Reduced uptake of liposomal idarubicin in the perfused rat heart

Pakawadee Sermsappasuk[a], Rafal Hrynyk[b], Jerzy Gubernator[c] and Michael Weiss[a]

Although idarubicin is one of the most important anthracyclines and liposomal formulations seemed less cardiotoxic than free drug formulations, there are no definite data on cardiac uptake of liposomal encapsulated idarubicin. This study has been designed to determine uptake and negative inotropic action of liposomal idarubicin in the single-pass isolated perfused rat heart. Idarubicin-bearing liposomes, composed of 1,2-distearoyl-sn-glicero-phosphocholine, 1,2-distearoyl-sn-glicero-phosphoethanolamine-*N*-[poly(ethylene glycol)2000], and cholesterol were administered as a 10-min constant infusion of 1 mg followed by a 70-min washout period. Outflow concentration and left ventricular-developed pressure were measured and compared with data of free idarubicin observed previously under the same experimental conditions. Liposomal encapsulation significantly reduced cardiac uptake of idarubicin to about 15% and extensively diminished its negative inotropic action to less than 5% of the values observed for free idarubicin. *Anti-Cancer Drugs* 19:729–732 © 2008 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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[a]Department of Pharmacology, Section of Pharmacokinetics, Martin Luther University Halle-Wittenberg, Halle, Germany, [b]Department of Personal Protective Equipment, Central Institute for Labour Protection, National Research Institute, Lodz and [c]Department of Lipids and Liposomes, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland

Correspondence to Michael Weiss, Department of Pharmacology, Section of Pharmacokinetics, Martin Luther University Halle-Wittenberg, 06097 Halle, Germany
Tel: +49 345 557 1657; fax: +49 345 557 1835;
e-mail: michael.weiss@medizin.uni-halle.de

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Introduction

Anthracyclines are highly effective in the treatment of various types of cancer. However, the dose-dependent cardiotoxicity limits their therapeutic use. As there are indications that cardiotoxicity is related to the cardiac levels of anthracyclines [1], liposomal encapsulation seems to be an effective way to reduce cardiotoxicity ([2–4] for review). The fact that encapsulation diminishes cardiac uptake of drugs was shown for doxorubicin [5,6] and daunorubicin [7]. However, still there is very little information on single-pass uptake of liposomal anthracyclines by the perfused rat heart, especially on liposomal idarubicin (IDA-L), where no data on cardiac uptake are available yet. In previous studies on the cardiac pharmacokinetics of IDA, we demonstrated that saturable uptake of IDA [8] was inhibited by doxorubicin [9] and enhanced by caffein [10]. Here, we used the same method [8] to evaluate the cardiac uptake of IDA administered as a liposomal formulation.

Materials and methods

Materials

IDA was purchased from Pharmacia & Upjohn (Erlangen, Germany). 1,2-distearoyl-sn-glicero-phosphocholine, 1,2-distearoyl-sn-glicero-phosphoethanolamine-*N*-[poly(ethylene glycol)2000], and cholesterol were purchased from Northern Lipids Inc. (Vancouver, British Columbia, Canada). Sodium dihydrogen phosphate, disodium hydrogen phosphate, sodium chloride, ammonium sulfate, Sephadex G-50 fine, and Dowex 50W resin were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Methanol high-pressure liquid chromatography (HPLC) grade was supplied by J.T. Baker (Deventer, Holland).

