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Body Distributioin of RGD-mediated Liposome in Brain-targeting Drug Delivery

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RGD conjugation liposomes (RGD-liposomes) were evaluated for brain-targeting drug delivery. The flow cytometric *in vitro* study demonstrated that RGD-liposomes could bind to monocytes and neutrophils effectively. Ferulic acid (4-hydroxy-3-methoxycinnamic, FA) was loaded into liposomes. Rats were subjected to intrastriatal microinjections of 100 units of human recombinant IL-1β to produce brain inflammation and caudal vein injection of three formulations (FA solution, FA liposome and RGD-coated FA liposome). Animals were sacrificed 15, 30, 60 and 120 min after administration to study the body distribution of the FA in the three formulations. HPLC was used to determine the concentration of FA *in vivo* with salicylic acid as internal standard. The results of body distribution indicated that RGD-coated liposomes could be mediated into the brain with a 6-fold FA concentration compared to FA solution and 3-fold in comparison to uncoated liposome. Brain targeted delivery was achieved and a reduction in dosage might be allowed.

Key words——brain-targeting; body distribution; flow cytometric; ferulic acid

INTRODUCTION

Many drugs fail to enter the brain following systemic administration because of the highly lipophilic nature of the blood-brain barrier (BBB), the presence of efflux transport processes, endothelial cell metabolism and plasma protein binding. Hence the management of brain-related diseases with presently available therapeutic strategies is often very difficult.[1–3] In many neurological diseases, leukocytes including monocytes and neutrophils can cross an intact BBB and be delivered to the site of injury or infection of the brain.[3–7] Then, one of the strategies to deliver drugs into the brain under pathological conditions is to exploit these inflammation cells as a target delivery system.[8] RGD peptide (Arg-Gly-Asp) can combine with integrin receptors which are expressed on the surface of leukocytes (neutrophils and monocytes).[9,10]. Then, RGD-liposomes can be devised for selective and preferential presentation to blood monocytes/neutrophils, and taken into the brain in response to the inflammation recruitment.[11] Brain targeted delivery can be achieved by the exquisite design. FA, one of the most important active components of several TCMs (Traditional Chinese Medicines), is applied to the treatment of neurovascular and cardiovascular diseases for many years.[12–16] However, its poor penetration of brain limits the application of FA in treating neurodegenerative diseases. Among various approaches to improve the body distribution, RGD-liposome appears to be a more promising strategy.

The purpose of this study was to investigate the brain targeting of RGD-liposome. RGD was introduced to the carboxylic end group cholesterol of esterified cholesterol with succinic anhydride. The flow cytometric study demonstrated that RGD-liposomes could bind to monocytes and neutrophils effectively. The results of body distribution indicated that RGD-coated FA liposomes could be mediated into the brain with a 6-fold FA concentration compared to FA solution and 3-fold in comparison to uncoated liposome.

EXPERIMENTAL

Materials and Reagents RGD, succinic anhydride, purchased from sigma. IL-1β are products of Pierce (USA). 1-Palmitoyl-2[12-[(7-nitro-2–1, 3-benzoxadiazol-4-yl) amino] dodecanoyl]-Sn-Glycero-3-Phosphoethanolamine (NBD-PE) was purchased from Avanti Polar Lipids Inc. (Alabaster, AL). OptiPrep™ was from AXIS-SHIELD PoC AS (Norway). Ferulic acid (FA) and salicylic acid were products of Wanma Synthetic Drug Corp. (Zhejiang, China). All other chemicals were of analytical grade.

Evaluation on Cell Binding Ability of RGD-lipo-

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some *in Vitro* Monocytes or neutrophils were separated from fresh human peripheral blood within 2 h of collection according to density gradients by centrifugation method with OptiPrep™. Monocytes and neutrophils were incubated with NBD-labeled RGD-liposomes or NBD-labeled control liposomes for 1 h at 37°C. Flow cytometer was used to assay the cell binding of washed, unfixed monocytes and neutrophils. A total of 10000 counts within the unlabeled cell-gated population were collected for each example.

Body Distribution Studies *In vivo* studies, the concentration of FA was determined by HPLC with salicylic acid as internal standard. Male Wistar rats (12 weeks, 250±20 g) provided by China Medical University Animals Center were divided randomly into three groups, each of three rats (the experiments complied with the requirements of the National Act on the use of experimental animals, People's Republic of China). Animals were subjected to intrastriatal microinjections of 100 unit of human recombinant IL-1β in order to produce brain inflammation[17] and caudal vein injection of three formulations including FA solution, plain FA liposomes and FA RGD-liposomes (equivalent FA 5 mg/kg body weight[18]).

