J. Pharm. Pharmacol. 1995, 47: 1053–1054
Received February 8, 1995
Accepted September 11, 1995

Letter to the Editor

Proliposome Targeting to Rabbit Brain Tissue

JIANG-YAN WANG, YU-RU XU, KAI HUANG AND LIAN-YUN SUN

Department of Biochemistry, Shijiazhuang Medical Higher Training School of Chinese PLA, Hebei 050081, China

The application of liposomes as drug carriers critically depends on controlling their disposition in-vivo. The pronounced tendency in-vivo of a single injection of liposomes to localize in the reticuloendothelial system (liver, spleen, bone marrow and blood phagocytes) has long been recognized (Gregoriadis & Neerunjun 1974; Jonah et al 1975; Kimelberg et al 1976). If the objective is to target drugs to tissues other than phagocytic macrophages and blood monocytes, three factors affecting disposition need to be considered; the affinity of liposomes for phagocytes; the ability of liposomes to escape into the extravascular space; and the affinity and specificity of liposomes for the target tissue. These factors affect the way that drugs which are incorporated into the brain cross the blood-brain barrier. This paper concerns the development of proliposomes to target the brain using mannose on the surface of liposomes.

Materials and Methods

Preparation of liposomes

The procedure of Szoka & Papahadjopoulos (1978) was followed. Egg phosphatidylcholine, cholesterol, polyvinylpropylene and mannose (5:5:1:2·5) were dissolved in chloroform and dried in a rotary evaporator to make a thin film. Penicillin in phosphate-buffered saline (200 mg mL^{-1}) was added and the system was purged with nitrogen gas and sonicated for 5 min in a water-bath sonicator.

Preparation of mannitol-base proliposomes

Mannitol was placed in a 100-mL round-bottomed flask and the rotary evaporator assemble. The unit was evacuated (80–96 kPa) and the rotating flask lowered into a water bath at 35°C. A weighed amount of the liposome solution equivalent to half the weight of the mannitol in the flask was added. After drying, the remaining liposome solution was added. Evaporation was continued to dryness, and the material was dried overnight in a desiccator under reduced pressure at room temperature, then packaged under nitrogen in unit-dose vials.

Animal experiments

Twelve New Zealand White rabbits, 2·5–3 kg from the animal Breeding Unit of Pharmacy Factory of North China, were divided into three groups. The first group was injected with proliposome, the second with penicillin and unloaded liposomes, and the third with penicillin only. Each rabbit was injected in the ear vein with 26 mg kg^{-1} liposome and 12 mg kg^{-1} penicillin in 2 mL kg^{-1}. After 30 min, cerebrospinal fluid was withdrawn from the foramen occipitale magnum, and blood from the heart. Grey matter and liver were also removed and extracted. Penicillin content of samples was measured by UV spectrometry. Values are expressed as means ± s.d. for four animals, and results analysed for significant differences by Student's *t*-test.

Results and Discussion

Table 1 shows the incorporation of proliposome-loaded penicillin into normal rabbit tissues. There were significant differences in the penicillin content of brain and cerebrospinal fluid in the liposome-treated animals compared with the controls. Umezawa & Eto (1988) have suggested that the blood-brain barrier cells and glial cells recognize mannose molecules on the surface of the membrane. Our results also

Table 1. Tissue distribution of rabbits receiving penicillin in liposomes.

Experiment	Cerebrospinal fluid ($\mu g\ mL^{-1}$)	Brain ($\mu g\ g^{-1}$)	Plasma ($\mu g\ g^{-1}$)	Liver ($\mu g\ g^{-1}$)
Penicillin-loaded liposomes	$11·2 \pm 1·6^{*++}$	$129·5 \pm 11^{*+++}$	$105·6 \pm 9·9$	$246·6 \pm 47·4^{+}$
Mixed liposomes and penicillin	$5·5 \pm 0·6^{+}$	$60·3 \pm 5·3^{++}$	$130·5 \pm 11·9$	$272·9 \pm 15·4^{++}$
Free penicillin	$3·3 \pm 0·3$	$42·1 \pm 3·7$	$121·8 \pm 22·2$	$147·9 \pm 23·9$

$^{*}P < 0·01$ compared with penicillin mixed with liposomes. $^{+}P < 0·05$, $^{++}P < 0·01$, $^{+++}P < 0·001$ compared with free penicillin.

