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Uptake of dexamethasone incorporated into liposomes by macrophages and foam cells and its inhibitory effect on cellular cholesterol ester accumulation

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Abstract

To confirm the efficacy of dexamethasone incorporated into liposomes in the treatment of atherosclerosis, the uptake of dexamethasone–liposomes by macrophages and foam cells and its inhibitory effect on cellular cholesterol ester accumulation in these cells were investigated in-vitro. Dexamethasone–liposomes were prepared with egg yolk phosphatidylcholine, cholesterol and dicetylphosphate in a lipid molar ratio of 7/2/1 by the hydration method. This was adjusted to three different particle sizes to clarify the influence of particle size on the uptake by the macrophages and foam cells, and the inhibitory effect on cellular cholesterol ester accumulation. The distribution of particle sizes of dexamethasone–liposomes were 518.7 ± 49.5 nm (L500), 202.2 ± 23.1 nm (L200), and 68.6 ± 6.5 nm (L70), respectively. For each size, dexamethasone concentration and dexamethasone/lipid molar ratio in dexamethasone–liposome suspension were 1 mg dexamethasone mL^{-1} and 0.134 mol dexamethasone mol^{-1} total lipids, respectively. The zeta potential was approximately –70 mV for all sizes. Dexamethasone–liposomes or free dexamethasone were added to the macrophages in the presence of oxidized low density lipoprotein (oxLDL) and foam cells, and then incubated at 37°C. The uptake amount of dexamethasone by the macrophages and foam cells after a 24-h incubation was L500 > L200 > free dexamethasone > L70. The macrophages in the presence of oxLDL and foam cells were incubated with dexamethasone–liposomes or free dexamethasone for 24 h at 37°C to evaluate the inhibitory effect on the cellular cholesterol ester accumulation. The cellular cholesterol ester level in the macrophages treated with oxLDL was significantly increased compared with that in macrophages without additives. L500, L200 and free dexamethasone significantly inhibited this cholesterol ester accumulation. L500, L200 and free dexamethasone also significantly reduced cellular cholesterol ester accumulation in foam cells. In addition, the relationship between the area under the uptake amount of dexamethasone–time curve (AUC) and the inhibition rate of cholesterol ester accumulation in macrophages and foam cells was evaluated. The inhibition rate of cholesterol ester accumulation (%) was related to the AUC in both types of cell. These results suggested that dexamethasone–liposomes would be a useful approach to the development of a novel drug delivery system for atherosclerotic therapy. Furthermore, the prediction of the inhibitory effect of dexamethasone on cellular cholesterol ester accumulation may become possible by using the results of this study.

Introduction

In the progressive process of atherosclerosis, the atherosclerotic lesions develop by accumulation of foam cells derived from macrophages via uptake of oxidized low density lipoprotein (oxLDL) (Ross 1993). Consequently, in atherosclerotic lesions, the macrophages and foam cells accumulate an excess of cholesterol ester in the subendothelial space (Yla-Herttuala et al 1989; Witztum & Steinberg 1991; Ryu et al 1995). The progressive process of atherosclerosis is similar to that of chronic inflammation (Tedgui & Bernard 1994). Several studies have reported that the anti-inflammatory drug dexamethasone inhibits the development of atherosclerosis in-vivo (Makheja et al 1989; Naito et al 1992; Asai et al 1993; Van Put et al 1995; Tauchi et al 2001), and the cellular cholesterol ester accumulation in macrophages and foam cells in-vitro (Tauchi et al 1999, 2000). Therefore, the efficient delivery of dexamethasone to the macrophages and foam cells in the atherosclerotic lesions

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is important for producing an efficient inhibitory effect of dexamethasone on cellular cholesterol ester accumulation.

