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CYCLOPHOSPHAMIDE LOADED ALBUMIN MICROSPHERES: LIVER ENTRAPMENT AND FATE IN MICE

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ABSTRACT

Microspheres offer the possibility of target selectivity through choice of appropriate size or surface charecteristics, slow release of drug and also minimize systemic toxicity. The active substance of this investigation, cyclophosphamide (CP), interferes with the growth of cancer cells which are eventually destroyed. Since side effects of CP are frequently dose related, by incorporating low dose of CP to human serum albumin (HSA) microspheres, the normal body cells are not affected while the tumour cells are destroyed.

Cyclophosphamide microspheres were prepared by the modification of the method of Scheffel et al and Gürkan et al. 2,3-butanedione was used as a cross-linking agent. The albumin microspheres containing CP were labelled by ^{99m}Tc by incorporating $SnCl_2.2H_2O$ at a concentration of 5% of the matrix material. All the microspheres used in this study ranged between 1-5 μm.

A suspension of ^{99m}Tc labelled cyclophosphamide microspheres was injected into swiss albino mice intravenously. At 15 min,30 min,

6 h and 24 h mice were killed and the organs assayed for radioactivity accumulated in each organ. 1 hour later the radioactivity in the liver increased to 4.73 percent. By 24 hours, 2.68 percent of the radioactivity was found in the liver. Whereas the percentage of free cyclophosphamide at 1 and 24 hours was 2.22 and 2.57 percent, respectively. Based on the evidence obtained from these results, the application of CP loaded HSA microspheres seems advantages in accumulation in liver.

INTRODUCTION

Microspheres offer the possibility of target selectivity through choise of appropriate size or surface characteristics, slow release of drug and also minimize systemic toxicity. The advantage of formulating drugs so that they are physically trapped in the capillary bed of certain tumour bearing organs and there slowly release drug is that high concentration of drugs are maintained locally (1).

One approach to decrease the host toxicity of anticancer agents, while maintaing or even increasing the cytotoxic effect on the tumour cells, is to target the drug to the specific location of the tumour. A way to reach this goal is to use drug - loaded, biodegradable, injectable microspheres which are targeted to specific organs on the basis of their size.

The active substance of this investigation, Cyclophosphamide (CP), belongs to the group of medicines called alkylating agents. It is taken by mouth or given by injection to treat some kinds of cancer. Cyclophosphamide interferes with the growth of cancer cells which are eventually destroyed. Since side effects of CP are frequently dose related, by incorparoting low dose of CP to HSA microspheres the normal body cells are not affected, while the tumour cells are destroyed.

In the present study, biodegradable albumin microspheres of ^{99m}Tc labeled cyclophosphamide were designed to fall approximatly in 5 μm size range and their entrapment and fate in the liver of mice have been examined.

EXPERIMENTAL

Materials

The active substance was cyclophosphamide (İbrahim-Etem İstanbul-Turkey). The matrix material is human serum albumin (HSA) (Behringwerke AG, Marburg). The hardening agent used is 2,3-butanedione (BASF). Technetium (^{99m}Tc) was obtained from a generator (Amersham). Swiss albino mice of about 30-35 g in weight were used.

Methods

Preparation of HSA microspheres

Cyclophosphamide microspheres were prepared by the modification of the method of Scheffel et al (2) and Gürkan et al (3). The aqueous solution containing 25% (w/v) HSA, 10 mg $SnCl_2.2H_2O$ and 30 mg cyclophosphamide were mixed with 130 ml cottonseed oil and homogenized. The resulting homogenate was added into continuously stirred contonseed oil at 25°C. The albumin microspheres were washed with diethyl ether to remove the oil phase and the microspheres were stabilized by using 2,3-butanedione as a crosslinking agent. The resulting microspheres were kept at 4°C after drying.

Labelling with ^{99m}Tc

Free Cyclophosphamide: Cyclophosphamide was labelled with ^{99m}Tc by the tin reduction method (Ercan 1976) 10 mg of cyclophosphamide was dissolved in 2 ml distilled water, and stirred for 5 min after adding 0.5 mg $SnCl_2.2H_2O$. Later it is adjusted to pH 7.0. It was filtered through a 0.22 um Millipore filter into a sterile vial containing 2 ml $^{99m}TcO_4^-$ of the 2 mCi radioactivity in 2 ml saline.

Albumin microspheres: Microspheres containing cyclophosphamide were labelled by modifying the method of Knop et al, (4). As incorporation material, 10 mg $SnCl_2.H_2O$ was used while preparing the microspheres. 2 ml saline solution containing 10 mg of CP microsphere and 2 mCi TcO_4 containing 0.05 ml 5% (v/v) Tween 80 were mixed and were settled for 10 min.

