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Eye-concentrated distribution of dexamethasone carried by sugar-chain modified liposome in experimental autoimmune uveoretinitis mice

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ABSTRACT

Corticosteroid is generally accepted as a standard therapeutic agent for active inflammatory (and) autoimmune eye diseases. In an attempt to develop a system to deliver corticosteroid most efficiently to the target eye, a sialyl-Lewis X (sLe^x)-conjugated liposome was adopted as a candidate for a carrier of dexamethasone (Dexa) and tissue distribution of intravenous Dexa with the modified liposome as well as Dexa alone as control was studied in normal and experimental autoimmune uveoretinitis (EAU) mice. Intravenous Dexa (1 mg) was widely distributed in all the tissues (eye, brain, heart, lung, liver, kidney, spleen and intestine) examined in similar manner in both mice and Dexa concentration was lowest in the eye except the brain. The tissue concentrations of Dexa in EAU group were all significantly lower than those in the corresponding tissues in normal group. Intravenous Dexa (2 μg) in the modified liposome was almost concentrated to the eye in EAU mice, reaching 13.84 ng/mg tissue in contrast to 2.34 ng/mg tissue in Dexa (1 mg) alone administered EAU mice. In normal mice, Dexa was undetectable in any tissues examined and thus the effect of the modified liposome was not observed. The result supported the potentiality of sLe^x-conjugated liposome for target-delivering of corticosteroid to inflamed eye.

Systemic administration of corticosteroid is now generally accepted as a standard therapy for active inflammatory (and) autoimmune eye diseases (12, 16), particularly for exacerbated uveitis. However, the use of corticosteroids in such cases is often in high doses for long periods of time and it causes significant increase in the incidence of severe adverse reactions that have commonly been observed in therapeutic use of the steroids. This is considered to be attributable, at least in part, to non-specific wide systemic distribution of the agents administered.

In the current study, in an attempt to develop a system to deliver systemically administered corticosteroids most efficiently to the target inflamed eye, we adopted a sialyl-Lewis X (sLe^x)-conjugated liposome, newly developed by Hirai *et al.* (10), as a candidate for drug carrier agent. Intravenous sLe^x-conjugated liposome was shown to accumulate significantly in inflammation regions in the arthritis mouse and the accumulation showed a shift of the liposome from blood vessels to the surrounding tissue (10). This observation suggested potential use of the modified liposome for carrying the drug to inflamed eye most efficiently.

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We analyzed the effect of sLe^x-conjugated liposome on tissue distribution of systemic dexamethasone (Dexa), one of the most often used corticosteroids for severe inflammatory (and) autoimmune eye diseases, in normal and experimental autoimmune uveoretinitis (EAU) model mice (8, 14). Since eye concentration of systemic Dexa in the mouse, especially in the EAU model mouse was extremely low, first of all we had to develop a micro-quantitation method with high enough sensitivity, specificity and efficiency. This was satisfied by radioimmunoassay (RIA). The current results obtained by using the RIA system clearly demonstrated an almost eye-specific Dexa delivering effect of sLe^x-conjugated liposome in EAU mice.

EAU (clinical grade 2) was induced in C57BL/6 mice (8–10 weeks old, about 20 g body weight; Japan SLC, Shizuoka, Japan) by subcutaneous immunization with human IRBP (interphotoreceptor retinoid binding protein) peptide, as reported previously (2, 15). Treatment of the animals conformed to the Statement for the Use of Animals in Ophthalmic and Visual Research of the Association for Research in Vision and Ophthalmology (http://www.arvo.org/eweb/). The sLe^x-conjugated liposome containing Dexa (sodium phosphate form) was prepared according to the method reported previously (9, 10). The obtained liposome included 2 μg of the drug in 100 μL of the preparation. Normal and EAU mice were sacrificed 1 h after intravenous administration from the tail of 100 μL of Dexa (sodium phosphate form 1 mg/100 μL of saline) or liposomal Dexa preparation (sodium phosphate form 2 μg/100 μL of saline). Tissues (about 50 mg each) were excised immediately and homogenized with a homogenizer at 4°C for 2 min (× 2) in 450 μL of 10 mM phosphate buffer (pH 7.4) containing 1% Triton X with TissueLyser (Qiagen, Valencia, CA, USA), followed by centrifugation at 15,000 × *g* at 4°C for 10 min. The supernatant was used as samples for RIA determination. Serum samples for RIA were prepared as described previously (3, 17). The final sample volume was 150 μL which corresponded to 40 mg of each tissue. Labeled antigen, [1,2,4-^{3}H]-Dexa and sheep anti-Dexa antiserum were purchased from Amersham Pharmacia Biotech (Little Chalfront, Buckinghamshire, England) and Biogenesis (Kingston, NH, USA), respectively. Normal sheep serum and rabbit anti-sheep IgG (H + L) antiserum were obtained Jackson ImmunoResearch Laboratories (West Grove, PA, USA). Other reagents for RIA were in chemical grade. RIA was performed as reported previously (1) with some modifications. Briefly, the standard diluent consisted of 100 mM sodium phosphate buffer (pH 7.4) containing 0.5% bovine serum albumin and 0.1% NaN_3. In each assay tube, 0.4 mL of standard diluent, 0.05 mL of standard antigen (Dexa) or unknown sample, 0.1 mL of labeled antigen (about 20,000 cpm) and 0.1 mL of sheep anti-Dexa antiserum (diluted 1 : 3,200) were mixed and the mixture was incubated at 4°C for 40 h. To the mixture, 0.05 mL of normal sheep serum (diluted 1 : 50), 0.05 mL of anti-sheep antiserum (diluted 1 : 10) and 0.75 mL of 5% polyethylene glycol 6000 were added, followed by further incubation at 4°C for 2 h and centrifugation at 3,000 rpm at 4°C for 45 min. The supernatant was aspirated and the precipitate was dissolved in 100 μL of 5% SDS, mounted on a Ready Cap (Beckman Instruments, Fullerton, CA, USA) and dried in an oven at 70°C for 1 h. The radioactivity of the solid scintillator was measured with a scintillation counter. Triplicate assays were performed. The data obtained were analyzed using unpaired two-tailed Student's t-test and presented as mean with standard deviation (SD). A probability of $p < 0.05$ was considered to be statistically significant.

