International Journal of Pharmaceutics, 88 (1992) 313–317

IJP 02943

Effect of liposomal encapsulation of chloramphenicol on its transfer across the human placenta in a dual in vitro perfusion system

M.A. Onur [a], C.J. Kirby [b], A. Isimer [c], S. Beksac [d], N. Basci [c], R. Pamir [a], T. Coskun [a] and A. Tumer [a]

[a] *Department of Biology, Hacettepe University, Beytepe, 06532 Ankara (Turkey),* [b] *AFRC Institute of Food Research, Reading Laboratories, Shinfield, Reading RG2 9AT, (UK),* [c] *Department of Pharmacology, Gulhane Military Medical Academy, 06018 Ankara (Turkey) and* [d] *Department of Obstetrics and Gynaecology, Faculty of Medicine, Hacettepe University, 06125 Ankara (Turkey)*

(Received 7 January 1992)
(Modified version received 18 May 1992)
(Accepted 15 June 1992)

Key words: Chloramphenicol; Dehydration-rehydration vesicle; Liposome; Human placenta; Placental perfusion; Placental transfer

Summary

This study attempts to demonstrate that drug transfer from the maternal to the fetal side across the placenta can be controlled by means of drug modifications. For this we encapsulated a teratogenic drug, chloramphenicol, in liposomes of dehydrated-rehydrated type and applied these to an in vitro dual perfusion system in which human placenta was perfused under controlled conditions. Results were compared with those obtained in similar perfusions performed with a solution of free chloramphenicol We observed a statistically significant decrease in the transfer from the maternal to the fetal side when the drug was applied in liposomal form.

Introduction

Most of the widely used drugs are able to pass across the placenta in vivo (Charles and Larsen, 1984; Sadler, 1990) and carry the risk of teratogenesis, especially if taken during the trimester of the pregnancy. The only way to exclude such a possibility completely is to avoid taking drugs during this period. However, this may place the health of the mother at risk. The present study describes preliminary investigations aimed at developing an alternative route to administration of drugs during pregnancy. We looked to see whether presentation of the drug in a liposome-encapsulated form would affect its transfer across the human placenta in an in vitro system.

Liposomes have been widely used as drug delivery vehicles (Fendler and Romero, 1977; Gregoriadis, 1977; Szoka and Papahadjopoulos, 1981) and can selectively target their contents to particular tissues. In the present study, however, the aim was for the liposomes to prevent, or at least decrease the transplacental transport of the drug they carry. A teratogenic antibiotic, chloram-

Correspondence to. A Tumer, Dept of Biology, Hacettepe University, Beytepe, 06532 Ankara, Turkey.

phenicol, was encapsulated in the liposomes and the effect of this formulation on the transplacental transport of the drug was investigated in the human placenta placed in a dual in vitro perfusion system.

Materials and Methods

Preparation of liposomes

Liposomes containing chloramphenicol were prepared by the dehydration-rehydration procedure of Kirby and Gregoriadis (1984). For this, 625 mg of egg phosphatidylcholine (Lipid Products, S. Nutfield, Surrey, U.K.), 312.5 mg of cholesterol (Sigma Chemical Co., Poole, U.K.) and 3.5 mg of α-tocopherol (Roche Products Ltd), initially dissolved in chloroform : methanol (2 : 1 v/v), were dried by rotary evaporation to form a thin film round the inside of a 1 l round-bottomed flask. Solvent traces were removed by drying for 30 min under a stream of nitrogen and the lipid was dispersed by swirling with 50 ml of distilled water, again in an atmosphere of nitrogen. The lipid suspension was then pumped through a microfluidizer (Microfluidics Corp, model 110T) at a working pressure of 12500 lb/inch^2 to form small unilamellar vesicles (SUVs). After mixing with 78 ml of chloramphenicol solution (2.5 mg/ml) the suspension was divided equally between seven large (200 × 3.5 mm) glass tubes. The contents of each tube were shell frozen by swirling in a bath of liquid nitrogen. The frozen mixtures were freeze-dried overnight, in an Edwards EF6 Freeze Drier, at an operating pressure of 0.08 Torr. Dehydration-rehydration vesicles (DRV) were formed by adding 0.6 ml distilled water to each tube, with the aid of vortexing to give complete suspension of the material. The liposomes were resuspended in Earl's solution to a volume of 10 ml and added to the perfusion fluid.

