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Preclinical study

Comparative cardiotoxicity of idarubicin and doxorubicin using the isolated perfused rat heart model

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Attempts to reduce the incidence of congestive heart failure following anthracycline therapy include the replacement of the parent compounds (especially doxorubicin) by less cardiotoxic analogs. Among these analogs, idarubicin (4-demethoxy-daunorubicin) was shown to be less cardiotoxic than doxorubicin in phase II clinical trials, but its actual cardiotoxicity has never been evaluated in large series and has never been compared to that of doxorubicin in relevant experimental models. Using the isolated perfused rat heart model, we compared the cardiac effects (developed pressure, contractility and relaxation of the left ventricle) induced by idarubicin to those induced by doxorubicin. Drugs were administered i.v. every other day for 11 days at doses of 1, 2, 2.5 and 3 mg/kg per injection for doxorubicin and 0.5, 0.75 and 1 mg/kg per injection for idarubicin. We confirmed that similar general toxicity symptoms were obtained for a dose ratio of 1:4 (idarubicin:doxorubicin). However, at the maximum tolerated doses of both drugs (3 mg/kg per injection for doxorubicin and 0.75 mg/kg per injection for idarubicin), the cardiac toxicity of idarubicin remained significantly lower than that of doxorubicin. Anthracycline cardiac accumulation was evaluated in parallel and revealed a lower cardiac accumulation of idarubicin, which could explain the reduced cardiac toxicity of this analog. Direct perfusion of the drugs in the isolated hearts of untreated animals revealed that idarubicin was taken up more readily than doxorubicin in the cardiac tissue, despite the fact that it had less deleterious effects on cardiac function. This indicates that idarubicin also had less intrinsic cardiotoxicity than doxorubicin in this model. [

Key words: Cardiotoxicity, doxorubicin, idarubicin, preclinical models.

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Introduction

Doxorubicin is widely used in cancer chemotherapy, both for the treatment of hematological malignancies and advanced or metastatic solid tumors such as breast cancer. Unfortunately, its clinical use is limited to a maximum cumulative dose of 500–550 mg/m^2,[1] due to the significant risk of development of congestive heart failure. In order to reduce this cardiotoxicity, several anthracycline derivatives have been synthesized. Idarubicin (4-demethoxy-daunorubicin) is one of these analogs, derived from research aimed at the increase of the therapeutic index of anthracyclines. Idarubicin is now widely used in the induction and consolidation treatments of acute leukemias.[2] Early *in vivo* studies indicated that idarubicin had a somewhat higher therapeutic index than doxorubicin and daunorubicin.[3] Phase II studies have then shown that idarubicin displayed a similar activity to doxorubicin in advanced breast cancer without[4,5] or with low[6,7] incidence upon cardiac function. However, the exact level of cardiotoxicity of idarubicin as compared to doxorubicin is as yet unknown, as no epidemiologic large-scale study has been undertaken to evaluate the risk of congestive heart failure in patients treated with idarubicin.

We have recently developed and validated an *ex vivo* model for the evaluation of anthracycline cardiotoxicity in rats.[8] This model relies on the alteration of cardiac functional parameters upon repetitive administrations of anthracyclines for a period of 12 days. This model allowed us to correctly predict the relative cardiotoxicity of several anthracyclines, types of formulation and cardioprotectors. It also allowed us to correlate the cardiac effects of anthracyclines with their accumulation in the left ventricle. In this study we wanted to evaluate the cardiotoxicity of idarubicin following multiple i.v. injections in our rat model. Indeed, previous pre-

clinical studies used single injections and did not directly measure heart functional parameters.[9]

For experimental studies, the dose equivalence of idarubicin and doxorubicin has to be taken into account to make comparisons valid. In fact, idarubicin has been mostly prescribed as three to five daily repetitive administrations,[4,5,10] which hinders precise and definitive comparisons. In terms of efficacy as well as of general toxicity, the equivalent dose ratio is around 1:4 or 1:5, 15 mg of idarubicin being roughly equivalent to 60–75 mg doxorubicin.[4] We have, therefore, chosen the doses to be administered as a function of this equivalence ratio.

Our results show that idarubicin is less cardiotoxic than doxorubicin at equipotent doses, using a schedule already validated for the evaluation of cardiac toxicity of anthracyclines.