The dexamethasone–low density lipoprotein complex is efficiently taken up by macrophages and foam cells, and effectively inhibits their cellular cholesterol ester accumulation in-vitro (Tauchi et al 1999, 2000, 2003). Tauchi et al (2001) showed that this dexamethasone complex selectively accumulates in atherosclerotic lesions and effectively inhibits the development of atherosclerosis in a mouse model of atherogenesis. Unfortunately, because low density lipoprotein is a biogenic component, it is very difficult to obtain. Thus, an artificial drug carrier is needed to deliver dexamethasone to macrophages and foam cells. Recently, artificial drug carriers such as liposomes and nanospheres have been used widely for therapeutic applications, because of their ability to alter the pharmacokinetics and reduce the toxicity of the associated drugs. In particular, liposomes have attracted great interest as a potential drug carrier system, because they are easy to prepare, their particle size can be altered easily, and lipid formulations and surface modification are possible. The liposomes accumulate in atherosclerotic lesions (Hodis et al 1991) and, therefore, may be useful as carriers for dexamethasone delivery to the macrophages and foam cells in these lesions. The uptake of a drug carrier, such as liposomes, by the macrophages is generally particle size-dependent (Rahman et al 1982; Heath et al 1985; Harashima et al 1994, 1995; Huong et al 1998). However, there is little information on the uptake of drug carrier by the cells contributing to the progressive process of atherosclerosis, such as macrophages in the presence of oxLDL and foam cells. The particle size of the drug carrier may also be an important factor in the efficient uptake of dexamethasone by macrophages in the presence of oxLDL and foam cells. Thus, liposomes are suitable for the detailed evaluation of the uptake of dexamethasone by these cells because their particle size can be modified.

In this study, dexamethasone was incorporated into liposomes (particle sizes: 500, 200 and 70 nm). The uptake of the dexamethasone–liposomes by macrophages and foam cells, and their inhibitory effect on cellular cholesterol ester accumulation in these cells, were investigated in-vitro to evaluate the efficacy of dexamethasone–liposomes used in atherosclerotic therapy. In addition, the relationship between the pharmacokinetics and pharmacodynamics of dexamethasone in the macrophages and foam cells was evaluated.

Materials and Methods

The Committee of Laboratory Animal Center (No. 05-004) approved the animal experimental plan, which conformed to the Guiding Principles for the Care and Use of Experimental Animals in Hokkaido Pharmaceutical University. Informed consent was obtained from all volunteers and all procedures were conducted in accordance with the Declaration of Helsinki.

Materials and animals

Dexamethasone was purchased from Wako Pure Chemicals Co., Ltd (Osaka, Japan). Egg yolk phosphorylcholine was purchased from NOF Co. (Tokyo, Japan), cholesterol from Wako Pure Chemicals Co., Ltd. Dicetylphosphate was obtained from Sigma Chemical Co. (St Louis, MO). [^{3}H]Dexamethasone and [^{3}H]cholesterylhexadecylether ([^{3}H]CHE) were from NEN Life Science Products, Inc. (Boston, MA). All other reagents were of analytical grade and commercially available.

Male ICR mice were obtained from Japan SLC, Inc. (Hamamatsu, Japan), and used for experiments at six-weeks of age.

Preparation of dexamethasone–liposomes

Preparation of dexamethasone–liposomes was performed by modification of a conventional lipid film hydration method (DiMatteo & Reasor 1997). Briefly, egg yolk phosphorylcholine, cholesterol and dicetylphosphate in a lipid molar ratio of 7/2/1 and dexamethasone were dissolved in chloroform/methanol (9/1), followed by evaporation to obtain a thin film. The film was completely hydrated by phosphate-buffered saline (PBS, pH 7.4) to obtain dexamethasone–liposomes. The dexamethasone–liposomes were extruded five times through polycarbonate filters with pore sizes of 600, 200 and 100 nm (Nuclepore, CA). The particle size of the dexamethasone–liposomes was determined by photon correlation spectroscopy using Coulter N4 plus a submicron particle analyser (Coulter Co., Miami, FL). The distributions of particle sizes of dexamethasone–liposomes (means ± s.d.) were 518.7 ± 49.5 nm (L500), 202.2 ± 23.1 nm (L200), and 68.6 ± 6.5 nm (L70) after extrusion. The zeta potential of dexamethasone–liposomes was determined by a laser Doppler method using a zeta potential analyser (Zeta Plus, Nikkiso Co., Ltd, Tokyo, Japan). The zeta potential was approximately –70 mV for all sizes of dexamethasone–liposomes. [^{3}H]Dexamethasone and [^{3}H]CHE as a non-exchangeable lipid phase marker (Pool et al 1982; Derksen et al 1987) were used as a label for the dexamethasone–liposomes. According to the HPLC analysis as described below, the dexamethasone concentration and dexamethasone/lipid molar ratio in all sizes of dexamethasone–liposomes were 1 mg dexamethasone mL^{-1} and 0.134 mol dexamethasone (mol total lipids)$^{-1}$, respectively.