Estimation of the level of drug encapsulated in the liposomes

Before each placental perfusion, the liposomes were separated from non-encapsulated chloramphenicol by diluting with phosphate-buffered saline (PBS) (0.14 M NaCl, 3 mM KCl, 10 mM sodium phosphate, pH 7.4) and washing twice (12000 × g, 40 min, Beckman J2-21). Samples taken from each batch were assayed for their chloramphenicol content by high-performance liquid chromatography (HPLC) and the encapsulation efficiency of the liposomes was expressed as the percent of the initial amount added at the beginning of the preparation procedure that became encapsulated.

Perfusions

Each placenta, obtained at term immediately after vaginal delivery, was transported to the laboratory near the delivery room and washed gently with physiological saline solution at room temperature. A nontraumatized cotyledon was selected and chorionic artery and vein were cannulated. The fetal circulation was immediately begun at a relatively slow flow rate of 3–5 ml/min. The arterial inflow and venous outflow were checked and their equivalence was accepted as a confirmation of the absence of a gross leak in the cotyledon. The perfused cotyledon was then trimmed away from the rest of the placenta and placed in a perfusion chamber slightly modified from that used by Schneider et al. (1972). Perfusions were performed under the conditions described by those authors. Maternal and fetal flow rates were maintained between 10–15 and 6–12 ml/min, respectively. Pressures were 50–90 and 80–140 mmHg for the maternal and fetal circulations, respectively. The perfusate consisted of phosphate-buffered Earl's solution (pH 7.4) containing 4% dextran (Reomacrodex, Pharmacia, Mol. Wt 40000) and 2500 U/ml heparin (Liquemine, Hoffmann). After each perfusion the cotyledon was removed and weighed.

Five perfusions were performed with liposome-encapsulated and six with free chloramphenicol. All perfusions were of the open circuit type in which perfusates were not recirculated.

Analysis of chloramphenicol in the perfusates

At the fetal side, perfusates were collected in separate tubes at 10 min intervals over a total perfusion time of 60 min. Each sample was analysed for its chloramphenicol content by HPLC, using a procedure adapted from that of Sample

et al. (1979). Thus 4 ml of chloroform : isopropanol (50 : 50, v : v) containing 7.5 mg sulphamethazine was added to 1 ml of the perfusate. After vortexing for 30 s it was centrifuged at 3000 × *g* for 10 min. The organic phase was collected and the solvent was completely evaporated under a stream of nitrogen. After addition of 500 μl of methanol and filtering, a volume of 12 μl was injected into the HPLC column. The instrument was Waters (Model ALC205) HPLC equipped with a UV detector set at 278 nm. The μBondapak C_{18} column (8 mm i.d. × 10 cm, 10 μm particle size) was eluted with acetonitrile : acetic acid : water (19.5 : 10 : 79.5, by vol.) at 3 ml/min.

Determination of transferred chloramphenicol

Chloramphenicol transfer from the maternal to the fetal side was determined by using the following equation:

$$\%T = \frac{[(C_{FV} - C_{FA})/(C_{MA} - C_{FA})]}{W} \times Q_R \times 100 \quad (1)$$

where $\%T$ is the percent of the initial chloramphenicol present in the maternal pool at the beginning of a time point that appeared in the fetal side per g weight of the cotyledon, C_{FV}, C_{FA} and C_{MA} denote the concentration of chloramphenicol in fetal venous outflow, fetal arterial inflow (zero in open circuit type perfusions) and maternal arterial inflow, respectively, Q_R is the fetal/maternal ratio of flow rates and W represents the weight of cotyledon in 9.