Preparation of liposomes

IDA-L was produced from IDA hydrochloride using a previously described procedure resulting in liposomes with a mean size of about 100 nm and a very high encapsulation efficiency [11]. In brief, empty or IDA-bearing 1,2-distearoyl-sn-glicero-phosphocholine/cholesterol/1,2-distearoyl-sn-glicero-phosphoethanolamine-*N*-[poly(ethylene glycol) 2000] (6.5 : 3 : 0.5) liposomes were prepared by lipid freeze-drying method from tert-butanol followed by extrusion on Thermobarrel Extruder (Lipex Biomembranes, Vancouver, British Columbia, Canada). The mean diameter of the vesicles was measured (multimodal analysis, volume weighted) on a Zetasizer 5000 (Malvern Instruments Ltd, Malvern, UK). IDA was subsequently encapsulated within liposomes via ammonium sulfate method. The drug encapsulation efficiency was close to 90% and nonencapsulated drug was removed by incubation of the resulting liposomal suspension with cation exchange resin (Dowex 50WX, Sigma-Aldrich). After the incubation, resin was removed by centrifugation and the

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drug concentration was determined colorimetrically. The initial drug-to-lipid ratio was 1 : 6.7. After drug incorporation, nonencapsulated drug was removed and final drug-to-lipid ratio was calculated and was 1 : 7.45.

Perfused rat heart

In rat hearts, Langendorff perfusion with Krebs–Henseleit bicarbonate buffer at a constant flow of 9.5 ml/min was performed as described previously [8]. A latex balloon was placed in the left ventricle and connected to a pressure transducer line. The balloon was inflated with 50% methanol to create a diastolic pressure of 5–6 mmHg. After 30-min stabilization, the hearts were beating spontaneously at an average rate of 255 beats/min. Coronary perfusion pressure, the left ventricular pressure, and heart rate were measured continuously. Left ventricular developed pressure (LVDP) was calculated from systolic and end-diastolic pressures as LVDP = left ventricular systolic pressure – left ventricular end-diastolic pressure. Coronary resistance was obtained by dividing coronary perfusion pressure by flow. The liposomal formulation containing 1 mg of IDA [0.66 ml of IDA-L (1.54 mg/ml)] was infused over 10 min using the same experimental design (as well as rats of the same strain and weight range) as used for IDA [8,12]. IDA concentration in the outflow samples was measured by HPLC [13]. For HPLC determination, 250 µl of the sample was deproteinized with 1 ml acetonitrile. After centrifugation (16000*g*/5 min), 50 µl of the supernatant was injected into HPLC column. An external calibration curve with five points was established by using IDA concentration range of 10.0–250.0 ng/ml. The standard solutions were prepared daily by adding IDA to the modified Krebs–Henseleit buffer and were deproteinized as described above. This investigation conformed to the National Institutes of Health 'Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 85-23, Revised 1996). Prior approval was obtained by the Animal Protection Body of the State of Sachsen-Anhalt, Germany.

Data analysis

Myocardial uptake of IDA (amount A_{uptake}) was calculated from the outflow concentration versus time data, $C(t)$,

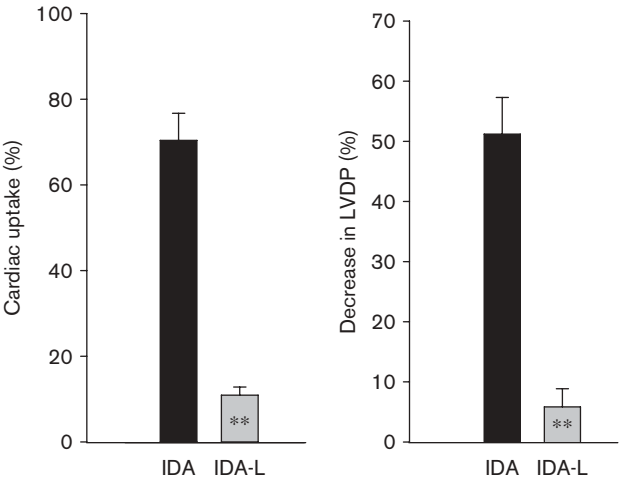
$$A_{uptake} = D - Q\int_0^{70} C(t)dt,$$

that is, as dose (D) minus the amount of IDA recovered in outflow perfusate within the sampling period of 70 min (Q denotes perfusate flow rate). A numerical integration method was used to calculate the area under the outflow curve. The mean (time-averaged) value of decrease in LVDP, during the interval 1–10 min after start of infusion (i.e. after reaching the plateau level), was used as measure of the negative inotropic effect of IDA. Differences in uptake and inotropic response between the two formulations were analyzed by the Mann–Whitney rank sum test for independent groups.