Determination of dexamethasone concentration in dexamethasone–liposomes

The dexamethasone concentration in dexamethasone–liposomes was measured by HPLC. A mixture of the dexamethasone–liposomes (50 μL) and d-equilenin (as an internal standard, 50 μL) was extracted with CH_2Cl_2 (5 mL). A 4-mL sample of the organic phase was evaporated at 30°C. The residue was dissolved in 120 μL CH_3CN/water (42/58), and a 50 μL sample was subjected to HPLC using a system (Shimadzu Co., Kyoto) involving a 5 μm Mightysil RP-18 GP column (4.6 × 150 mm, Cica-Merck, Kanto Chemical). The mobile phase was CH_3CN/water (42/58) and the flow rate was 1.2 mL min^{-1}. The eluate was monitored at 254 nm and quantified using a model C-R6A chromatopac integrator (Shimadzu). The concentrations were determined with respect to a standard curve of dexamethasone.

Preparation of oxLDL

Low density lipoprotein (LDL, d = 1.019~1.063 g mL^{-1}) was isolated by sequential ultra-centrifugation (100 000 *g*) from the plasma of healthy volunteers (Hatch & Less 1968). The LDL fraction was dialysed against PBS (pH 7.4) at 4°C, and then concentrated to 1 mg protein mL^{-1} using Centriprep 30 (Millipore Co., Bedford, MA). The oxidized low density lipoprotein (oxLDL) was prepared by incubation with 5 μM $CuSO_4$ for 20 h at 37°C (Tauchi et al 1999). Oxidation of LDL was confirmed by electrophoresis.

Preparation of the macrophage and foam cell monolayer

The peritoneal cells from unstimulated mice were suspended at a concentration of 10^6 cells mL^{-1} in RPMI 1640 medium (Gibco, BRL, Life Technologies, Rockville, MD). Samples (1 mL) of cell suspension were transferred to 24-well culture plates (Becton Dickinson, Lincoln Park, NJ). The plates were incubated for 90 min at 37°C with 5% CO_2. After incubation, non-adherent cells were removed by washing three times with RPMI 1640 medium. The macrophage monolayer was then placed in fresh RPMI1640 medium for 20 h at 37°C with 5% CO_2. The foam cells were induced from the macrophage monolayer by incubation with oxLDL (50 μg protein/well) for 20 h at 37°C with 5% CO_2. After incubation, the foam cell monolayer was washed three times with RPMI 1640 medium to remove the oxLDL. Foam cell formation was checked by determination of the cellular cholesterol ester level.

Uptake of dexamethasone–liposomes by macrophages and foam cells

[^{3}H]Dexamethasone incorporated into liposomes or dexamethasone incorporated into [^{3}H]CHE-labelled liposomes was added to macrophages or foam cells, and then the cells were incubated at 37°C with 5% CO_2. The concentrations in the medium were 25 nmol dexamethasone mL^{-1} and 186 nmol total lipid mL^{-1}. The uptake of dexamethasone–liposomes by macrophages was examined in the presence of oxLDL. In brief, dexamethasone–liposomes and oxLDL (50 μg protein/well) were added to the macrophages at the same time. Free dexamethasone was used as a comparison. At each time point after incubation, the medium was removed and the cells were washed three times with RPMI 1640 medium. The cells were extracted with 1 mL 0.1 M NaOH solution. The extract (100 μL) and 0.9 mL Hionic-Fluor (Packard BioSci. Co., Meriden, CT) were mixed and stored overnight. The radioactivity of [^{3}H]dexamethasone and [^{3}H]CHE was determined by scintillation counting. The cell protein level was determined by Coomassie Protein Assay reagent (Pierce Chemical Company, Rockford, IL) using bovine serum albumin as a standard (Bradford 1976).

Effect of dexamethasone–liposomes on cellular cholesterol ester accumulation in macrophages and foam cells

