Preliminary radiochemical and biological studies on the liposome encapsulated platinum-[125I]iodohistamine complex

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Abstract

BACKGROUND: The platinum-iodohistamine complex with in vitro cytostatic activity toward colon and mammary cancer cells has been synthesised recently in our laboratory. The pharmacokinetics of radioactive complex analogues, labelled with I-131 and I-125, has been examined in murine model of spontaneous mammary adenocarcinoma. The present work is devoted to the examination of the potential use of liposomes as a carrier system for the radioactive platinum-[125I]iodohistamine complex in vivo.

MATERIAL AND METHODS: Encapsulations of the Pt-[125I]iodohistamine were studied using a different molar ratio of the complex and liposomes with positive surface charge, as well as various incubation procedures. Biodistribution of the initial and the liposomal form of the complex were studied in C3H/16C tumour-bearing mice with spontaneously developed and transplantable (16C) mammary adenocarcinoma.

RESULTS: Comparative biodistribution studies in C3H/16C mice and in mice with spontaneously developed mammary tumour have shown that in the former model pharmacokinetics of the Pt-[125I]iodohistamine complex is more predictable and more similar to that observed for cisplatin. Therefore, the transplantable tumour model is more advantageous for the complex and its liposomal form evaluation.

In C3H/16C mice, significant differences in the biodistribution between the radioactive platinum complex and its liposomal form were observed. The concentration of the activity in blood after 2 h p.i.v. was two times lower for the encapsulated complex, and the uptake of the radioactivity by liver, spleen, and lungs was twice as high as that obtained for the free Pt-[125I]iodohistamine preparation. The radioactivity in tumour was almost constant for liposomal platinum complex (ca. 2% ID/g), although it was two times lower compared to the initial platinum complex.

CONCLUSIONS: The results of the present study indicate that platinum-[125I]iodohistamine can be efficiently incorporated into cationic liposomes (c. 40%). However, the uptake of the encapsulated complex by the liver and spleen macrophages demands further modification of the lipid membrane.

Key words: platinum-[125I]iodohistamine complex, cationic liposomes, spontaneous mammary adenocarcinoma in C3H mice, tumour-bearing C3H/16C mice

Introduction

It has been demonstrated both in the laboratory and in clinical trials that a concomitant combination of irradiation and chemotherapeutic agents enhance the efficacy of the tumour cells killing [1–5]. Cisplatin, as well as other platinum-based anticancer drugs, is an excellent radiosensitiser, therefore in combination with external irradiation or with low-dose continuous internal radiotherapy it produces significant supra-additive treatment effects toward several tumour cells. Recently, we have synthesised the new
Material and methods

The platinum(II)-[125I]iodohistamine complex was synthesised according to the developed procedure [6], by heating the solution of platinum(II) tetrachloride ion with the mixture of radioiodinated histamine (i.e. [125I]-histamine) and the carrier-added iodohistamine. The stock solutions of the platinum-iodohistamine complexes were prepared in dimethylformamide (DMF). The estimated specific activities of the Pt(II)-[125I]iodohistamine preparations used for biodistribution studies were in the range of 0.8 to 5 MBq/μmol of Pt. For in vivo studies, the injections of platinum [125I]iodohistamine complex were prepared in 0.9% saline containing 25% of DMF.

Liposomes with a cationic charge of the surface were formed in the presence of stearylamine (18 μmol), phosphatidylcholine (63 μmol), and cholesterol (9 μmol) (Sigma kit L4395). Hydration of the lipid mixtures was done in saline at 65°C followed by downsizing the lipid suspensions by filtration through 0.2 μm polycarbonate membrane, and finally by sonication using ultrasonic bath. With this procedure, typically, small unilamellar vesicles with diameters in the range of 50–100 nm are formed [9, Avantilipids: Technical Information]. Encapsulation of the Pt-[125I]iodohistamine was studied using a different molar ratio of the complex and lipid, as well as various incubation procedures. The yield of the complex entrapment into liposomes was monitored by TLC, radioelectrophoresis and RP HPLC. The liposomal platinum complexes were isolated by gel filtration using a glass column (15/1 cm) filled with Sephadex G-25F, and/or by solid phase extraction using SPE C18 cartridges and gradient of EtOH concentration in saline.

The comparative biodistribution studies of the preparations of the platinum complex, i.e. free Pt-[125I]iodohistamine and the complexes encapsulated within liposomes, were done in two models of mammary adenocarcinoma in C3H mice. The experiments were performed using female C3H/W mice with developed spontaneous mammary adenocarcinoma (purchased from the Department of Genetics and Laboratory Animal Breeding Cancer Centre and Institute of Oncology, Warsaw, Poland), and using tumour-bearing C3H mice about 20 days after subcutaneous injection of the stabilised 16C cells of mammary adenocarcinoma (Institute of Immunology and Experimental Therapy of Wroclaw). Mice with tumour diameter of c. 0.8–1 cm were used.

The platinum-[125I]histamine complex under study and its liposomal forms were administered intraperitoneally in a volume of 0.1 ml and dose of about 0.5 MBq per one animal. At selected time, 2 and 24 hrs after dosing, the animals were anaesthetised and killed, and then the organs were taken out to determine the distribution of radioactivity. The radioactivity of blood pool, urine, and samples of weighted tissues and carcass were measured using a gamma counter supplied with an adapter for whole body measurement. The results were calculated as a percentage of the injected dose in organs (%ID) or a percentage of dose in gram of tissue (%ID/g). An unpaired Student t-test (at 95% confidence interval) was applied for data evaluation. The animal experiments were approved by The 4th Local Animal Ethics Committee in Warsaw (authorisation number ZT/27/2002), and were carried out in accordance with the principles of good laboratory practice.