Results

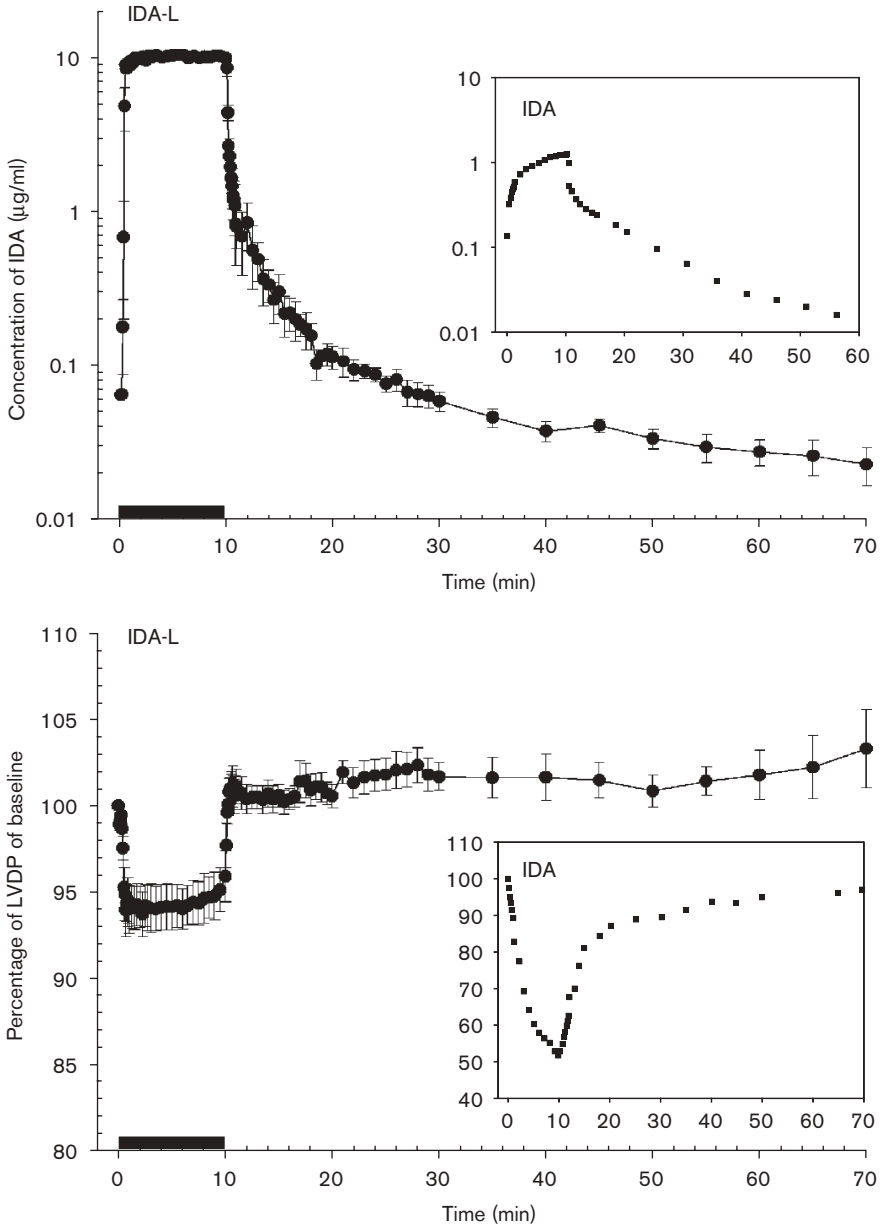
A cardiac uptake of 10.9% of the dose was estimated for IDA-L compared with 70.4% observed previously for free IDA [8] (Fig. 1). Thus, liposomal encapsulation significantly reduced cardiac uptake to about 20% of that of free IDA. The induced negative inotropic effect (percentage decrease in baseline) was also significantly lower (5.8% for the 1 mg dose of IDA-L versus 49% for the 0.5 mg dose of free drug [8], Fig. 1). Outflow concentration, $C(t)$, increased and inotropy, LVDP, decreased rapidly immediately after start of infusion to reach a maximum within less than 1 min in both cases (Fig. 2). These plateau values were maintained until the end of infusion. This is in contrast with the response after administration of unencapsulated drug, where $C(t)$ and LVDP reduction reach their maxima at the end of infusion [8]. IDA-L increased the coronary vascular resistance by 14.2 ± 12% with high interindividual variability over time. This maximum value occurred about 3 min after the start of infusion and few minutes after the end of infusion, no statistically significant change can be detected. The maximal vasoconstrictive effect was significantly lower ($P < 0.01$) than the 75 ± 16% increase in coronary resistance caused by free IDA at the end of the 10 min infusion interval (then decreasing to 20% after 20 min and increasing to 40% after 80 min) [12].