The macrophages in the presence of oxLDL and foam cells were incubated with dexamethasone–liposomes (1 μM as a final dexamethasone concentration in medium) for 24 h at 37°C with 5% CO_2. The effect of dexamethasone–liposomes on cellular cholesterol ester accumulation in macrophages was examined in the presence of oxLDL. In brief, the macrophages were incubated with dexamethasone–liposomes and oxLDL (50 μg protein/well) was added at the same time. Free dexamethasone was used as a comparison. After incubation, the medium was removed and the cells were washed three times with RPMI 1640 medium. Lipids and proteins in the cells were extracted with 0.5 mL hexane/isopropanol (3/2) and 1 mL 0.1 M NaOH solution, respectively. The cellular cholesterol was determined by a fluorometric enzymatic method (Tauchi et al 2001). Briefly, 200 μL extract and 2.5 mg Triton-X100 were mixed and evaporated. The residue was suspended in 200 μL enzyme mixture and the suspension was incubated for 30 min at 37°C. The enzyme mixture for free cholesterol assay contained 0.12 U mL^{-1} cholesterol oxidase, 45 U mL^{-1} peroxidase, 0.5 mg mL^{-1} homovanilic acid and 2 mg mL^{-1} Triton-X100 in 100 mM sodium phosphate buffer (pH 7.0). The enzyme mixture for total cholesterol assay contained 0.36 U mL^{-1} cholesterol esterase in the above mixture. To stop the reaction following incubation 2.8 mL 0.1 M NaOH solution was added. The fluorescence intensity in the suspensions was determined at an excitation wavelength of 323 nm and an emission wavelength of 420 nm. The cell protein level was determined as described above. The cellular cholesterol ester level was calculated by subtracting the free cholesterol from the total cholesterol.

Data analysis

The area under the uptake amount of dexamethasone–time curve (AUC) was determined by a trapezoid formula. The inhibition rate of cholesterol ester accumulation (%) was determined as follows: Inhibition rate of cholesterol ester accumulation (%) in macrophages = 100 × (cholesterol ester level in macrophages treated with oxLDL − cholesterol ester level in macrophages treated with dexamethasone–liposomes or free dexamethasone)/cholesterol ester level in macrophages treated with oxLDL.

Inhibition rate of cholesterol ester accumulation (%) in foam cells = 100 × (cholesterol ester level in foam cells treated without additives − cholesterol ester level in foam cells treated with dexamethasone–liposomes or free dexamethasone)/cholesterol ester level in foam cells treated without additives.

Statistics

Statistical analysis was performed by a one-way analysis of variance followed by Dunnett's test using Stat View software (Abacus Concepts Inc., Berkeley, CA).

Results

Uptake of dexamethasone–liposomes by macrophage and foam cells

The uptake of dexamethasone–liposomes by macrophages in the presence of oxLDL and foam cells was examined.

The time courses of the uptake amount of dexamethasone and liposomes are shown in Figures 1 and 2. The uptake amount of dexamethasone by macrophages after addition of dexamethasone–liposomes was particle-size dependent at each time point (Figure 1A). The uptake amount of dexamethasone by macrophages after addition of L70 was lower than that after addition of free dexamethasone at each time point (Figure 1A). The uptake amount of liposomes by macrophages was also particle-size dependent

