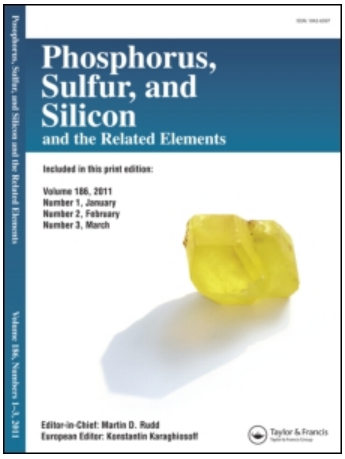

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Liposomes their fate in vivo and their possible therapeutic use (1.V. route). Efficiency of liposome-entrapped ATP in cerebral ischemia

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Liposomes their fate in vivo and their possible therapeutic use (I.V. route). Efficiency of liposome-entrapped ATP in cerebral ischemia.

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Abstract The therapeutic potentialities of liposomes after IV administration are discussed on the light of theirs *in vivo* fate. Studies performed with ATP-loaded liposomes illustrate their ability to increase ATP stability in blood and efficiency in the treatment of experimental brain ischemia in rats.

INTRODUCTION

Liposomes are usually described as passive colloidal drug carriers in which an aqueous space is surrounded by one or several phospholipid bilayers. Initially used as membrane models, liposomes have been, in the early seventies, suggested as potential drug carriers (1). During the last decade, research on therapeutic uses of liposomes has considerably gained interest integrating data from biological studies among which their behavior in the presence of biological fluids and theirs *in vivo* distribution.

IN VIVO FATE OF LIPOSOMES

The *in vivo* fate of liposomes after IV administration depends upon a number of events that take place in the blood compartment. After IV administration, liposomes interact strongly with serum components mainly with opsonins or other serum proteins (2). This interaction increases considerably the uptake of liposomes by RES cells (2). This uptake occurs by an endocytosis process that strongly depends upon opsonization. Liver Kupffer cells are the main macrophages involved in liposomes uptake (3). However, according to their

composition liposomes can avoid, at least to a certain extent, RES uptake and remain in the blood compartment for a long time (4).

Liposomes are unable to pass across most of endothelium capillary walls unless capillaries are discontinuous and liposome small enough to go through the fenestrations. The foregoing properties can be responsible for many applications of liposomes in therapeutics (5). Indeed, liposomes can target drugs to macrophages and improve for example the treatment of lysosomal diseases. Conversely, liposomes may be used to re-route away drugs from tissues particularly sensitive to their side-effects and inaccessible to this type of carrier (heart, kidney). Furthermore liposomes are able to protect drugs from degradation and therefore to increase their half-life in blood.

ATP, a very hydrophilic drug, is unable to cross the blood brain barrier (BBB) after IV injection. In addition, it is very quickly degraded *in vivo* and has a very short half-life in blood. This paper shows that its encapsulation within liposomes allows high plasma level and might lead to an improvement in the treatment of brain ischemia.

ATP-LOADED LIPOSOMES IN EXPERIMENTAL BRAIN ISCHEMIA

Preparation of ATP-loaded liposomes

Liposomes were prepared according to a Reverse Phase Evaporation (REV) method modified to obtain liposomes with a diameter of about 200 nm (6). Liposomes were composed of phosphatidylcholine, cholesterol and sulfatides (molar ratio: 7/2/1). The entrapment efficiency, expressed as the ratio µmol of ATP/µmol of phospholipid, was of 0.28 (6).

Induction of experimental brain ischemia in Rats

Rats were submitted to iterative cerebral ischemia. Ischemia was achieved by clamping the right carotid artery and reducing the mean arterial blood pressure to 50 Torr by withdrawing blood from the femoral artery. At the end of the ischemia, circulation was resumed. Ischemic episodes, lasting 3 minutes were triggered every 15 minute. Intracarotidal (I.C.) administration of the test preparations was performed 6 minutes before the third episode.
As shown in table I, ATP entrapped into liposomes compared to free ATP significantly increased the number of ischemic episodes tolerated before the first irreversible ECoG silence and before death (7).