Fig. 1



Cardiac uptake (percentage of dose) and negative inotropic effect (percentage decrease from baseline) after a 10-min infusion of 1 mg liposomal idarubicin (IDA-L) and 0.5 mg unencapsulated idarubicin (IDA) [8] in perfused rat hearts. Values present mean ± SD; $n = 5$ in IDA and $n = 6$ in IDA-L groups; $^{**}P < 0.01$ versus IDA. LVDP, left ventricular developed pressure.

Fig. 2



Idarubicin outflow concentration profile (top) and the induced decrease in left ventricular developed pressure (LVDP) (bottom) after a 10-min infusion of 1 mg liposomal idarubicin (IDA-L) in perfused rat hearts. Points represent means ± SEM ($n = 6$). The insets show the data obtained for free idarubicin (IDA) [8] (note the different scale on the vertical axis).

Discussion

On the basis of a kinetic model of IDA uptake and inotropic effect in the perfused rat heart, we predicted the negative inotropic effect as a function of drug amount at the cardiac effect site or biophase [8]. Given the reduction in cardiac uptake described above, the model (Fig. 3d in Ref. [8]) predicts a 3% decrease in LVDP from baseline for 0.5 mg IDA-L (instead of the 49% for free drug [8]). Taking into account that the 5.8% decrease in LVDP shown in Fig. 1 was observed after the double dose (1 mg IDA-L vs. 0.5 mg free IDA), our result is in accordance with the prediction of the model. The characteristic shape of the $C(t)$ curve (resembling that of a vascular indicator) suggests that IDA-L predominately remains in the vascular space, in accordance with the fact that intact liposomes are too large to extravasate in normal tissue. The uptake reduction to 20% of the free form observed here for IDA-L in the rat heart is in good

agreement with the same value reported for liposomal doxorubicin in the single-pass perfused rat liver [14]. As it has been suggested that the increase in coronary resistance may contribute to cardiotoxicity [1], it is important to note that compared with free IDA, IDA-L also induced lower coronary vasoconstriction. Similar to the negative inotropic effect, the maximum (14% increase in coronary resistance) occurred already about 3 min after start of infusion, and not at the end of infusion, as observed for unencapsulated IDA [12]. It remains an open question whether idarubicinol formed in the vascular space (or vessel wall) is involved in this effect [15], as the increase in coronary resistance induced by idarubicinol was 1.6-fold higher than after IDA [12]. As the IDA concentration measured here represents the total concentration in perfusate, including the liposomal fraction, protein-bound fraction, and free fraction, we have no direct information on the concentration of free IDA. However, our results are consistent with the high encapsulation efficiency and stability of this liposomal formulation of IDA reported in Slifirski *et al.* [11].

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