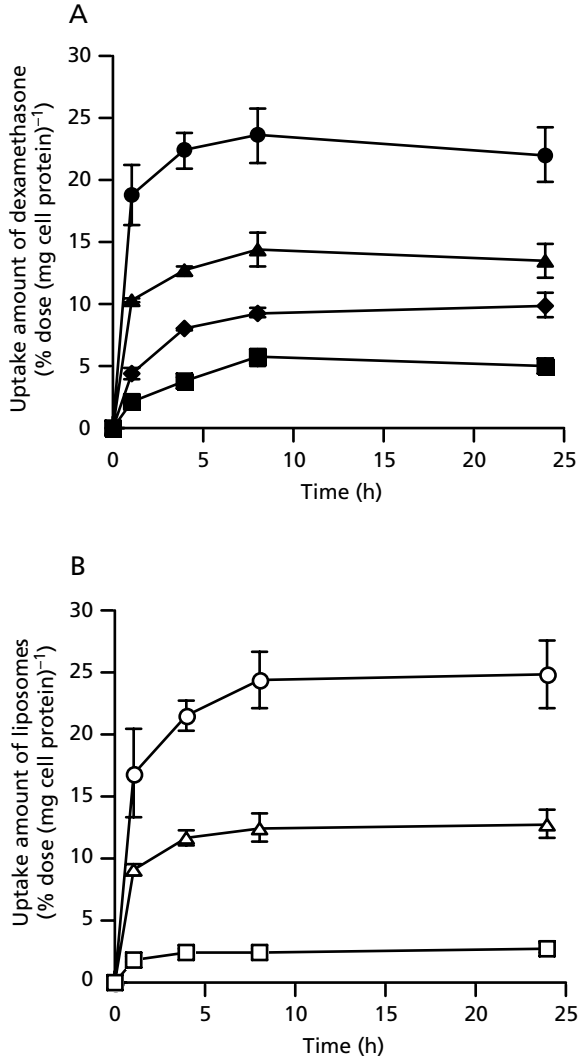

Figure 1 Uptake of dexamethasone (A) and liposomes (B) by macrophages after addition of dexamethasone–liposomes. [^{3}H]Dexamethasone incorporated into liposomes (25 nmol dexamethasone/well, 186 nmol total lipid/well) or dexamethasone incorporated into [^{3}H]CHE-labelled liposomes (25 nmol dexamethasone/well, 186 nmol total lipid/well) and oxLDL (50 μg protein/well) were added to macrophages at the same time, and then macrophages were incubated at 37°C. At each time point, cell extracts were collected. [^{3}H]Dexamethasone and [^{3}H]CHE in cell extracts were determined by scintillation counting. Cell protein was determined as described in Materials and Methods. Each value represents the mean ± s.e. of four experiments. Symbols: closed circle or open circle, L500; closed triangle or open triangle, L200; closed square or open square, L70; closed diamond, free dexamethasone.

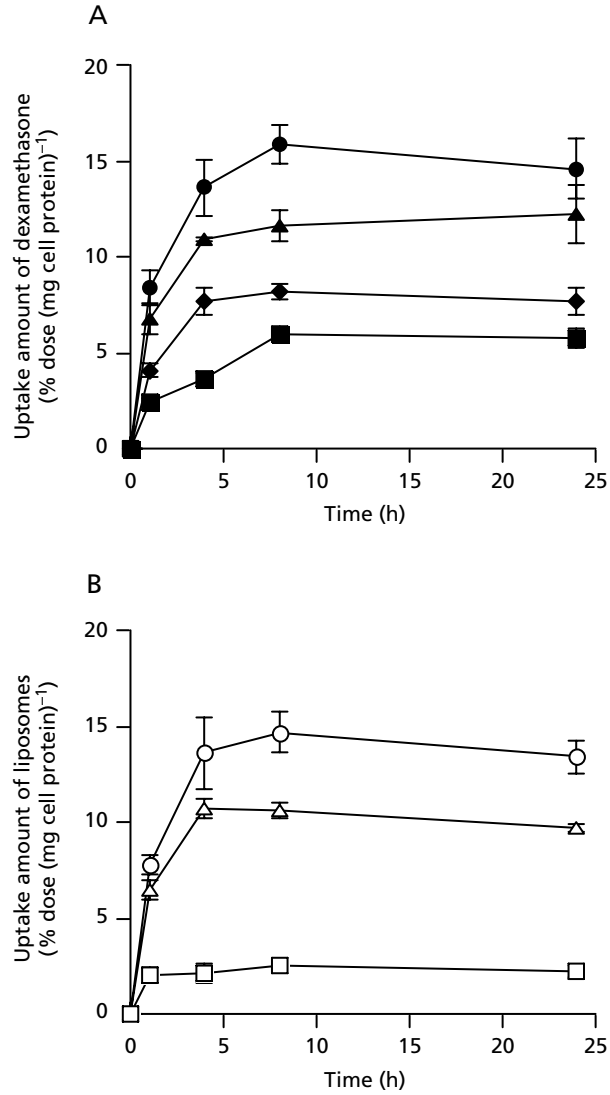

Figure 2 Uptake of dexamethasone (A) and liposomes (B) by foam cells after addition of dexamethasone–liposomes. [^{3}H]Dexamethasone incorporated into liposomes (25 nmol dexamethasone/well, 186 nmol total lipid/well) or dexamethasone incorporated into [^{3}H]CHE-labelled liposomes (25 nmol dexamethasone and 186 nmol total lipid/well) were added to foam cells, and then foam cells were incubated at 37°C. Other details are the same as those in Figure 1.

at each time point (Figure 1B). The uptake of dexamethasone and liposomes by the foam cells after addition of dexamethasone–liposomes was similar to the uptake by macrophages (Figure. 2).

Effects of dexamethasone–liposomes on cellular cholesterol ester accumulation in macrophages and foam cells

The effects of dexamethasone–liposomes on cellular cholesterol ester accumulation in macrophages produced by

incubation with oxLDL and foam cells were examined. The cellular cholesterol ester level in macrophages with added oxLDL was significantly increased compared with that in macrophages without additives (Figure 3). L500, L200 and free dexamethasone significantly inhibited this cholesterol ester accumulation (Figure 3). L500, L200 and free dexamethasone also significantly reduced cellular cholesterol ester accumulation in foam cells (Figure 4). In addition, effects of unbound liposomes (not enclosing dexamethasone) on the cholesterol ester accumulation in both cells were examined. The size of the unbound liposomes (500, 200 and 70 nm) did not affect the amounts of cholesterol ester accumulated in macrophages and in foam cells (data not shown).