A typical standard curve of the newly developed RIA system, transformed in logit-log scale, showed a good linearity over the range of 1.25 to 160 ng/mL of the standard antigen (Fig. 1). Precision and accuracy of intra- and interassay in the RIA system are summarized in Table 1. In the range of 3.1–

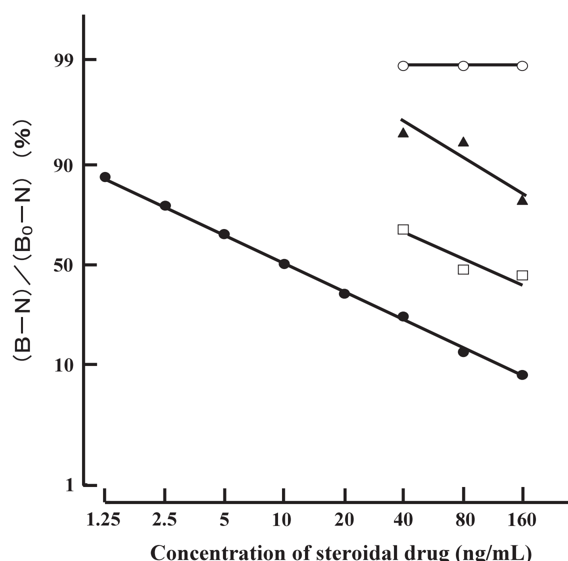

Fig. 1 A typical standard curve (●) of dexamethasone radioimmunoassay system and dilution curves of other steroidal hormones, cortisol (○), prednisolone (▲) and betamethasone (□).

Table 1 *Precision and accuracy of intra- and interassay in dexamethasone radioimmunoassay*

Expected values (ng/mL)	Day 1 assay			Day 5 assay			Significance in difference between day 1 and day 5 assay values
	Observed values* (ng/mL)	Recovery (%)	CV (%)	Observed values* (ng/mL)	Recovery (%)	CV (%)	
100.0	100.5 [5.8]	100.5	5.8	98.2 [12.2]	98.2	12.4	N.S.
50.0	50.4 [2.0]	100.8	4.0	51.1 [0.7]	102.2	1.4	N.S.
25.0	24.6 [1.0]	98.4	4.1	25.8 [1.6]	103.2	6.2	N.S.
12.5	12.7 [0.6]	101.6	4.7	12.2 [0.4]	97.6	3.3	N.S.
6.3	6.4 [0.3]	102.4	4.7	6.4 [0.2]	102.4	3.1	N.S.
3.1	3.4 [0.4]	108.8	11.8	3.3 [0.2]	105.6	6.1	N.S.

