materials and methods

Buserelin acetate (Hoechst Japan Ltd, Saitama, Japan) was used without further purification. DE-β-CyD was kindly donated by Nisshin Flour Milling Co. Ltd (Saitama, Japan). The solid complex of buserelin acetate with DE-β-CyD in a 1:100 weight ratio was prepared by the kneading method (Tsuruoka et al 1981). For example, 25 mg of buserelin acetate and 2·5 g of DE-β-CyD were wetted in 10 mL of water, kneaded thoroughly for 90 min and then dried under reduced pressure at room temperature (20°C) for 1 day. Detailed characterization of the complex will be reported elsewhere. After being passed through 100 mesh screen, buserelin acetate or its DE-β-CyD complex (equivalent to 5 mg of buserelin acetate) was dispersed in 10 mL of arachis oil, which was used as a vehicle for the sustained release preparation. The drug release from the oily suspension into 10 mL of water was measured at 37°C. The drug concentrations were analysed by high-performance liquid chromatography under the following conditions; pump and detector: Hitachi L-6000 machine with 650-10 LC Fluorescence spectrophotometer (Tokyo, Japan), column: Waters μBondapak C18 (3·9 × 300 mm, Tokyo, Japan), mobile phase: 0·8% KH_2PO_4 solution-acetonitrile (2:1), excitation: 280 nm, emission: 355 nm.

Sprague-Dawley male rats, 350–420 g (about 9 weeks old), were used. The oily suspension containing buserelin acetate or its DE-β-CyD complex was injected subcutaneously at a dose of 1 mg kg^{-1} as buserelin acetate. Blood samples were taken periodically from the tail and jugular veins. The plasma levels of buserelin were determined by the double-antibody radio-immunoassay and the lower limit of detectability of buserelin with 95% confidence was 30 pg mL^{-1} in plasma (Saito et al 1985).

The maximum plasma buserelin level (C_{max}) and the time required to reach the maximum plasma drug level (T_{max}) were extracted from the plasma data. The area under the plasma drug level-time curve (AUC) and the mean residence time of the drug in plasma (MRT) were calculated from the plasma drug levels up to 7 weeks post-administration by moment analysis (Yamaoka et al 1981).

Results and discussion

Fig. 1 shows the release profiles of buserelin from the oily suspension containing the drug or its DE-β-CyD complex into the aqueous phase. It is apparent that the interfacial transfer of buserelin was significantly retarded by the complexation with DE-β-CyD. The release of drug from a vehicle is known to be influenced by various factors including drug-vehicle interactions, solubility, partition coefficient, and particle size of drug in the vehicle (Roseman et al 1981). Buserelin was practically insoluble in arachis oil as a vehicle (less than 2 ng mL^{-1} at 37°C). DE-β-CyD was found to increase the solubility of buserelin in the vehicle, the solubility of buserelin in the complexed form was 47 ng mL^{-1} at 37°C. This indicates that the complex has higher affinity to the vehicle than the drug alone. However, it seems unlikely that the increased affinity of the complex to the vehicle largely contributes to the observed duration of drug release into the aqueous phase, because the amount of buserelin dissolved in the vehicle, even in the complexed form, was less than 0·01% of the total amount (5 mg in 10 mL of the vehicle), the major fraction existing as well-dispersed fine particles. Therefore, the

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Table 1. Some pharmacokinetic parameters of buserelin following the subcutaneous administration of the oily suspension containing buserelin acetate or its ethylated β-cyclodextrin (DE-β-CyD) complex (equivalent to 1 mg kg^{-1} as buserelin acetate) to 5 rats.

System	C_{max}[a] (ng mL^{-1})	T_{max}[b] (h)	AUC[c] (ng day mL^{-1})	MRT[d] (day)
Buserelin acetate	166·3 ± 20·0	0·95 ± 0·30	24·7 ± 4·3	0·13 ± 0·02
DE-β-CyD complex	9·9 ± 1·7*	1·45 ± 0·65	27·1 ± 2·0	9·18 ± 0·38*

[a] The maximum plasma drug level; [b] The time required to reach the maximum plasma drug level; [c] The area under the plasma drug level-time curve up to 7 weeks post-administration; [d] The mean residence time of the drug in plasma. Each parameter is expressed as the mean ± s.e. of 5 rats.
* Significantly different from buserelin acetate ($P < 0·01$, Student's t-test).