Relationship between the pharmacokinetics and pharmacodynamics

The relationship between the pharmacokinetics and pharmacodynamics of dexamethasone in macrophages and foam cells was evaluated. The relationship between the AUC and inhibition rate of cholesterol ester accumulation (%) is summarized in Figure 5. The inhibition rate of cholesterol ester accumulation (%) was related to the AUC in both types of cell.

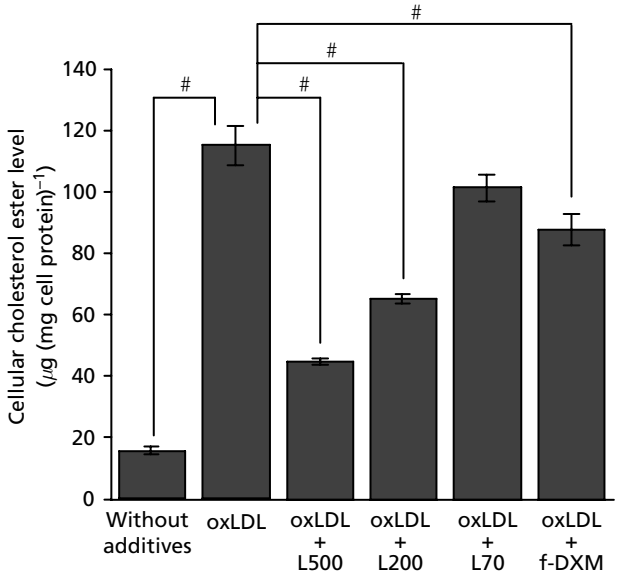

Figure 3 Effects of dexamethasone–liposomes on cellular cholesterol ester accumulation in macrophages produced by incubation with oxLDL. Dexamethasone–liposomes (1 μM as a final dexamethasone concentration in medium) and oxLDL (50 μg protein/well in medium) were added to macrophages at the same time, followed by incubation for 24 h at 37°C. After incubation, cells were washed and cell extracts were collected. f-DXM, free dexamethasone. Cholesterol ester and protein in cell extracts were determined as described in Materials and Methods. Each value represents the mean ± s.e. of six experiments. #$P < 0.0001$, in Dunnett's test.

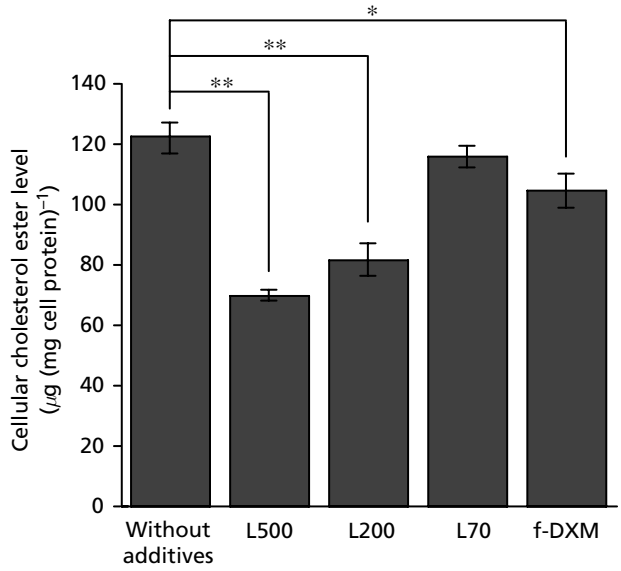

Figure 4 Effects of dexamethasone–liposomes on cellular cholesterol ester accumulation in foam cells induced from macrophages by preincubation with oxLDL. Dexamethasone–liposomes (1 μM as a final dexamethasone concentration in medium) were added to foam cells, followed by incubation for 24 h at 37°C. Other details are the same as those in Figure 3. *$P < 0.05$, **$P < 0.0001$, in Dunnett's test.

Discussion

In this study the uptake of dexamethasone–liposomes by macrophages and foam cells in-vitro, and their inhibitory effect on cellular cholesterol ester accumulation in these cells in-vitro were investigated in a pilot animal experiment in-vivo to evaluate the efficacy of dexamethasone–liposomes on atherosclerotic therapy. In addition, the relationship between the AUC, reflecting the pharmacokinetics, and the inhibition rate of cellular cholesterol ester accumulation, reflecting the pharmacodynamics, was evaluated in macrophages and foam cells.