Determination of FA in serum Animals were sacrificed after 15, 30, 60, 120 min of administration. Blood was collected into heparinized tubes following decapitation and separated immediately by centrifugation (10000 rpm 10 min). Ten μl of internal standard (salicylic acid), 1 ml of 5% trichloro acetic acid and 1 ml of acetoacetate were added to 150 μl of serum and were mixed by vortexing for 30 s. The mixture was then centrifuged (4000 rpm 15 min). The abstract procedure was repeated with another 1 ml acetoacetate. The organic phase was collected and blown to dryness under Nitrogen protection. 200 μl of mobile phase was used to redissolve the residue for determination of HPLC.

Drug Determination in Different Organs Different organs (heart, liver, spleen, lung, kidney and brain) were washed with PBS and dried, followed by weighted. Every organ sample was homogenized and treated in similar manner of serum. Whole organ was homogenized in case of less than 1.0 g.

Statistical Analysis All results and data were analyzed with the SPSS statistical package using one-way analysis of variance (ANOVA). Probability values of less than 0.05 were considered significant.

RESULTS AND DISCUSSION

The Results of Cell Binding Ability of RGD-liposome *in Vitro* Figure 1 showed the results of cell binding ability of RGD-liposomes to monocytes/neutrophils at flow cytometer. RGD-coated and uncoated formulations showed the same level of background staining at concentration of 270 μM total lipid (equivalent to 5.5 μM peptide accessible for binding). The results showed a significant increase in fluorescence intensity of RGD-coated liposomes compared to uncoated liposome formulations. It indicated that RGD, a ligand for the integrin receptors on neutrophils and monocytes, facilitated receptor-mediated endocytosis which resulted in higher uptake of the liposomes.[19] Also, this indicated that the application of succinic anhydride facilitated to the cell selectivity property of RGD-liposome.

Body Distribution Figure 2 showed the rat body distribution of FA solution, plain FA liposomes and RGD coated liposomes after 15, 30, 60, 120 min of administration, respectively. As shown in Figs. 2 (A) and (B), FA was found mainly in the kidney in FA solution formulation, with concentrations of 22.7 ±1.83 and 25.8±1.96 μg/g after 15 and 30 min of administration, respectively. There was a clear reduction in FA concentration for FA solution in all organs after 60 min of administration according to Fig. 2 (C).

For the two liposome formulations, a continuous

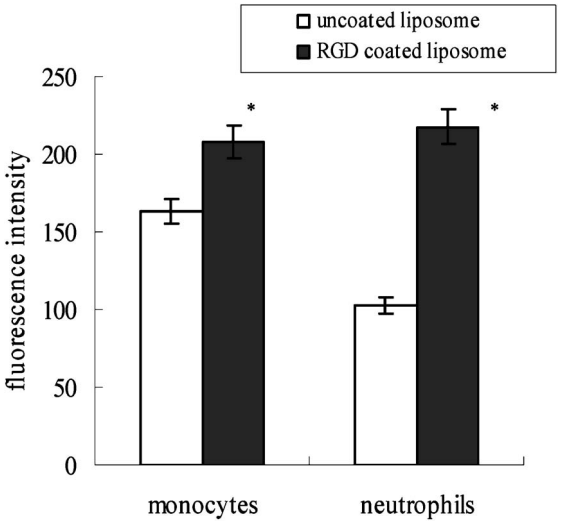

Fig. 1. The Results of Flow Cytometer of Cells Binding Study *in Vitro*.
*represents $p<0.05$.