Results

Five open circuit type perfusions were performed with liposome-encapsulated chloramphenicol. Prior to each perfusion, the encapsulation efficiency (expressed as the fraction of total drug that became entrapped) of the liposomes was calculated. This gave a mean value of 25.4 ± 3.2%. Throughout the 60 min perfusion period, perfusates at the fetal side were collected at 10 min HPLC. Values for percent transfer of drug, were calculated by substituting these concentra-

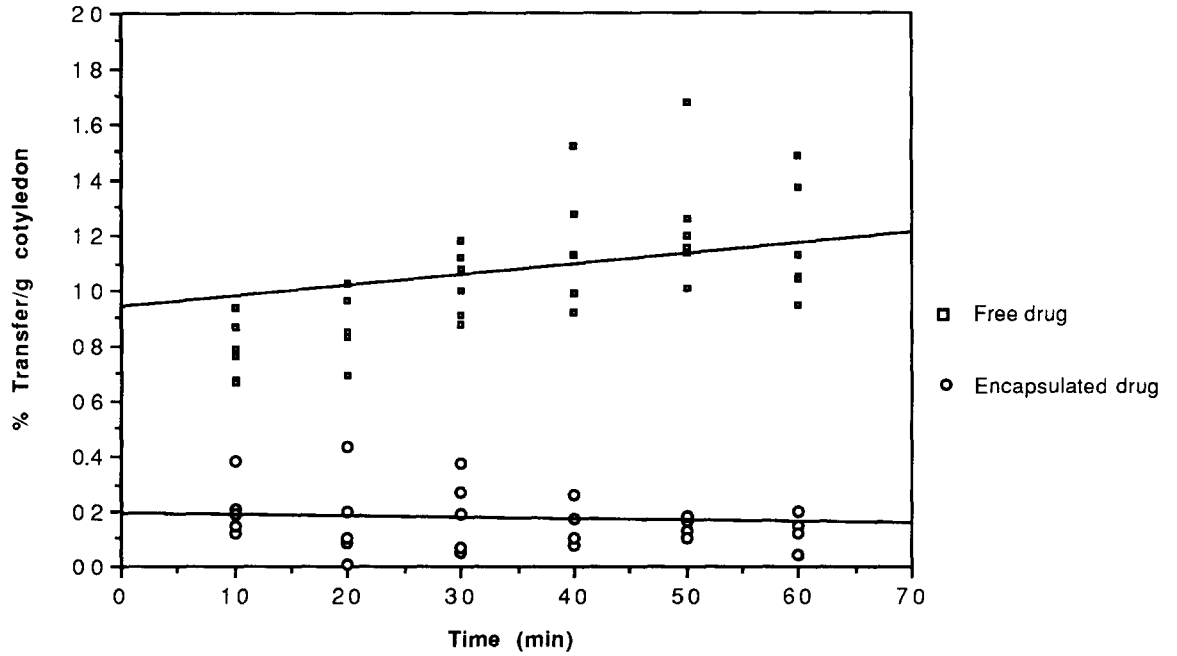

Fig 1 Transfer of chloramphenicol from the maternal to the fetal side in five perfusions performed with liposome-encapsulated (○) and six perfusions with free (□) drug. Solid lines are simple curve fits. Values at each time point are expressed as percent of the initial amount added into the maternal pool that passed to the fetal side per g weight of each cotyledon

tions into Eqn 1. In Fig. 1, the results of these five perfusions are presented together with those of six similar perfusions performed with free (non-encapsulated) drug at an initial concentration of 100 μg/ml. Fig. 1 shows that chloramphenicol transfer to the fetal side is higher when it is given in free form than when liposome-encapsulated. It can also be seen in Fig. 1 that the rate of transfer of free drug is maintained throughout the first 50 min of perfusion, whereas drug transfer decreased throughout this period, when presented in an encapsulated form.

The mean total (%) transfer of chloramphenicol was defined as the proportion of the total drug in the maternal circulation at zero time, that subsequently appeared in the fetal circulation after 60 min. When liposome-encapsulated drug was used, this gave a value of $0.166 \pm 0.07\%$, compared to $1.044 \pm 0.13\%$ when drug was given in a free form. This means that approx. 6-times less drug was transferred to the fetal side when presented in a microencapsulated form. Statistical analyses (t-test) revealed that transfer of drug presented in encapsulated form is significantly lower than that of free drug ($t = 13.6$ at $p < 0.0001$).

Discussion

The dual in vitro perfusion system was selected as the most suitable method for this study, since it is being used by several others and is claimed as a system which eliminates the safety and ethical considerations, while avoiding unwanted effects of maternal and fetal metabolism (Schneider et al., 1972, 1981; Fortunato et al., 1988). The perfusion time was limited to 60 min because of the possibility of degeneration of placental tissue (Hearse, 1985; Kaufmann, 1985).