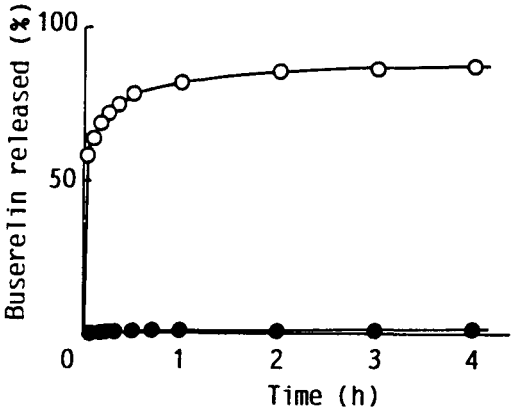

FIG. 1. Release profiles of buserelin from the oily suspension containing buserelin acetate or its DE-β-CyD complex (equivalent to 5 mg as buserelin acetate) in water at 37°C. ○: buserelin acetate; ●: DE-β-CyD complex.

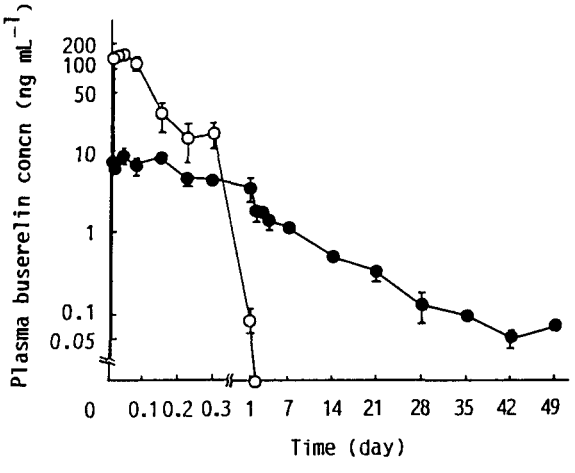

FIG. 2. Plasma levels of buserelin following the subcutaneous administration of the oily suspension containing buserelin acetate or its DE-β-CyD complex (equivalent to 1 mg kg^{-1} as buserelin acetate) to rats. ○: buserelin acetate; ●: DE-β-CyD complex. Each value represents the mean ± s.e. of 5 rats.

extended duration of drug release could be explained by the slow dissolution of the complex at the oil/water interface, owing to the poor water solubility of the DE-β-CyD complex (Uekama et al 1987).

To ascertain whether DE-β-CyD acts as a sustained-release drug carrier in-vivo, the oily suspension containing buserelin acetate or its DE-β-CyD complex was administered subcutaneously to rats at a dose of 1 mg kg^{-1} as buserelin acetate. The suspensions could be injected easily subcutaneously into rats using a standard syringe without any additives. Fig. 2 shows the plasma concentrations of buserelin in rats following the subcutaneous administration of the suspension, and some pharmacokinetic parameters are summarized in Table 1. There was no appreciable difference in the area under the plasma drug level-time curve (AUC) between the two suspensions; the DE-β-CyD complexation does not alter the extent of drug bioavailability. It is evident that the suspension containing the DE-β-CyD complex provided plasma levels of buserelin lasting for at least 7 weeks, giving a mean residence time of the drug in plasma (MRT) for the complex about seventy times longer than that for the drug alone. While the biotransformation and the local tissue tolerance of the DE-β-CyD complex should be further investigated before its practical use, the present data support the possible use of DE-β-CyD as an injectable sustained-release type carrier for buserelin acetate; the simple manufacturing process and no need for additives may be a significant advantage of this system over other sustained-release devices.