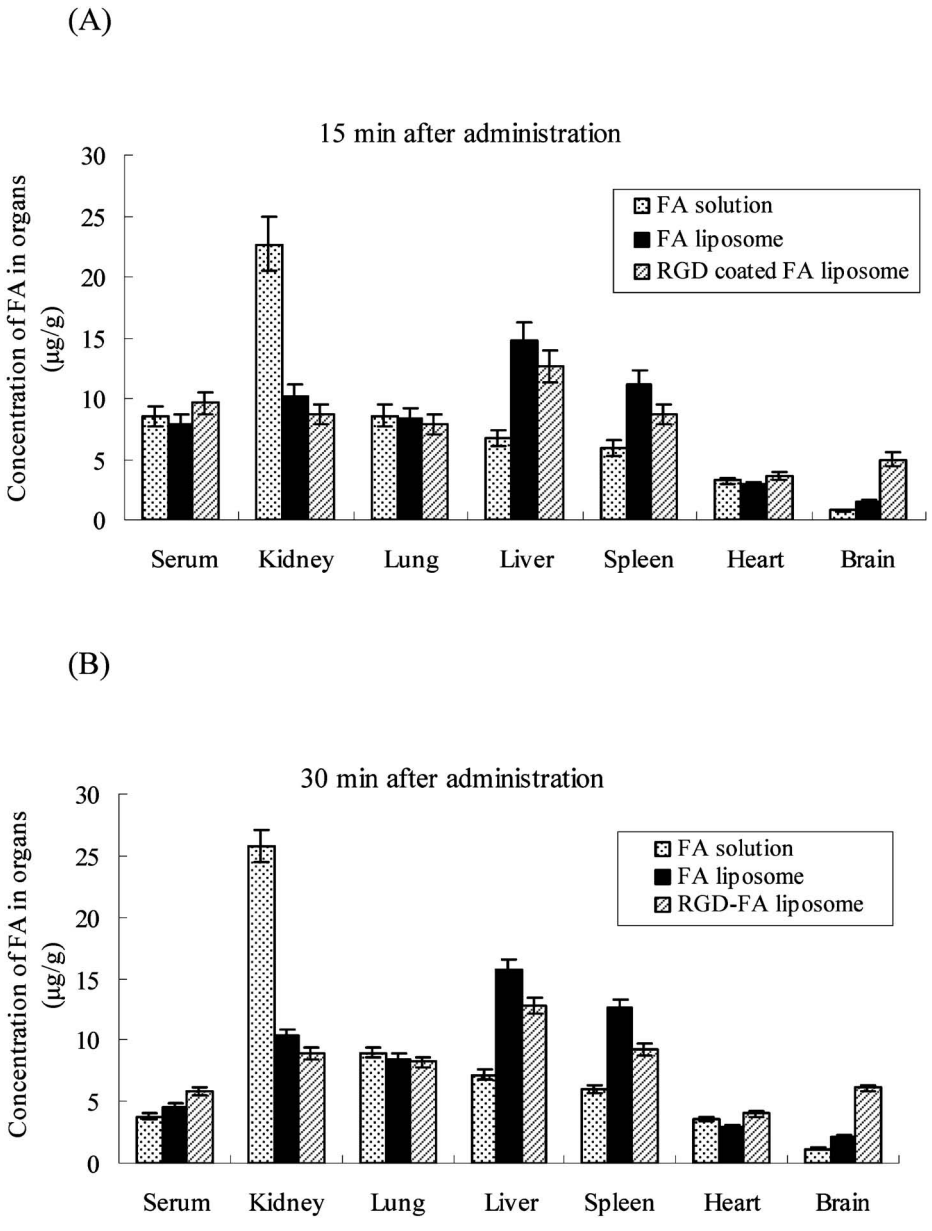

Fig. 2.

increase in FA concentration in some organs was observed after 60 min of administration (Fig. 2(C)) and the elimination of FA in organs showed remarkable slow compared with FA solution (Fig. 2(D)). There is a significant reduction in the FA kidney concentration of the two liposome formulations according to Figs. 2(A)–(D). Hence, it indicated that the body distribution of FA liposomes mainly depends on the distribution behavior of the liposomes *in vivo*. The increase of plain FA liposomes in liver and spleen concentration might due to absorption by the reticuloendothelial system (RES). Thus, liposomes can greatly improve the distribution behavior of FA.

As shown in Fig. 2(A), very little ($0.8\pm0.07\ \mu g/g$) of FA solution formulation reached the brain after 15 min of administration. About six times the concentration of FA ($5.0\pm0.22\ \mu g/g$) of RGD coated liposomes reached the brain target site via cell selectivity (monocytes and neutrophils). Less than twice the concentration of FA ($1.2\pm0.05\ \mu g/g$) of uncoated liposomes reached the brain.

Figure 2(B) showed results of the body distribution after 30 min of administration. RGD coated liposome exhibited a 5.08-fold FA concentration in brain ($6.1\pm0.56\ \mu g/g$) compared to FA solution although a maximum concentration ($1.2\pm0.10\ \mu g/g$) in brain

