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Effects of verapamil on cardiac uptake of radiolabeled doxorubicin and iodo-doxorubicin in rabbits

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Since verapamil has been shown to increase cellular concentrations of anthracyclines in resistant tumor cells, this study was designed to investigate the effect of verapamil on myocardial accumulation of radiolabeled doxorubicin (DOX) and iodo-doxorubicin (IDOX) in rabbits, and to estimate the risk of increasing anthracycline-related cardiotoxicity. After intravenous administration of either I-123 DOX or I-123 IDOX alone, and in combination with a single dose of 0.3 mg or 3 mg verapamil/kg body weight scintigraphic imaging was performed up to 100 min p.i. and cardiac uptake of the radiolabeled anthracyclines was calculated by ROI-technique in percent of total body activity. Cardiac uptake was decreasing in a monoexponential manner for both DOX and IDOX, and there were no significant differences due to verapamil in either dosage. However, cardiac uptake of DOX was nearly 2-fold higher than of IDOX confirming the lower cardiotoxic potential of IDOX. In conclusion, our results in rabbits do not show a significant increase of myocardial accumulation of anthracyclines following intravenous injection of verapamil suggesting no increased risk of cardiotoxicity for a combined therapy of DOX or IDOX and verapamil.

Key words: heart radionuclide imaging; doxorubicin; verapamil; myocardium-drug effects; rabbits

Introduction

Anthracyclines like the widely used doxorubicin (DOX) or the more lipophilic derivative iodo-doxorubicin (IDOX) are efficient and well-established anticancer drugs in various tumors, e.g. breast or lung cancer.[1-5] However, drug resistance to anthracyclines is a well-known phenomenon limiting effective anti-cancer treatment. Multidrug resistance related to an overexpression of transmembranous glycoprotein p170 decreases the intracellular concentration of several antineoplastic drugs including anthracyclines such as DOX or IDOX by actively enhancing their efflux out of the cells.[6-9]

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Recently, the so-called chemosensitizers have been shown to modulate the effects of p170 glycoprotein on drug efflux; e.g. calcium channel-blockers like verapamil increase the intracellular concentration of anthracyclines in resistant tumor cells by reducing their efflux via p170 glycoprotein, thus, enhancing cytotoxicity.[10-13] Since clinical studies testing anthracyclines in combination with verapamil also suggested an improved therapeutic efficacy of this antineoplastic regimen,[14, 15] a potential increase of toxic side effects of anthracyclines to normal tissue has special interest.[11] In particular, an increase of the well-known cardiotoxicity[16-18] of anthracyclines would be of great clinical impact.

The aim of this study, therefore, was to evaluate the impact of verapamil on in-vivo myocardial uptake of radiolabeled DOX and IDOX in rabbits in order to estimate the potential risk of increasing cardiotoxicity.

Materials and methods

The anthracycline antibiotics doxorubicin and its derivative 4'-deoxy-4'-iododoxorubicin (Farmitalia, Italy) were radioiodinated with I-123 (Amersham, Braunschweig, Germany) by electrophilic substitution by the Iodogen method as recently published in detail.[19–21] Radiochemical purtiy as confirmed by Sep-Pak RP-C 18 cartridges (Millipore, Eschborn, Germany) was more than 98 % in all cases.

Six 3-month old male New Zealand white rabbits weighing approximately 2.5–3 kg were investigated in two subsets of three animals each. One subset was treated with I-123 labeled DOX, the other with I-123 labeled IDOX. In both subsets the animals first recieved the radiolabeled anthracycline alone, then in combination with low-dose verapamil after one week, and thirdly in combination with high-dose verapamil after another week. In each animal 40 MBq of I-123 labeled DOX or IDOX were administered via an intravenous butterfly catheter in a central ear vein. Verapamil (Knoll, Ludwigshafen, Germany) was administered slowly 5–10 min prior to injection of the radiolabeled anthracycline. Thereby, a dose of 0.3 mg verapamil per kg body weight intravenously applied was considered as low-dose, 3 mg per kg body weight as high-dose injection.