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Supersensitivity of atherosclerotic rabbit aorta to ergometrine is mediated by 5-HT_2 receptors

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Abstract—The concentration response curve of ergometrine in aortae from rabbits fed a high cholesterol diet for 12 weeks is biphasic. The first phase of the biphasic curve is antagonized by ketanserin, spiperone and cyproheptadine, but not by prazosin. pK_B values are compatible with a 5-HT_2 receptor mediated effect. The second phase is shifted to the right by prazosin, ketanserin and spiperone but not by cyproheptadine. In this case the pK_B values are compatible with an α_1-adrenoceptor mediated effect. The concentration response curves for ergometrine and phenylephrine in aortae from control rabbits are monophasic and pK_B values again indicate an α_1-adrenoceptor mediated response. Thus, ergometrine contracts the aortae of normal and cholesterol-fed rabbits via activation of α_1-adrenoceptors. The supersensitivity observed in atherosclerotic strips seems to reflect the appearance of a high affinity component mediated by 5-HT_2 receptors.

Persistent hypercholesterolaemia in rabbits is known to induce atherosclerosis and to alter vascular responsiveness to ergometrine (ergonovine) (Henry & Yokoyama 1980; Yokoyama et al 1983; Heric & Tackett 1985). The hypersensitivity of atherosclerotic aortae to ergometrine was selectively antagonized by cyproheptadine (Yokoyama et al 1983). Since cyproheptadine binds with high affinity to the recognition site designated as 5-HT_2 (Leysen et al 1982), and since the receptor subtype mediating contraction of the rabbit aorta has been characterized as 5-HT_2 (Maayani et al 1984), these results would be consistent with hypersensitivity to ergometrine being mediated by 5-HT_2 receptors. This suggestion is based, however, on the results with a single 5-HT receptor antagonist, which apart from 5-HT_2 receptors also antagonizes 5-HT_{1C} receptors (Hoyer 1988). We now report the antagonistic effects of cyproheptadine and other 5-HT receptor antagonists on the contractile response to ergometrine of atherosclerotic rabbit aortae. The choice of the antagonists was such, that a distinction between a 5-HT_2 and a 5-HT_{1C} receptor mediated event would be possible. Ergometrine is used in clinical practice as an oxytocic agent and in a provocation test for Prinzmetal-type of angina. Since in both cases the receptor subtype involved in the clinical effect has not been defined, the present investigation could contribute to the understanding of the mode of action of ergometrine.

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Materials and methods

Male New Zealand white rabbits, with an initial weight between 2·0 and 2·5 kg, were randomized to two dietary groups of 15 animals each. One group was fed normal chow while the other group received pellets containing 2% cholesterol for 12 weeks.

The rabbits were killed with an overdose of pentobarbitone. The thoracic aorta was promptly excised and cleaned of surrounding tissue. Spiral strips of approximately 2·5 cm length and 3 mm width were cut and the endothelial layer removed. Strips were mounted under 2 g tension in Krebs solution bubbled with 95% O_2 and 5% CO_2 at 37°C. The composition of the Krebs solution was as follows [mM]: NaCl 118, KCl 4·7, $CaCl_2$ 2·5, $NaHPO_4$ 1·2, $NaHCO_3$ 25, glucose 11. Changes in tension were recorded with Gould force-displacement transducers attached to a pen-recorder (Texas Instruments).

After equilibration (2 h), the organs were repeatedly stimulated with a submaximal concentration of noradrenaline (0·1 μM) until constant contractions were obtained. Acetylcholine (3 μM) was administered during the last contraction to verify the absence of a functional endothelium (Furchgott & Zawadzki 1980). The tension was reduced to 1·5 g and a cumulative concentration response curve to ergometrine was determined. From one aorta 8 strips were obtained. One was used as control; the remaining 7 were randomly allocated to be incubated in cyproheptadine, ketanserin, spiperone (all at 0·1 μM) or prazosin (0·01 μM) 15 min before ergometrine. This parallel set-up had to be chosen, since between killing the animal and the completion of the ergometrine concentration response curve it took one complete working day. A variation in sensitivity across the 8 different strips was noted and therefore a full randomization of the experimental protocol was ensured. Responses to ergometrine were plotted as % of the contraction to the last administration of noradrenaline.

In a separate experiment, ergometrine (0·1 μM) was tested as an antagonist of 5-HT in aortae from control rabbits. In this case, prazosin (0·1 μM) was added to the Krebs solution, since it has been reported that in rabbit aortae high concentrations of 5-HT stimulate α_1-adrenoceptors (Purdy et al 1987).

Curves were fitted by computer using Feldman's equations for complex ligand-binding systems at equilibrium (Feldman 1972). These equations allow the determination of pD_2 values and