Quality control of ^{99m}Tc-HSA, ^{99m}Tc-microsphere, and ^{99m}Tc-cyclophosphamide: The labelling efficiency was determined by impregnated

thin-layer chromatography using ready plates. Acetone was used as a solvent. The labelled compound stayed at the origin and the free pertechnetate ($^{99m}TcO_4^-$) moved with an Rf of 1.0 (5).

Characterisation of microspheres

All the cyclophosphamide microspheres used in this study ranged around 5 μm. Most of the particles have considerable sphericity. The surface of the spheres appeared smooth with solid structures. Very few agregates were observed.

Histological evaluation of microspheres

Lungs and liver were removed after intravenous injection of HSA microspheres of cyclophosphamide. Tissues were processed through formaldehyde fixative and paraffin embedding. Sections of 1 um thickness were prepared and stained with hematoxylin and eosin and examined microscopically.

Tissue distribution

in vivo distribution of free cyclophosphamide: 0.2 ml ^{99m}Tc cyclophosphamide was injected to the tail vein of the mice. After 15, 30, 60 min and 6 and 24 h the mice were killed and the percentage of radioactivity accumulated in each organ was calculated.

in vivo distribution of microspheres: 0.2 ml suspension of ^{99m}Tc labelled cyclophosphamide microspheres were injected in to mice intravenously. At 15, 30, 60 min and 6 24 h, mice were killed and the organs assayed for radioactivity. The organs were weighed and also counted in a scintillation counter for the percentage of radioactivity accumulated.

To investigate loss of particles from the mouse liver and lungs microspheres were administered intravenously; at intervals, animals were killed, and the organs processed for histology.

RESULTS and DISCUSSION

In the present study it is attempted to prepare a biodegradable, biocompatable drug carrier that by virtue of particle size will become entrapped in the liver. The size of CP loaded microspheres is an important factor affecting their fate when administered. Microspheres smaller than 5 um in diameter injected intravenously passes through the lung and concentrates in liver and

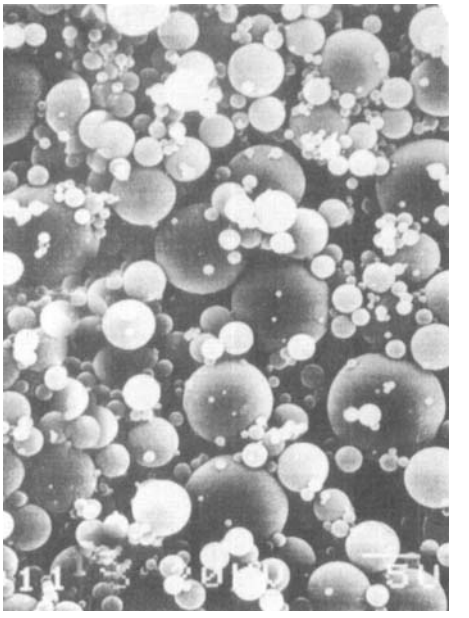

Figure 1. Electrophotomicrograph of HSA Microspheres of Cyclophosphamide.

in the RES. HSA microspheres of CP prepared in this study were all smaller than 5 μm and all spherical in shape (Figure 1). By obtaining monodispersed spheres with perfect spherical geometry of less than 5 μm in diameter, it was possible to target CP microspheres to liver. Human serum albumin was chosen as matrix material because of its low toxicity and biodegradability (2, 3, 6 - 11).

Observations of ours indicate that the distribution and accumulation of free and microsphere-entrapped cyclophosphamide were quite different (Figure 2 and 3). Thus in the free form, the drug was detected at very low level as 2.22 % and 0.57 % at 1 and 24 hours, respectively. This is in accord with our own previous studies and also with the observations in experimental animals (2, 3, 12, 13).