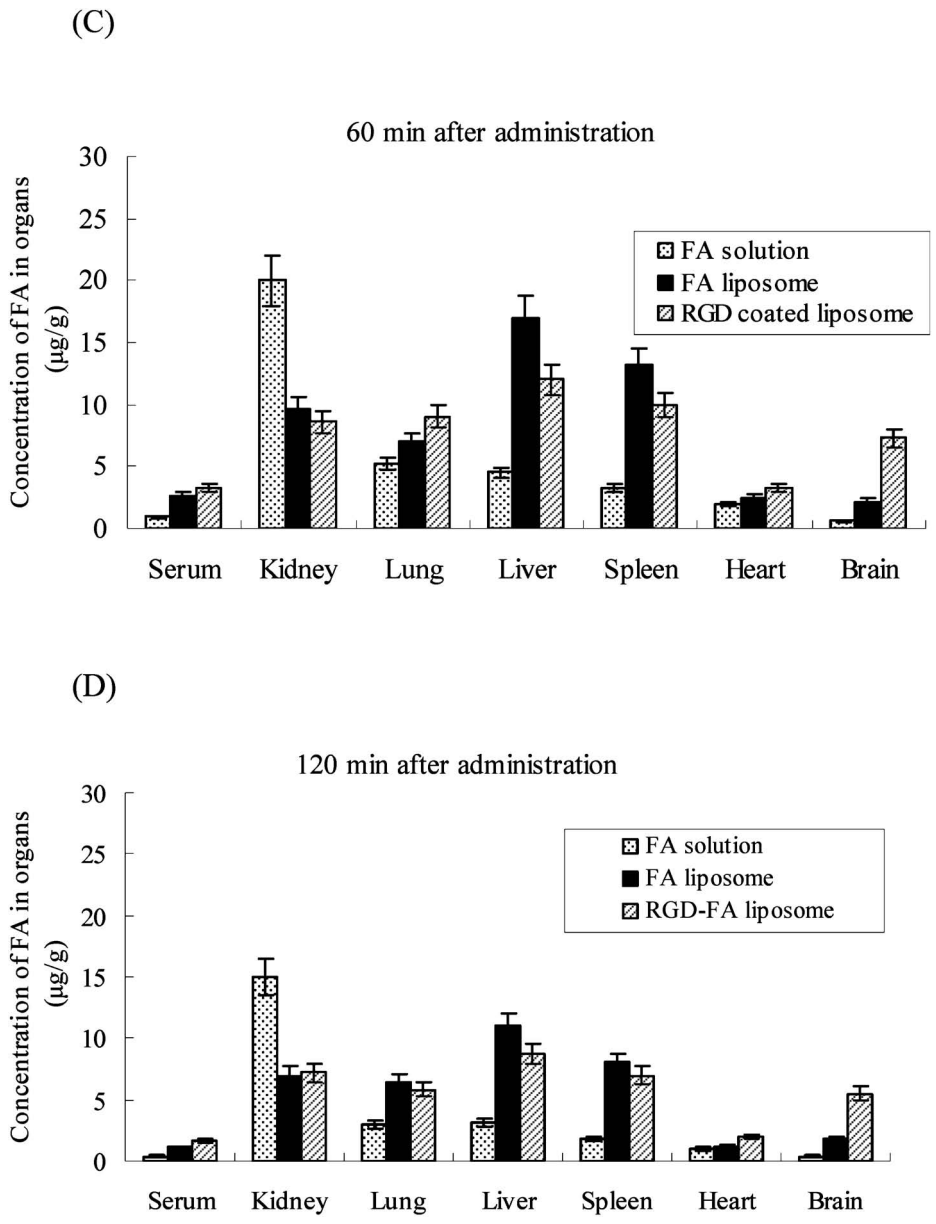

Fig. 2. The Results of Body Distribution of FA Solution, FA Liposome and RGD Coated Liposome after *i.v.* Administration 15 min (A), 30 min (B), 60 min (C) and 120 min (D)

was obtained for FA solution. The maximum brain concentration of FA ($7.3 \pm 0.65\ \mu g/g$, Fig. 2(C)) in RGD coated liposome was 6.1-fold compared with the maximum brain concentration ($1.2 \pm 0.10\ \mu g/g$ Fig. 2(B)) in FA solution. As shown in Fig. 2(D), the remarkable increase in residue of FA in brain in RGD-liposome formulation revealed that the elimination of FA in brain in RGD-liposome formulation was significantly slow compared the other two formulations. Thus, it might allow a reduction in dosage.

Although FA liposomes were also accumulated on liver and spleen, the FA concentration of RGD liposomes showed a significant reduction in liver and spleen and a great increase in brain. Furthermore, RGD coated liposomes could be mediated into the brain with three times FA concentration in comparison to uncoated liposome (Figs. 2(A)–(D)). It indicated that RGD-liposome could be efficiently anchored to monocytes and neutrophils to avoid the uptake of liver and spleen. Hence, RGD-liposome could significantly enhance the concentration of FA in brain. It strongly suggested that the anchor effect of RGD on monocytes and neutrophils can efficiently delivery RGD-liposome into target site.[8]

CONCLUSIONS

In the present study, the molecular weight and chain length of succinic anhydride is suitable for RGD binding to monocytes and neutrophils. RGD-liposomes exhibit a satisfying brain targeting ability *in vivo* study. Hence, this strategy is a promising approach because it can deliver drug directly to the inflammatory site in the brain following the recruitment of leukocytes and allow a reduction in dosage.

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