Materials and methods

Drugs

Doxorubicin and idarubicin were provided by Pharmacia & Upjohn (Saint-Quentin-en-Yvelines, France) as clinical formulations, which was reconstituted in 0.9% NaCl at the concentration of 1 mg/ml, divided into aliquots and kept frozen until use.

All other chemicals and solvents were of the highest grade commercially available. Special care was taken concerning the water used for the perfusion medium; we used sterile apyrogenic water specially prepared for parenteral injections in humans.

Experimental animals

All experiments described in this report were done in accordance to the guidelines of Institut National de la Santé et de la Recherche Médicale. Male Sprague-Dawley rats aged 10–12 weeks and weighing 300–350 g were obtained from CERJ (Le Genest-Saint-Isle, France). All experiments included controls receiving 0.9% NaCl. Drugs (doxorubicin at 1, 2, 2.5 or 3 mg/kg per injection and idarubicin at 0.5, 0.75 or 1 mg/kg per injection) were administered i.v. via the tail vein on days 1, 3, 5, 7, 9 and 11 after weighing of the rats and assessment for possible general toxicity symptoms. On day 12 the rats were killed, their hearts were removed and perfused, cardiac functional parameters were monitored as described below, and the hearts were weighed at the end of the experiment.

Independent series of animals were treated similarly with idarubicin (0.5 mg/kg per injection) and doxorubicin (1 mg/kg per injection) but, after removal of their heart, a portion of about 120 mg of the left ventricular myocardium was sampled and kept frozen for the evaluation of the anthracycline accumulation in these organs as described below.

Finally, the hearts of untreated rats were also isolated and perfused as described below with a solution containing either doxorubicin at concentrations of 10^{-6} and 10^{-5} M or idarubicin at concentrations of 10^{-6} and 2×10^{-6} M. Cardiac functional parameters were monitored as described below and anthracycline accumulation in the perfused heart was estimated after sampling of a 120 mg portion of the left ventricular myocardium.

Perfusion of isolated rat hearts

Rats were heparinized i.p. (500 IU per 100 g body weight) and anesthetized with diethylether. The heart was quickly excised and soaked in Krebs-Henseleit solution at 4°C. Coronary perfusion was initiated through a short cannula in the aortic root and maintained at a constant pressure of 90 mmHg in a non-recirculating way by the Langendorff technique as described by Lorell *et al.*[11] Perfusion pressure was measured by a P23Db transducer (Bentley Trantec, Irvine, CA) connected to the aortic infusion cannula. The heart was electrically paced at a rate of 300 b.p.m. (5 Hz) through stimulator-activated stainless steel electrodes placed on the heart. A latex balloon attached to one end of a polyethylene catheter was placed in the left ventricle through the mitral valve. The catheter was filled with water and the other end was linked to an electronic amplifier (Thomson Medical, Saint-Cloud, France) via a second P23Db transducer.

The coronary perfusion pressure and the left ventricular pressure were recorded on a computer that allowed continuous monitoring of heart rate, left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular developed pressure (LVDP=LVSP − LVEDP) and the maximal and minimal first derivatives of LVDP as a function of time [$LV(dp/dt)_{max}$ and $LV(dp/dt)_{min}$], respectively. The perfusate consisted in a modified Krebs-Henseleit buffer, pH 7.4, containing NaCl (118 mM), KCl (4.7 mM), $MgSO_4$ (1.2 mM), KH_2PO_4 (1.2 mM), $NaHCO_3$ (25 mM), glucose (11 mM), $CaCl_2$ (0.95 mM) and insulin (10 IU/l). It was continuously bubbled with a mixture of 95% O_2/5% CO_2 and maintained at 37°C. The latex balloon inserted in the left ventricle was periodically dilated with distilled water in order to produce a LVEDP of 5–6 mmHg. After 30–45 min stabilization, necessary to reach the maximal func-

tional cardiac values, the above parameters were recorded.