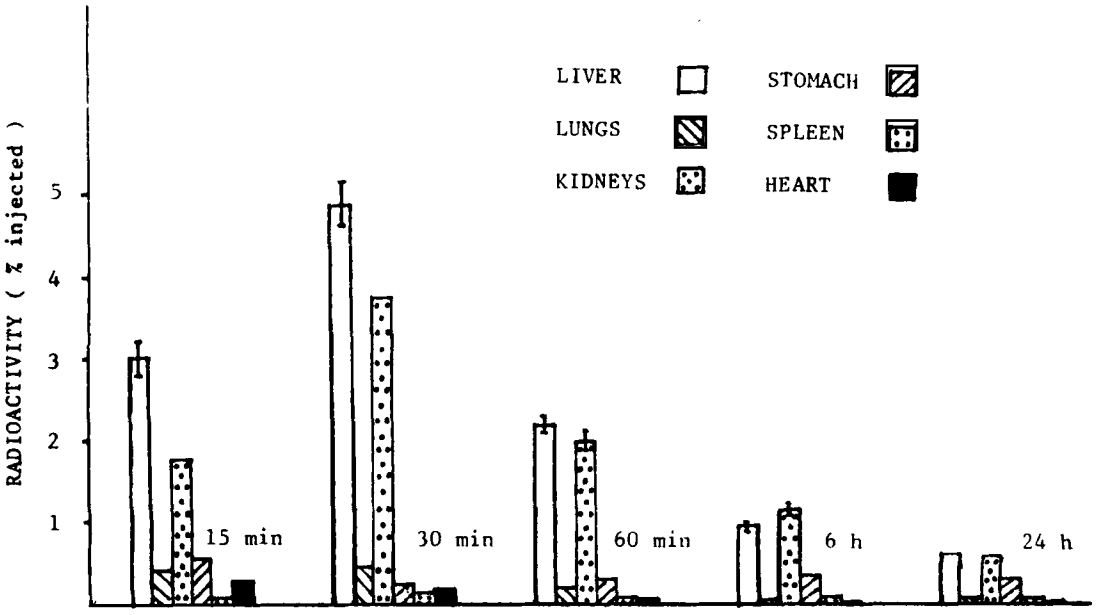

Figure 2. In Vivo Distribution of Free Cyclophosphamide Labelled with ^{99m}Tc.

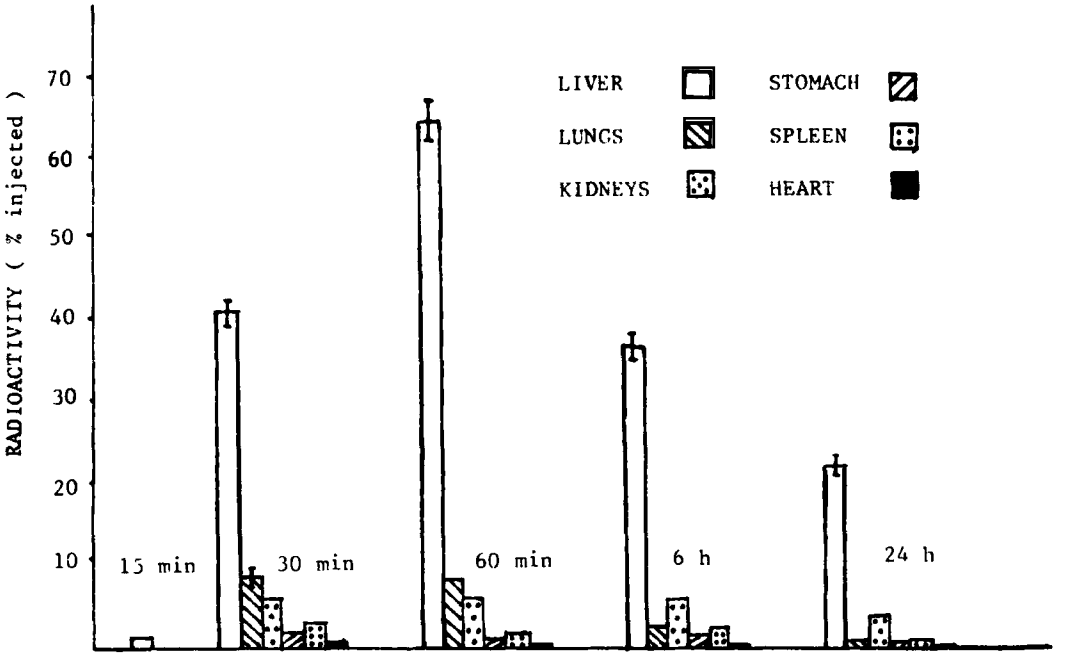

Figure 3. In Vivo Distribution of HSA Microspheres of Cyclophosphamide Labelled with ^{99m}Tc.