Immediately after tracer application, simultaneous planar whole body images from anterior and posterior views with a zoom factor of 1.2 were acquired initially in five 1-min intervals and then in 5-min intervals up to 100 min p.i. using a double head gamma camera system (Bodyscan, Siemens Gammasonics) equipped with high resolution parallel hole collimators for low energy. In order to avoid artefacts due to movements the animals were anesthesized with thiopental (Trapanal®; Byk Gulden, Konstanz, Germany) during scintigraphy. Cardiac uptake of radiolabeled DOX and IDOX in percent of total body activity was calculated for each time interval by conventional ROI-technique.

Quantitative data are given as mean ± one standard deviation. Two-tailed students t-test was used to evaluate differences, with $p < 0.05$ considered to be statistically significant.

Results

Time-to-uptake curves of cardiac uptake of I-123 labeled DOX, DOX in combination with low-dose verapamil (DOX/LD) and DOX in combination with high-dose verapamil (DOX/HD) are shown in Figure 1. The corresponding curves of IDOX, IDOX in combination with low-dose verapamil (IDOX/LD) and IDOX in combination with high-dose verapamil (IDOX/HD) are depicted in Figure 2.

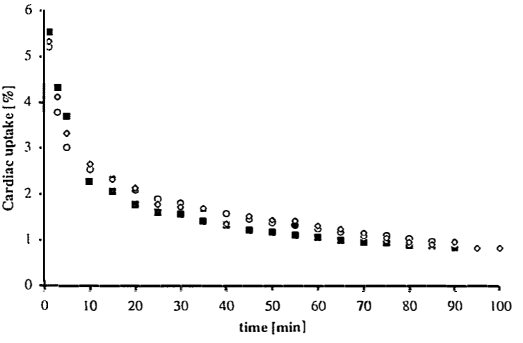

Figure 1. Cardiac uptake in percent of total body activity of I-123 DOX (filled squares), I-123 DOX in combination with low-dose verapamil (open circles) and I-123 DOX in combination with high-dose verapamil (open diamonds) in rabbits up to 100 min p.i.. Symbols represent means, standard deviation bars are omitted for clearness.

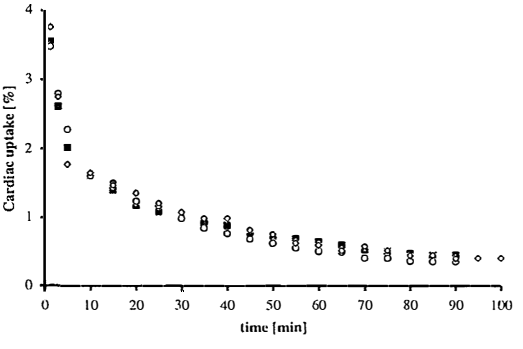

Figure 2. Cardiac uptake in percent of total body activity of I-123 IDOX (filled squares), I-123 IDOX in combination with low-dose verapamil (open circles) and I-123 IDOX in combination with high-dose verapamil (open diamonds) in rabbits up to 100 min p.i.. Symbols represent means, standard deviation bars are omitted for clearness.

Cardiac tracer uptake was decreasing in a roughly mono-exponential manner during measurement from 0–100 min p.i. for both iodine-123 labeled DOX and IDOX. Verapamil in either low-dose or high-dose administration did neither significantly affect the myocardial time-to-uptake curves of DOX (Figure 1) nor of IDOX (Figure 2). Although both DOX/LD and DOX/HD values were slightly higher than DOX values, there were no significant differ-

ences between these curves ($p > 0.05$). Furthermore, there were no significant differences between DOX/LD and DOX/HD and between IDOX/LD and IDOX/HD ($p > 0.05$).

However, myocardial uptake of DOX was significantly higher ($p < 0.05$) as compared to IDOX. DOX values were nearly twice as high as the corresponding values of IDOX during the whole time period. Similiar results were obtained for DOX/LD and IDOX/LD as well as for DOX/HD and IDOX/HD confirming the higher cardiac accumulation of DOX.

Discussion

Since an effective anti-cancer treatment with cytotoxic agents like the widely used anthracyclines is often hampered by multidrug resistance, drug combinations with chemosensitizers have been investigated in order to restore drug sensitivity in resistant cancer cells. Recently, calcium channel-blocking agents like verapamil have been shown to effectively improve the cellular uptake of anthracyclines by reducing their efflux in p170 glycoprotein-associated multidrug resistant cancer cells both in experimental animals and in first clinical studies.[10–15] However, since a severe and dose-related cardiotoxicity is a well-known and treatment-limiting side effect of anthracyclines,[16–18] it is necessary to evaluate whether verapamil would also increase the toxicity of anthracyclines in normal tissues.

