A targeted liposomal delivery system to liver endothelial cell based on a new peptide motif present in the Apo B-100 sequence

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ABSTRACT SUMMARY

We herein describe the development of a system targeting liver endothelial cell. The RLTRKRGLK sequence (3359-3367) from Apo B-100, which mediates the association of LDL with LDL receptor, was utilized as a ligand for achieving this. In vivo biodistribution and confocal microscopy analysis showed that this peptide and also its reverse peptide modified PEG-LP successfully targets Liver EC. Distribution of RLTR-PEG-LPs were greatly reduced with a pretreatment of unlabeled RLTR-PEG-LPs, not cationic LPs, indicating that the sequence is important for targeting LECs. This is suggesting that the inherited sequence RKR or RXXR is the key motif for targeting LEC.

INTRODUCTION

Liver is the largest organ of the body. Two major types of cells populate liver, namely parenchymal and non-parenchymal cells. Different types of liver diseases are associated with different types of liver cells. So selective drug delivery system would become a good approach to achieve a subsequent efficient therapy in different types of liver diseases. Liposomes are suitable nano-carriers that have the capacity to deliver drug particles to various target cells in vitro or diseased tissues in vivo. Based on these considerations, we selected the ApoB segment RLTRKRGLK (3359-3367) abbreviated here as RLTR for use as a novel ligand in designing a selective targeting system for hepatocytes. But surprisingly this carrier system was accumulated through the blood vessels in liver. In order to examine the targeting ability of this RLTR modified liposomes, we focused on two parameters, one is the cationic nature of this peptide and second is the essential peptide sequence in the following experiments.

EXPERIMENTAL METHODS

Here we examined the cellular uptake of Rhodamine labeled peptide modified PEGylated liposome in liver sinusoidal endothelial cells (LSECs) both in vitro by using FP-750 Spectrofluorometer and in vivo by taking image by confocal microscope and the data was compared with the cellular uptake of a control cationic liposome (DOPE/Chol/DC6-14). We also did some inhibition experiment by using unlabeled peptide modified PEG-LP as inhibitor to check the LSEC selectivity of peptide modified PEG-LP. ICR mice were injected with unlabeled LPs and after 15 min, they were injected with cationic LP or RLTR modified PEG-LP or KLGR (reverse peptide sequence of RLTR) modified PEG-LP. After another 25 min the mice were sacrificed and the livers were perfused with 10 ml of a 40% heparin-PBS solution. The mice livers were then collected, sliced into 0.5 mm x 0.5 mm pieces, stained with Hoechst 33342 and isolectin B4 and then observed by confocal microscopy (A1 Confocal Laser Microscope System, Nikon Instruments Inc., Tokyo, Japan).

RESULTS AND DISCUSSION

In the biodistribution study it was observed that most of the RLTR modified PEG-LP has been accumulated in liver rather than other organs like lung or spleen (Figure 1). There is very few report of targeting liver endothelial cells using cationic or neutral nano carrier. But those systems failed to achieve high accumulation in liver endothelial cells.

![Figure 1: Biodistribution of $^3$H-CHE labeled RLTR-PEG-LPs, RLTR-PEG-LP and LPs in different organ. % of ID is expressed as the mean ± SD (n=4).](image-url)
develop this nanocarrier to target liver EC. As an effort to describe the high accumulation of RLTR modified PEG-LP in liver EC we thought either high cationic charge of the peptide is causing its high accumulation or the peptide sequence itself has specificity to the liver endothelial cell. In vitro and in vivo inhibition study demonstrated that both the RLTR-PEG-LPs and KLGR-PEG-LPs uptake were inhibited by unlabeled peptide modified PEG-LPs. But cationic LPs did not affect its uptake in vivo (Figure 2). So this data rejects the possibility of higher accumulation in LEC due to higher cationic charge. Then we tried to describe the second possible reason. We found that the sequence contain a motif RKR which remains same in both peptide (Figure 3).

Moreover, both the peptide has RXXR motif which is essential for cell penetration (Figure 3).

Both the peptide, RLTR and KTLR, modified LPS seem to have similar targeting ability for liver EC. But we were not able to identify the receptor or the key motif responsible for this targeting. Further study will be required to identify the motif or the receptor.

CONCLUSION
The RLTR or KLGR modified liposomes have the potential for use as a carrier system for the delivery of drugs to liver endothelial cells.

REFERENCES

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