The uptake of dexamethasone–liposomes by macrophages in the presence of oxLDL and foam cells was examined (Figures 1 and 2). The time course of the uptake amount of dexamethasone by both cell types after addition of L500 and L200 were similar to that of liposomes (Figures 1 and 2). This result indicated that intact L500 and L200 incorporating dexamethasone were taken up by both cells. Interestingly, the uptake amount of dexamethasone by both cells after addition of L70 was approximately half that after the addition of free dexamethasone at each time point (Figures 1A and 2A). L70 exhibited approximately 50% initial release against the total encapsulated amount in an in-vitro release test (data not shown). Thus, in the culture medium after addition of L70, it was thought that 50% of the total addition was in the form of free dexamethasone. The uptake amount of liposomes after the addition of L70 was minor (Figures 1B and 2B), and so the addition of L70 was almost the same as half the addition of free dexamethasone. This explained why the time course of the uptake amount of dexamethasone by both cells after

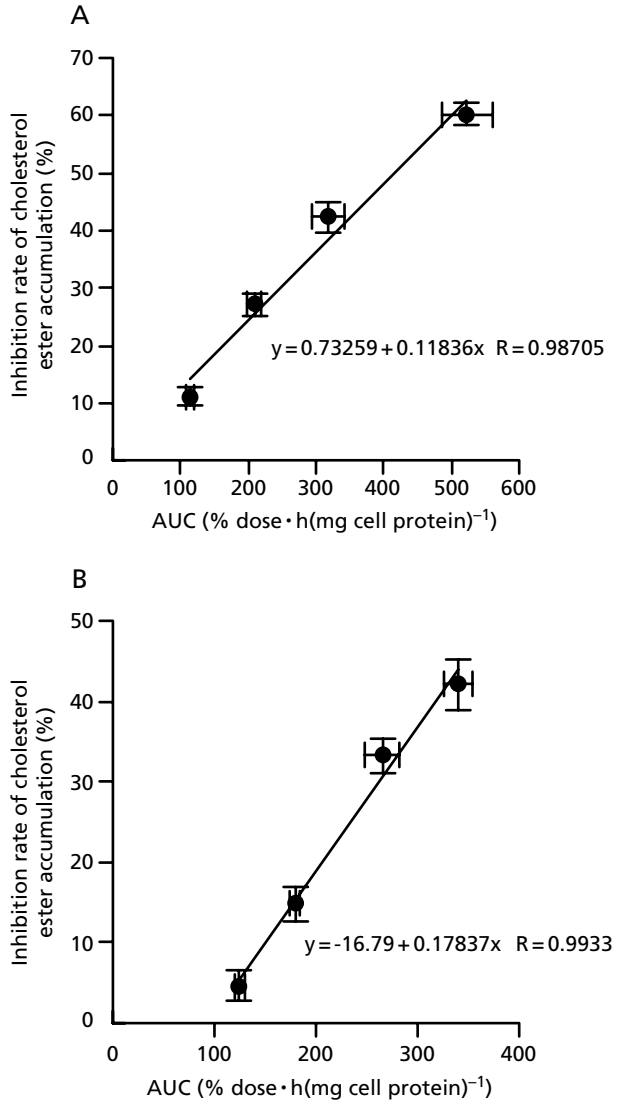

Figure 5 Relationship between the AUC and inhibition rate of cholesterol ester accumulation. A. AUC and inhibition of cholesterol ester accumulation in macrophages. B. AUC and inhibition of cholesterol ester accumulation in foam cells.

addition of L70 was approximately 50% of that after addition of free dexamethasone. The uptake amount of liposomes by foam cells after addition of L500 was lower than that by macrophages at each time point (Figures 1B and 2B). On the other hand, the uptake amount of liposomes by foam cells after addition of L200 and L70 was similar to that by macrophages at each time point (Figures 1B and 2B). These results indicated that the uptake of large particles by foam cells was difficult. However, the uptake of L500 by foam cells may be improved markedly by surface modification of L500 using a ligand which is specifically recognized by foam cells. Although the uptake amount of dexamethasone by macrophages following incubation for 48 h after addition of dexamethasone–LDL complex was approximately 40% of the dose/mg cell protein, the uptake amount of dexamethasone following incubation for 24 h was approximately 10% of the dose/mg cell protein (Tauchi et al 2003). The uptake amounts of dexamethasone by macrophages following incubation for 24 h after addition of L500 and L200 was approximately 22% of the dose/mg cell protein and 13% of the dose/mg cell protein, respectively (Figure 1). Although the uptake amount of dexamethasone by macrophages following incubation for 24 h after addition of L200 was similar to the uptake amount under the same conditions after addition of dexamethasone–LDL complex, the uptake amount of dexamethasone by macrophages under the same conditions after addition of L500 was approximately 2-fold that after addition of dexamethasone–LDL complex. These results indicated that L500 was more effective in delivering dexamethasone to macrophages than dexamethasone–LDL complex in-vitro. The uptake amount of dexamethasone by foam cells following incubation for 24 h after addition of dexamethasone–LDL complex was not reported. Thus, under the same conditions, comparison of dexamethasone–liposomes and dexamethasone–LDL complex was not possible as far as the uptake by foam cells was concerned.