Chloramphenicol is an antibiotic which is known to be capable of producing pronounced teratogenic effects, especially on the fetal liver, and can cause a condition known as the 'gray baby syndrome' (Keller, 1984). Our results revealed a statistically significant decrease in transfer of chloramphenicol from the maternal to the fetal side, when the drug was presented in a liposome-encapsulated form, as compared to drug in free form. This would be expected to translate to a decreased toxicity of the drug to the fetus.

It should be borne in mind that chloramphenicol was studied here as a model drug, and any benefit would be expected to apply also to other drug systems. It will of course be necessary to confirm that the encapsulated drug has the same efficiency towards the mother, as the free drug would have had. However, many studies have demonstrated improved therapeutic benefits of drugs delivered in this way (Gregoriadis, 1984).

It is very likely that liposomes are acting here as slow release depots, and so the amount of the free (released) drug in a given period of time is kept low. Therefore, the amount of the drug crossing the placenta during the same period is also low. These results suggest that presenting the drug in a microencapsulated form can control the amount crossing the placenta, and thus enable therapy of the mother during pregnancy, with substantially reduced risk to the unborn baby.

Acknowledgements

This work was supported by the Turkish Scientific and Technical Research Council. We wish to thank the AFRC Institute of Food Research Laboratory.

References

Charles, D. and Larsen, B., Placental transfer of antibiotics In Ristuccia, A.M. and Cunha, B.A. (Eds), *Antimicrobial Therapy,* Raven, New York, 1984, pp. 519–533.

Fendler, H.J., Romero, A., Liposomes as drug carriers. *Life Sci*, 20 (1977) 1109–1120

Fortunato, S.J, Bawdon, R.E. and Baum, M., Placental transfer of cefoperazone and sublactam in the isolated in vitro perfused human placenta *Am J Obstet. Gynecol*, 159 (1988) 1002–1006

Gregoriadis, G., Targeting of drugs. *Nature,* 265 (1977) 407–410.

Gregoriadis, G., *Liposome Technology,* vols 1–3, CRC Press, Boca Raton, FL, 1984.

Hearse, D.J., Detection and prevention of tissue injury during hypoxia and ischemia. In Schneider, H. and Dancis, J

(Eds), *Contributions to Gynaecology Obstetrics,* Vol 13, Kargel, Basel, 1985, pp. 48–58

Kaufmann, P , Influence of ischemia and artificial perfusion on placental ultrastructure and morphometry In Schneider, H. and Dancis, J. (Eds), *Contributions to Gynaecology Obstetrics,* Vol. 13, Kargel, Basel, 1985, pp 18–26

Keller, H , Miscellaneous antibiotics chloramphenicol and thiamphenicol In Dukes, M.N.G. (Ed.), *Meyler's Side Effects of Drugs,* 10th Edn, Elsevier, Amsterdam, 1984, Chap 27, pp. 492–494

Kirby, C. and Gregoriadis, G , Dehydration-rehydration vesicles a simple method for high yield drug entrapment in liposomes *Biotechnology,* 2 (1984) 979–984

Sadler, T W., *Medical Embryology,* Williams and Wilkins, Baltimore, 1990

Sample, R.H B., Gick, R M., Kleiman, B.M., Smith, W.J and Oei, P.O T., High-pressure liquid chromatographic assay of chloramphenicol in biological fluids *Antimicrob Agents Chemother*, 15 (1979) 491–493.

Schneider, H , Panigel, M and Dancis, J., Transfer across the perfused human placenta of antipyrine, sodium and leicinge. *Am J Obstet Gynecol.,* 114 (1972) 822–828.

Schneider, H., Challier, J C and Dancis, J., Transfer and metabolism of glucose and lactate in the human placenta studied by a perfusion system in vitro *Placenta,* (Suppl 2) (1981) 129–138

Szoka, F Jr. and Papahadjopoulos, D., Liposomes: preparation and characterization. In Knight, C.G (Ed), *Liposomes From Physical Structure to Therapeutic Applications,* Elsevier, Amsterdam, 1981, pp 51–82.