Anthracycline accumulation

The samples obtained from the hearts of rats treated with 1 mg/kg doxorubicin and with 0.5 mg/kg idarubicin were homogenized in physiological saline (2 ml for 100 mg tissue) with a tissue homogenizer (Ultra-Turrax; Bioblock Scientific, Strasbourg, France). After addition of an adequate amount of internal standard (daunorubicin) and of 0.5 ml borate buffer (50 mM, pH 9.8) to 0.5 ml of the homogenate, anthracyclines were extracted with 9 ml of chloroform/methanol 4/1 (v/v), according to Baurain *et al.*[12] After mixing and centrifuging (10 min at 3000 *g*), the solvent layer was recovered, evaporated to dryness and reconstituted in 200 μl methanol. Calibration curves were obtained after incubating heart homogenates with doxorubicin or idarubicin *in vitro* for 15 min at room temperature. A good linearity was obtained from 0.15 to 30 nmol/g tissue. Chromatography was performed on a Radial-Pack C18 column (Waters Associates, Milford, MA) inserted in a compression device. The solvent was a mixture of ammonium formate buffer (60 mM, pH 4.0) and acetonitrile (68/32) delivered at 3 ml/min. Detection was achieved with a laser-induced fluorescence recorder (Zeta Technology, Toulouse, France) with excitation and emission wavelengths set at 488 and 550 nm, respectively. Retention times and peak areas were recorded with a microcomputer using the PC1000 software (Thermo Quest, Les Ulis, France).

Statistical analysis

Statistical comparisons between untreated and treated groups were made by Student's *t*-test after ANOVA assumption of the validity of *t*-test; all data in the tables are expressed as mean value $\pm$ SEM. Statistical significance was determined as $p < 0.05$.

Results and discussion

We first compared the general toxicity symptoms provided by doxorubicin and idarubicin administered to rats every other day for 11 days (Table 1 and Figure 1). At the dose of idarubicin of 1 mg/kg per injection, all animals died before day 12, preventing any analysis.

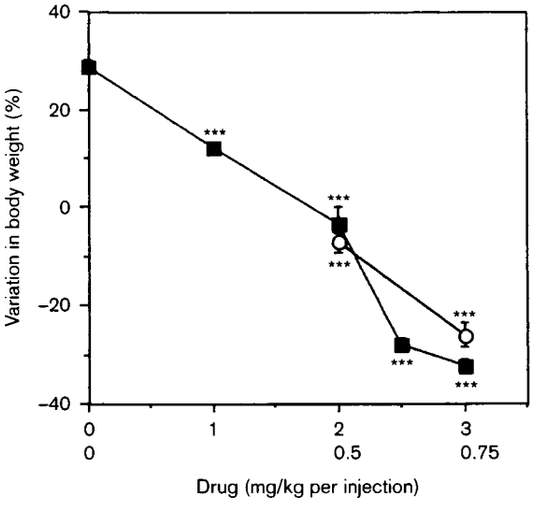

Figure 1. Effect of doxorubicin (■, upper scale) and idarubicin (O, lower scale) on body weight. Rats were treated by repetitive i.v. administrations every 2 days for 12 days. Doses of idarubicin were shifted in order to stay at a 1:4 ratio (idarubicin:doxorubicin). Bars represent SEM. Student's *t*-test: treated versus control rats: $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ idarubicin versus doxorubicin (at the 1:4 dose ratio); $^{+}p<0.001$.

Table 1. General toxicity induced by doxorubicin and idarubicin in rats treated by repetitive i.v. administrations every 2 days for 12 days

Drug dose (mg/kg per injection)		Weight change on day 12	Early death	Diarrhea	Epistaxis
Control		+28.9±3.7	0/10	0/10	0/10
Doxorubicin	1	+12.4±1.9	0/8	0/8	0/8
Doxorubicin	2	−3.2±9.2	0/9	0/9	4/9
Doxorubicin	2.5	−27.8±3.4	2/10	5/8	8/8
Doxorubicin	3	−32.4±3.1	0/11	7/11	11/11
Idarubicin	0.5	−7.1±5.8	0/9	0/9	8/9
Idarubicin	0.75	−26.0±5.2	8/13	2/5	5/5
Idarubicin	1	–	4/4	4/4	4/4

The dose of idarubicin of 0.75 mg/kg per injection was accompanied by a severe mortality, major side effects (diarrhea and epistaxis) and 26% weight loss. Apart from the mortality, this general toxicity corresponded to that obtained with doses of doxorubicin of 2.5 or 3 mg/kg per injection. The dose of idarubicin of 0.5 mg/kg per injection provided only epistaxis and a 10% weight loss, comparable to what was obtained with doxorubicin at 2 mg/kg per injection (Figure 1). We can confirm, therefore, that the equivalent dose ratio for general toxicity is around 1:4 (idarubicin: doxorubicin).