Correspondence: J.-Y. Wang, Department of Biochemistry, Shijiazhuang Medical Higher Training School of Chinese PLA, Hebei 050081, China.

demonstrate that the liposome-coated mannose reaches brain tissue, suggesting that the mannose coat assists transport of loaded drug through the blood-brain barrier.

Thus, these studies contribute to the possible treatment of brain infection and offer an effective method for the clinical application of other drugs which cannot normally cross the blood-brain barrier.

References

Gregoriadis, G., Neerunjun, E. D. (1974) Control of the rate of hepatic uptake and catabolism of liposome-entrapped proteins injected into rats. Possible therapeutic applications. Eur. J. Biochem. 47: 179-185

Jonah, M. M., Cerny, E. A., Rahman, Y. E. (1975) Tissue distribution of EDTA encapsulated within liposomes of varying surface properties. Biochim. Biophys. Acta 401: 336-348

Kimelberg, H. K., Tracy, T. F., Biddlecombe, S. M. (1976) The effect of entrapment in liposomes on the in vivo distribution of [^{3}H]methotrexate in a primate. Cancer Res. 36: 2949-2957

Szoka, F., Papahadjopoulos, D. (1978) Procedure for the preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc. Natl. Acad. Sci. USA 75: 4194-4198

Umezawa, F., Eto, Y. (1988) Liposome targeting to mouse brain: mannose as a recognition marker. Biophys. Res. Commun. 153: 1038-1044

Book Review

Prostaglandins and Related Compounds:
Ninth international conference, Florence, Italy

(Advances in Prostaglandin, Thromboxane, and Leukotriene Research. Volume 23)
Edited by Bengt Samuelsson, Peter W. Ramwell, Rodolfo Paoletti, Giancarlo Folco, Elisabeth Granström and Simonetta Nicosia
Published 1995 Raven Press Ltd, New York
608 Pages
ISBN 0 7817 0238 0 $95.00

Like the curate's egg, this book is excellent in parts. It is a compilation of more than 100 brief papers based on the main programme of lectures at the ninth International Conference on Prostaglandins and Related Compounds (June 1994), which was as usual held in Florence. The publishers and editors have done well to have produced such a weighty tome to this reasonably high standard in the time available. The price that has been paid is a lack of editorial uniformity (or in some cases, apparent total lack of editorial control), and a dissapointing variability in appearance of each contribution. I guess this might be because author's manuscripts (disk files?) have been used as supplied and not reformatted.

This familiar series of bright blue books now occupies an important place in the libraries of most institutions involved in the prostaglandins and lipid mediators area, and is regularly used by researchers for updating and casual browsing. However, these books are not usually of much use for detailed study of current research problems or for access to methodologies, for which primary journal articles and dedicated sources are necessary. Neither are they generally suitable for students wishing to study or research a specific topic (coverage is designed for bredth not depth), although there are many articles in the present volume which provide a satisfying entrée, giving some of the key references.

Thus to some extent books like these fall between two stools. Although valuable to scientists in the field, they are probably not essential. This latest volume is no exception.

However, it contains much interesting material, and many of the authors are to be congratulated on the brevity, accuracy and interest of their short papers. Hot topics within the last few years in this field and which are adequately represented here include molecular, biological, structural and expression studies on several of the important enzymes like 5-lipoxygenase, leukotriene C_4 synthase, prostaglandin H (PGH) synthase/COX-1 and COX-2 (though disappointingly there is nothing meaty on $cPLA_2$) as well as receptors for group I PLA_2 and various prostanoids (all reflecting the enormous impact cloning and sequencing techniques have had over the last few years for this field), as well as characterization of many of the relevant genes and the mechanisms whereby they are expressed (lots of attention on growth factors).

At a more practical level, it is interesting to note that hopes for pharmaceutical pay-offs have swung back in favour of prostaglandins (rather neglected in the eighties). This is shown here by work on a $PGF_{2\alpha}$ analogue introduced as an ocular hypotensive in glaucoma, PGE_1 in erectile dysfunction (suprisingly longwinded contributions here considering the topic!) and uterine stimulant prostanoid/anti-progestin combinations for early pregnancy termination. There are also several contributions showing that pharmaceutically-speaking the "COX-2 in inflammation should be inhibited" concept is beginning to bear useful fruit.

Taken overall and despite my caveats above, there are many strong points making this a reasonable purchase for aficionados. Perhaps you should add it to the series!

ROBIN HOULT
KINGS COLLEGE LONDON, UK