In experimental studies, a 30 % increase of DOX concentrations in normal rat myocardial cells perfused with verapamil has been reported, as well as a higher incidence and severity of degenerative changes in cardiac tissue of mice treated with verapamil and doxorubicin.[22–24]

Our results in rabbits, however, did not confirm significant differences of cardiac accumulation of I-123 labeled DOX as compared to either DOX in combination with low-dose (0.3 mg/kg body weight) or with high-dose (3 mg/kg body weight) verapamil. Moreover, there were no significant differences between radiolabeled IDOX and IDOX in combination with low-dose or high-dose verapamil.

This may probably be due to the dosage and the different ways of administration of verapamil. In experimental studies perfusion of isolated myocardial tissue with verapamil in a constant concentration will reach continuously higher and more effective plasma levels as compared to single intravenous bolus injections or oral application of verapamil as used in clinical settings.[25] Therefore, plasma concentrations of verapamil as achieved in this study – even using a high-dose regimen of 3 mg verapamil per kg body weight – may be too low to effectively modulate p170 glycoprotein function of myocardial cells resulting only in a small increase of intracellular anthracycline concentration. Similar observations with no increase in both tumor cytotoxicity and toxic side effects have been reported from a clinical phase II study.[25] The major problem thereby is most probably the dose-limitation of verapamil because of its severe acute cardiovascular toxicity when plasma concentrations reach those required for successful reversal of multidrug resistance in vitro.[26, 27]

Furthermore, in normal cells without overexpression of p170 glycoprotein, e.g. cardiomyocytes, verapamil may decrease the efflux of anthracyclines only slightly so it could not be detectable by scintigraphic means. Similar results are reported for normal human bone marrow cells in which myelotoxicity of anthracyclines was not increased by verapamil.[28]

Another important modifying factor to be considered is the labeling of DOX and IDOX with I-123. Adding iodine by the Iodogen method,[19–21] physicochemical and, thus, biological properties of the molecule may be altered resulting in different pharmacokinetics of the labeled drugs as compared to nonlabeled derivatives. Therefore, further investigations are necessary to define changes in biokinetics of iodinated DOX and IDOX as compared to the unlabeled drugs.

Total cardiac activity measured by scintigraphic means, i.e. ROI-technique, includes both myocardial uptake and cardiac blood pool activity. Therefore, our results of cardiac accumulation, as presented in this study, do not exclusively depend on myocardial uptake. However, it has been shown that less than 0.4 % of the injected dose of either DOX or IDOX is detectable in the plasma within a few minutes after administration.[29] This is in accordance to a fast blood clearance of DOX and IDOX as shown by a rapidly clearing activity from the large vessels and the lungs. The decreasing bloodpool activity has been demonstrated by a quick decrease of cardiac uptake in the first two minutes, too. Therefore, cardiac activity appears to be mainly related to myocardial uptake after two minutes, thus, confirming our results that single-dose verapamil has no major impact on myocardial uptake of both DOX and IDOX.

DOX showed a nearly 2-fold higher cardiac accumulation as compared to IDOX. This holds true either alone or in combination with verapamil. Although IDOX is the most lipophilic derivative of common anthracyclines,[30] cardiac accumulation in our study was significantly lower suggesting a less cardiotoxic potential of IDOX. This is consistent with several preclinical and clinical studies reporting a reduced cardiotoxicity of IDOX as compared to DOX.[30-32] Thus, this scintigraphic finding confirms the reliability of the experimental setup chosen and suggests no major differences in pharmacokinetics of the labeled drugs as compared to non-labeled derivatives.

Conclusion

The results in rabbits did not confirm a significant increase of in vivo cardiac accumulation of anthracyclines following a single bolus injection of verapamil. Therefore, no increased risk of cardiotoxicity for treatment of DOX or IDOX in combination with verapamil is suggested so far. The lower cardiac accumulation of IDOX confirmed its lower cardiotoxic potential as compared to DOX.

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