We then compared the cardiotoxicities of idarubicin and doxorubicin in animals treated on the basis of this 1:4 ratio. Doxorubicin treatment led to important alterations of the left ventricular function from a dose of 2.5 mg/kg per day (Figures 2-4). In comparison, idarubicin displayed a much weaker cardiotoxicity, since the left ventricular functional parameters were only altered at the highest dose we could study, 0.75 mg/kg per injection, and to a significantly lower degree than doxorubicin at the dose of 2.5 mg/kg per injection. From the dose-effect curves presented on Figures 2-4, the equivalent dose ratio for cardiotoxicity can be, therefore, estimated to be about 1:3 (idarubicin:doxorubicin).

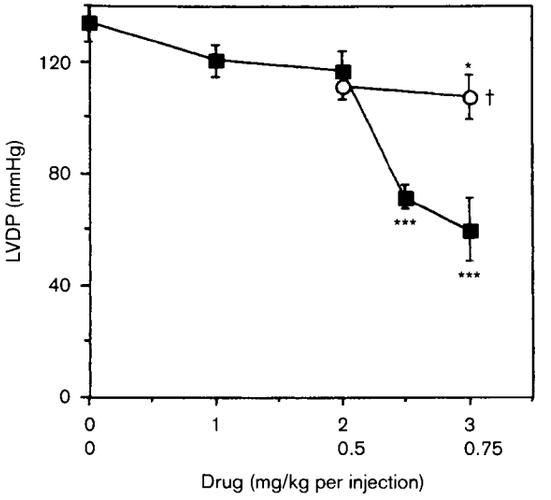

Figure 2. Effect of doxorubicin (■, upper scale) and idarubicin (O, lower scale) on the LVDP. *$p<0.05$; ***$p<0.001$ idarubicin versus doxorubicin (at the 1:4 dose ratio); †$p<0.001$.

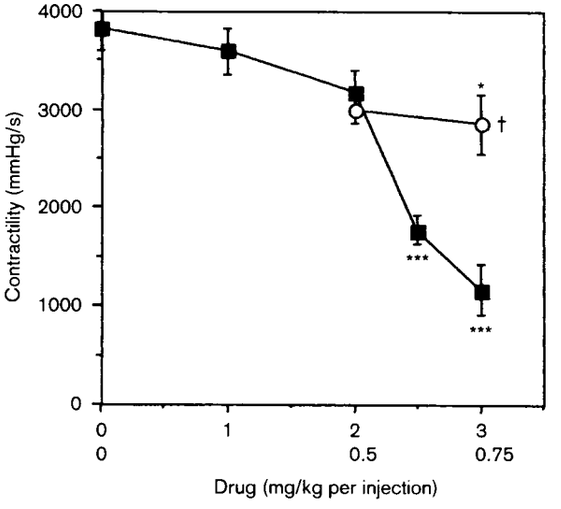

Figure 3. Effect of doxorubicin (■, upper scale) and idarubicin (O, lower scale) on the left ventricular contractility. *$p<0.05$; ***$p<0.001$ idarubicin versus doxorubicin (at the 1:4 dose ratio); †$p<0.001$.

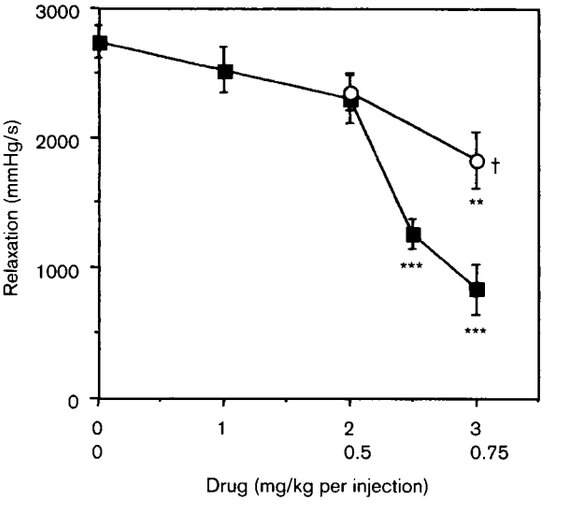

Figure 4. Effect of doxorubicin (■, upper scale) and idarubicin (O, lower scale) on the left ventricular relaxation. **$p<0.01$; ***$p<0.001$ idarubicin versus doxorubicin (at the 1:4 dose ratio); †$p<0.001$.