Results and discussion

Encapsulation of PtCl2-[125I]iodohistamine

The yields of the complex entrapment into liposomes varied in the function of applied method as follows: Method 1 — freeze-drying liposomes, rehydration with saline in the presence of the complex dissolved in 20% DMSO — yield c. 80%;
Method 2 — incubation of the solutions of liposomes and the complex (in 20% DMF) at 65°C with vortexing and sonication — yield 15 to 30%; Method 3 — freeze-drying liposomes with the complex following by rehydration with 10-percentaged DMF in saline — yield c. 40%. The highest yield of the platinum-[125I]iodohistamine encapsulation (c. 80%) was achieved using Method 1, i.e. after mixing the complex solution in DMSO with the freeze-dried liposomes in the molar ratio of the lipid to Pt 1.5:1, following rehydration with saline and incubation for 1 hr at 65°C with shaking and occasional sonication. However, further studies have shown that DMSO significantly affected the structure of the complex. Therefore, Method 3, which produced a satisfactory yield of the complex entrapment into liposomes (c. 40%; Fig. 2), has been used for the liposomal forms of the radioactive platinum-[125I]iodohistamine complex preparation.

Choice of the tumour model

Biodistribution study of the native radioactive platinum-[125I]iodohistamine complex on the two models of mammary adenocarcinoma revealed about four times higher accumulation of the complex in tumour than in normal muscular tissue (Fig. 3A, B). Anyhow, a significant difference of the complex pharmacokinetics has been observed for the two tumour models used. A high radioactivity retention in blood (over 14% ID/ml after 2 h p.i.) has been observed in mice bearing spontaneous mammary carcinoma, and that implied also a high radioactivity concentration in tumour and other tissues. However, decrease of radioactivity in tumour was observed after 24 h p.i. (3.75% ID/g after 2 h and 2.30% ID/g after 24 h p.i.). In contrast, a significantly lower blood concentration of the complex was observed 2 h p.i. in the transplantable tumour model (C3H/16C), although retention of the complex in the tumour increased with the time after administration (from 1.63% ID/g to 3.53% ID/g after 2 h and 24 h p.i., respectively, Fig. 3B).

The comparative biodistribution studies have shown that the pharmacokinetics of the complex in C3H/16C mice is more predictable and more similar to that observed for cisplatin [14, 15]. Thus, it can be assumed that retention of the complex undergoes the same mechanisms that are strictly connected with a higher metabolism and/or angiogenesis of the tumour masses. Other studies revealed also the fundamental differences between transplantable and spontaneously arising tumours in mice [16, 17], and these facts support our assumption. Therefore, it seems that the model with transplanted stabilised 16C cells of mammary adenocarcinoma is more convenient for drug evaluation than the murine model with spontaneously developed tumour. Considering accumulation of the complex in 16C tumour, which suggests penetration of the complex into the tumour cells, we have chosen this model for further evaluation of the complex encapsulated within liposome.

In vivo evaluation of the Pt(II)-[125I]iodohistamine liposomal form

In vivo study of the complex and its liposomal forms in C3H/16C tumour-bearing mice has shown differences in biodistribution between the two complex forms mainly after 24 h p.i. (Fig. 3B, Fig. 4). The encapsulated complex revealed a high and almost constant concentration in liver and spleen, and a significant clearance from GIT, whereas the retention of the free complex in these organs showed the opposite tendency. The liposomal platinum complex was also more rapidly cleared from the blood, and its almost two times higher excretion with urine was also noted. Some advantages of platinum-[125I]iodohistamine encapsulation in cationic liposomes could be observed in vivo (e.g. lower concentration in GIT), however, in general it has not produced significantly better pharmacokinetics compared to that of the free complex. The radioactive concentration of the liposome-encapsulated complex in tumour was constant (c. 2% ID/g) at the studied time points (Fig. 4), whereas for the “free” complex an increment of concentration was observed after 24 h p.i. to the level twice higher than those for the liposomal form.

Conclusions

The present study indicates that Pt(II)Cl₂-[1]iodohistamine complex can be efficiently incorporated into cationic liposomes. These types of liposomes have previously demonstrated efficacy in animal models of human diseases, and are currently being eval-
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Short communications

It has been shown that cationic liposomes can mediate efficient delivery of DNA and DNA/protein complex to mammalian cells in vitro and in vivo. Finally, human tumour cells selected for cisplatin resistance or isolated from patients who have failed cisplatin therapy are highly transfectable with cationic liposomes [11]. However, the observed uptake of the encapsulated complex by the liver and spleen macrophages, as well as its low concentration in the tumour tissue observed in our study, demands further modification of the lipid membrane. We also tested neutral in charge, large multilamellar liposomes (9.3 μmol phosphatidylycholine and distearoyl, 6.9 μmol cholesterol; pre-liposome formulation Sigma L3406) as in vivo carriers of the complex, although without any success. Taking into account a variety of the existing protocols for coating liposomes surface, which extend liposome blood circulation times and decrease uptake of encapsulated complex by the liver and spleen macrophages, future optimisation of the specific system for targeting of the platinum-radiopharmaceutical should be perhaps possible. We hope it may also increase the concentration of the radioactive platinum-[*I]iodohistamine complex in tumour cells significantly, but confirmation of these expectations demands further studies.

References


Figure 3. Comparison of the Pt(II)Cl2-[125I]iodohistamine complex biodistribution in tumour-bearing C3H mice: A. Spontaneous mammary tumour; B. Transplanted 16C cells of mammary adenocarcinoma (mean and 1SD of the % ID/g, nA = 10, nB = 5).

Figure 4. Accumulation of Pt(II)Cl2-[125I]iodohistamine complex encapsulated within cationic liposomes in the selected tissues of C3H/16C tumour-bearing mice (mean and 1SD of the % ID/g, n = 5).