ATP Plasma levels

Administration of the ATP preparations was performed immediately after the second ischemic episode in hypoxic animals. ATP level in plasma was measured by a bioluminescence method. Figure 1 shows a significant increase in plasma ATP when rats were treated with ATP-loaded liposomes

both after IV or IC injection compared to free ATP. One minute after IC injection, the level of ATP was 10 fold the basal ATP concentration. From then on it decreased slowly but after 60 minutes it was still 4 fold the initial ATP Plasma level. A similar pattern was observed in rats treated through the IV route (8).

TABLE I: Number of ischemics episodes tolerated before ECoG silence or before death after intracarotidal administration

	Before EEG silence	Before death
Sodium Chloride (0.9%)	6.00 ± 0.36	7.83 ± 0.47
Free ATP 1.86 mg/Kg	7.33 ± 0.21	9.00 ± 0.25
Liposomes + Free ATP1.86mg/Kg	8.50 ± 0.22	10.16 ± 0.30
ATP loaded liposomes 1.86 mg/Kg	14.00 ± 0.57	14.83 ± 0.75

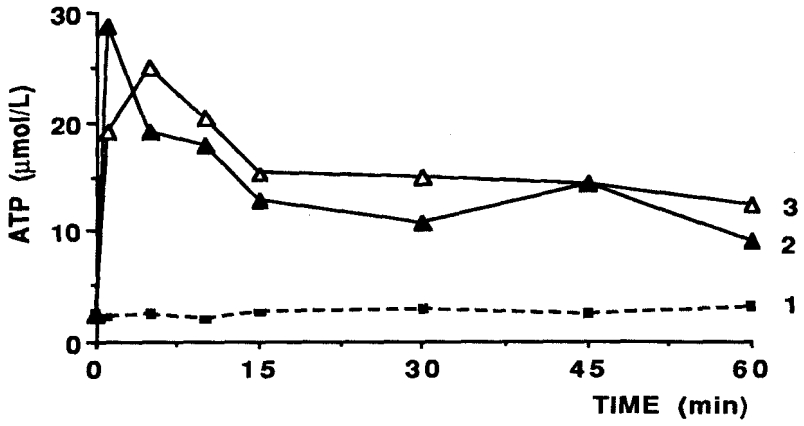

FIGURE 1: Plasma ATP level ($\mu mol.l^{-1}$) in nonischemic rats. Each point represents means of six rats. 1: Intracarotidal injection of free ATP (1.80 $\mu mol.Kg^{-1}$) 2: Intracarotidal injection of liposomally ATP (1.45 $\mu mol.kg^{-1}$). 3: Intravenous injection of liposomally ATP (1.45 $\mu mol\ kg^{1}$).

Brain Slices

Free or liposomally entrapped carboxyfluorescein (CF) were injected (IC) to ischemic rats. Brain slices (8 µm thick) were examined by fluorescence microscopy. After free CF injection, diffuse fluorescence was observed. After administration of ATP-loaded liposomes, brain fluorescence appeared forming small spots which sizes correspond to that of intact liposomes (8). These data suggest that under hypoxic conditions the BBB is opened allowing the liposomes to reach the cerebral parenchyma.

CONCLUSION

The major therapeutics possibilities of liposomes by the IV route are now well known. One of them is their ability to increase and maintain circulating concentrations of certain drugs. This paper clearly demonstrates this capacity in the case of ATP. After IC or IV, liposomes may protect ATP from its usual degradation mainly by endothelial ectonucleases. On the other hand, data obtained with the same liposomes containing carboxyfluorescein suggest that, under hypoxic conditions, the BBB is opened allowing the liposomes to reach the cerebral parenchyma. The mechanism of brain uptake is, however, still unclear: endothelial tight junction opening or endothelial trancytosis.

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