* Mean [S.D.], n = 4

Table 2 *Tissue distribution of dexamethasone in normal and EAU mice after administration of dexamethasone or dexamethasone in sLe^x-liposome*

	Dexamethasone (dose = 1 mg)				Dexamethasone in sLe^x-liposome (dose = 2 μg)			
	Normal mice (n = 4)		EAU mice (n = 4)		Normal mice (n = 5)		EAU mice (n = 5)	
	ng/mg tissue	ratio	ng/mg tissue	ratio	ng/mg tissue	ratio	ng/mg tissue	ratio
Eye	4.02 [0.12]	1	2.34 [0.32]	1	ND	–	13.84 [5.10]	1
Blood	9.44 [1.00]	2.35	3.20 [0.32]	1.37	ND	–	ND	~0
Brain	1.13 [0.17]	0.28	0.53 [0.08]	0.23	–	–	–	–
Heart	15.70 [2.43]	3.91	4.60 [0.65]	1.97	–	–	–	–
Lung	15.16 [1.69]	3.77	5.22 [0.63]	2.23	–	–	–	–
Liver	40.90 [4.16]	10.17	10.48 [1.70]	4.48	ND	–	1.40 [0.84]	0.101
Kidney	18.91 [2.62]	4.70	4.73 [0.19]	2.02	ND	–	ND	~0
Spleen	7.84 [1.67]	1.95	3.29 [0.32]	1.41	ND	–	ND	~0
Intestine	15.56 [3.55]	3.87	5.86 [0.80]	2.50	ND	–	1.98 [0.79]	0.143

Mean [S.D.], ND: not detectable, –: not measured or not calculated, ratio: to the level in eye

100 ng/mL, the analytical recoveries of Daxa spiked were 97.6–108.8% and the coefficients of variance (CV) of the system were 1.4–12.4%. There was no significance in difference between day 1 and day 5 assay values. The crossreactivities of cortisol (hydrocortisone), prednisolone and betamethasone in this system were < 1%, 0.5–1% and 10–20%, respectively, in the range of 20–80 ng/mL (Fig. 1). The data proved satisfactorily high sensitivity and specificity of the currently developed Dexa RIA, which validated the use of this assay system for measurement of extremely low concentration of Dexa, for example, in the tissues of mice administered the agent systemically.

Table 2 summarizes the tissue distribution of Dexa 1 h after intravenous administration of Dexa (1 mg) or Dexa (2 μg) in sLe^x-conjugated liposome to normal and EAU mice. In the case of Dexa administration, Dexa was widely distributed in all the tissues examined in both normal and EAU mice. Assuming the Dexa concentration in the eye as 1, the tissue distribution manners of Dexa in the tissues examined were not substantially different between normal and EAU groups. The concentration of Dexa in the eye was lowest among eight kinds of tissues examined except the brain. Very high concentration of Dexa in the liver was noticeable in both groups of mice. On the other hand, marked discrepancy between Dexa (1 mg)-administered normal and EAU groups of mice was observed in Dexa concentrations in the tissues examined. The tissue concentrations of Dexa in EAU group were all lower as compared with those in the corresponding tissues in normal group. The differences were statistically significant ($p < 0.05$ or $p < 0.01$). The reason for this observation can not be explained at present. Our previous paper has claimed that a systemic inflammation in EAU mice increased the clearance of prednisolone from the blood (7). Although the EAU mouse is commonly used as an animal model of inflammatory (and) autoimmune eye diseases, the whole aspect of physiology and pathology of EAU

still remains to be clarified. But it is still conceivable that the significantly lower concentrations of Dexa in the tissues of EAU group examined are due, at least in part, to the increased clearance of Dexa in EAU group of mice. The current RIA system is highly specific to the authentic type of Dexa, although the crossreactivities of Dexa metabolites were not examined yet in the system. It is thus supposed that the current RIA measured almost specifically authentic Dexa in mouse tissues. On the other hand, in the case of intravenous Dexa in sLe^x-conjugated liposome, Dexa was almost concentrated to the eye in EAU mice. The dose of Dexa (2 µg) in this case was only 1/500 of that (1 mg) of Dexa alone administration, and yet the concentration of the agent in the eye of EAU mice reached 13.84 ng/mg tissue in contrast to 2.34 ng/mg tissue in the case of Dexa alone administered to EAU mice. In normal mice given Dexa alone, the eye concentration of the agent was higher (4.02 ng/mg tissue) than that (2.34 ng/mg tissue) in EAU group, while it was as low as undetectable level in sLe^x-liposome-Dexa group.

Liposomes have been accepted as one of the most effective carriers for site-targeting delivery of drugs. Modification of the liposome surface has been reported to improve its target cell-specific binding and delivery (6, 13). The mutual recognition of E-selectin classified into the C-type lectin and sLe^x has been best clarified in the inflammation model (4, 5, 11). Hirai *et al.* noticed the specific recognition and binding between lectin and sugar chain for giving active targeting ability to the liposomes (18) and they prepared the liposome binding sLe^x on the surface in order to deliver substances to inflammation or tumor regions (10). We applied in the current study the sLe^x-conjugated liposome as a carrier for Dexa delivery and thus demonstrated highly efficient eye-directed delivering ability of sLe^x-conjugated liposome for systemic Dexa in EAU mice.

The current results obtained provided evidence of the potentiality of sLe^x-conjugated liposome as a carrier for eye-targeted delivery of corticosteroids in inflammatory eye diseases to exert its primary pharmacological effect most efficiently with minimum side effect.

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