The effects of dexamethasone–liposomes on cellular cholesterol ester accumulation in macrophages caused by incubation with oxLDL and foam cells were examined (Figures 3 and 4). The inhibitory effect of L500 on cellular cholesterol ester accumulation in both types of cell was the highest of all (Figures 3 and 4). These results corresponded to the uptake amount of dexamethasone after addition of dexamethasone–liposomes or free dexamethasone. Dexamethasone–LDL complex inhibited the cellular cholesterol ester accumulation in macrophages and foam cells (Tauchi et al 1999, 2000). The inhibition rates of cellular cholesterol ester accumulation in macrophages and foam cells after 48-h incubation with 1 μM dexamethasone–LDL complex were approximately 40% and 25%, respectively (Tauchi et al 1999, 2000). In addition, the inhibition rates of cellular cholesterol ester accumulation in macrophages and foam cells after 24-h incubation with L500 at the same dose were approximately 60% and 40%, respectively (Figures 3 and 4). In contrast, the drug efficacy of L500 was greater than that of the dexamethasone–LDL complex and the drug effect appeared faster compared with the dexamethasone–LDL complex. L200 also inhibited cellular cholesterol ester accumulation in macrophages and foam cells under the same conditions and produced an inhibition of approximately 42% and 33%, respectively (Figures 3 and 4). The uptake amount of dexamethasone by macrophages following incubation for 24 h after addition of L200 was almost the same as that after addition of dexamethasone–LDL complex. However, the effect of L200 appeared faster than for the dexamethasone–LDL complex. This result suggested that the release of dexamethasone from L200 in the cytosol of macrophages was faster compared with that from the dexamethasone–LDL complex. These results showed that liposomes were an efficient drug carrier for the delivery of dexamethasone to macrophages and foam cells. According to a previous report, dexamethasone inhibited the degradation of oxLDL in macrophages as well as the uptake of oxLDL by macrophages (Asai et al 1993). Although the inhibitory mechanisms of dexamethasone in foam cells are unknown, dexamethasone may accelerate the cholesterol esterase activity in foam cells. It is thought that L500 and L200 inhibited

cholesterol ester accumulation in macrophages and foam cells by the inhibitory mechanism much the same as dexamethasone as described above. Unbound liposomes of different size (500, 200 and 70 nm) did not affect the amounts of cholesterol ester accumulated in macrophages and in foam cells (data not shown). This indicated that the inhibitory effect of cholesterol ester accumulation by dexamethasone–liposomes was based on the uptake of dexamethasone, not the uptake of the liposomes themselves.

The relationship between the pharmacokinetics and pharmacodynamics of dexamethasone in macrophages and foam cells was evaluated. The inhibition rate of cholesterol ester accumulation (%) was related to the AUC in macrophages in the presence of oxLDL and foam cells (Figure 5). This indicated that it should be possible to predict the inhibitory effect of dexamethasone on the cellular cholesterol ester accumulation in macrophages and foam cells. Thus, the relationship between the pharmacokinetics and pharmacodynamics of dexamethasone in macrophages and foam cells may give useful information to help in the development of novel pharmaceutical preparations for atherosclerotic therapy.

Conclusion

Efficient delivery of dexamethasone to macrophages and foam cells in-vitro was possible using a liposomal preparation of dexamethasone. Furthermore, dexamethasone–liposomes effectively inhibited cellular cholesterol ester accumulation in macrophages and foam cells. These findings suggested that using liposomes as drug carriers would be an efficient drug delivery system for atherosclerotic therapy. In this study, the efficacy of dexamethasone–liposomes on atherosclerotic therapy in-vitro was confirmed in a pilot in-vivo experiment. It is now necessary to evaluate the efficacy of dexamethasone–liposomes in an animal model of atherogenesis in-vivo.

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