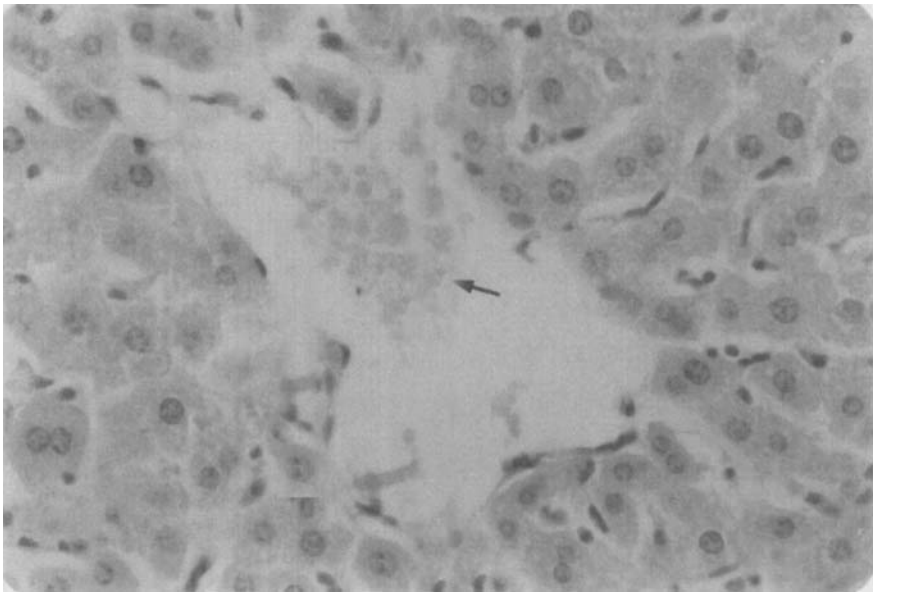

Figure 4. Photograph of HSA Microspheres of Cyclophosphamide in Liver After i.v. Injection.

The values are expressed as percentage of administered dose in terms of radioactivity. In the microsphere form, at 15, 30 and 60 minutes and 6 and 24 hours, mice were killed and the organs were assayed for radioactivity accumulated in each organ. 30 minutes after injection, 42.00 % of the radioactivity was found in the liver, 8.00 % in the lungs, 5.80 % in kidneys and 2.80 % in the spleen. Stomach and heart contained less that 1 % of the injected dose.

1 hour later, the radioactivity in the lungs and liver increased to 8.20 and 64.73 %, respectively. By 24 hours, 22.68 % of the radioactivity was found in the liver, where as 0.40 % was accumulated in the lungs. However, the percentage of free cyclophosphamide in the liver at 1 and 24 hours was 2.22 and 0.57 %, respec-

tively. Radioactivity of the free CP in all the other organs were much less than 1 % of the injected dose.

In mice, thi microspheres studied gave no sign of inflamatory reaction. They were located in liver (Figure 4) and very few in lungs by the histopatological evaluation.

CONCLUSION

The targeting of drugs to specific tissues has long been an attractive thought especially in cancer therapy. Based on the evidence presented in this investigation, the application of low dose cyclophosphamide-loaded HSA microspheres seems advantages in accumulation in the liver and also in the treatment of liver cancer.

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REFERENCES

- L.ILLUM, S.S.DAVIS, 1982, J.Parent.Sci.Tech., 36, 242.
- U.SCHEFFEL, B.A.RHODES, T.K.NATARAJAN, H.N.Jr.WAGNER, 1972, J. Nucl.Med., 13, 498.
- H.GÜRKAN, H.S.YALABIK-KAŞ, A.A.HINCAL, M.T.ERCAN, 1986, J. of Microencapsulation, 3 (2) 101.
- G.KNOP, 1984, J. of Microencapsulation, 1, 249.
- M.T.ERCAN, 1976, Hac.Bull. Med.Surg., 9, 85.
- K.F.ARONSEN, C.HELLEKANT, J.HOLMBERG, U.ROTHMAN, and H.TEDER, 1979, Eur.Sur.Res., 11, 99 (1979).
- R.W.BAKER, M.E.TUTTLE, and R.HELWING, 1984, Pharm.Tech., 8, 26.
- E.İŞERİ, M.T.ERCAN, H.S.KAŞ, A.A.HINCAL, 1988, APGI Association Pharmacie Galenique Industrielle-1989, Paris.
- T.K.LEE, T.D.SOKOLASKI, and G.P.ROYER, 1981, Science, 213, 233.

- K.SUGIBAYASHI, M.AKIMOTO, Y.MORIMOTO, T.NADAI and Y.KATO, 1979, J.Pharm.Dyn., 2, 350.
- K.J.WIDDER, A.E.SENYEI, and D.G.SCARPELLI, 1978, Proceedings of the Society for Experimental Biology and Medicine, 58, 141.
- D.REICH, 1978, Pharmacol and Ther., 2, 239.
- P.M.WILKINSON, M,ISRAEL, W.J.PEGG and E.FREI, 1979, Cancer Chem. Pharmacol., 2, 21.