Table 2. Cardiac accumulation of doxorubicin, idarubicin and their dihydrometabolite (doxorubicinol or idarubicinol) after repetitive i.v. administrations every 2 days for 12 days

		Cardiac concentration (nmol/g tissue)	
Drug dose (mg/kg per injection)		Parent drug	Dihydrometabolite
Doxorubicin	1	4.4±0.8	0.12±0.01
Idarubicin	0.5	0.11±0.05	0.68±0.14

Table 3. Cardiac functional parameters and accumulation of doxorubicin and idarubicin after 70 min perfusion of hearts isolated from untreated rats

Concentration	(M)	Developed pressure (mmHg)	Contractility (mmHg/s)	Relaxation (mmHg/s)	Cardiac concentration (nmol/g tissue)	
					Parent drug	Dihydrometabolite
Doxorubicin	10^{-6}	102±12	106±7	108±7	40±3	not detected
Doxorubicin	10^{-5}	75±4	42±4	71±6	320±30	not detected
Idarubicin	10^{-6}	102±8	102±8	104±8	360±50	9±1
Idarubicin	2×10^{-6}	83±11	73±8	83±8	470±30	17±2

This weak cardiotoxicity could be ascribed at least in part to a lower cardiac accumulation of idarubicin as compared to doxorubicin in animals treated every other day for 11 days (Table 2). This was studied at a 1:2 ratio (idarubicin:doxorubicin). In these conditions, the cardiac accumulation of doxorubicin plus doxo-rubicinol was 5 times higher than that of idarubicin plus idarubicinol. As already known,[13] and unlike what happens with doxorubicin, the concentration of idarubicinol was largely higher than that of the parent compound. Furthermore, the cardiac accumulation of idarubicinol was 6 times higher than that of doxo-rubicinol. Since doxorubicinol is known to display higher cardiac effects than doxorubicin, this indicates that idarubicinol must be devoid of such specific cardiotoxicity.

When the hearts of untreated animals were perfused with 10^{-6} M of doxorubicin or idarubicin, no alteration of the cardiac functional parameters was seen after 70 min perfusion (Table 3). At the dose of 10^{-5} M, doxorubicin provided a 25-60% decrease of developed pressure, contractility and relaxation after 70 min perfusion, whereas these alterations were significantly lower with idarubicin at 2×10^{-6} M, confirming that equivalent doses (ratio 1:5) were not followed by equivalent cardiotoxicity (Table 3). After 70 min perfusion with 10^{-6} M doxorubicin or idarubicin, the cardiac accumulation of idarubicin was 8- to 10-fold higher than that of doxorubicin (Table 3). At the dose ratio 1:5 (2×10^{-6} M idarubicin and 10^{-5} M doxorubicin), the cardiac accumulation of idarubicin was still 1.5-fold higher than that of doxorubicin (Table 3). These results indicate that reduced cardiac accumulation was not the only reason for the lower cardiotoxicity of idarubicin observed in treated animals, but that the two drugs have different intrinsic cardiotoxic properties, to the benefit of idarubicin. However, very little idarubicinol was formed during direct perfusion experiments, which may prevent comparison with the situation observed with treated animals.

In conclusion, idarubicin exerted a higher general toxicity than doxorubicin to rats treated by repetitive injections of the drug. However, at the maximum tolerated doses of both drugs the cardiac toxicity of idarubicin remained significantly lower than that of doxorubicin. There was a lower cardiac accumulation of idarubicin than of doxorubicin, which could explain the reduced cardiac toxicity of this analog. However, idarubicin was taken up more readily than doxorubicin in the cardiac tissue when directly administered in isolated hearts. This indicates that idarubicin also had less intrinsic cardiotoxicity than doxorubicin in this model.

Acknowledgments

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