Pharmacokinetic Interaction between 4′-Epidoxorubicin and the Multidrug Resistance Reverting Agent Quinine

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The serum and red blood cell (RBCs) disposition of epirubicin (EPR) after intravenous bolus injection without and with coadministered quinine (QUI) was investigated in patients undergoing a cyclic chemotherapy with EPR. QUI possesses a statistical significant influence on the EPR serum concentrations and, as a consequence, on the pharmacokinetic parameters for the initial distribution phase of EPR. Within the first 15 min after administration, EPR was distributed from the central compartment distinctly faster in compare to the control, when QUI was preadministered ($t_{1/2} = 6$ min for the control group and $t_{1/2} = 3$ min with QUI; −46%, p < 0.05). Yet, in the beta-phase when drug-elimination predominates, no statistical significant influence of QUI in regard to EPR serum and RBC concentrations could be observed. Half-life of elimination was 9.5 h for the control group and 8.6 h for the QUI group (−10%). The mean initial serum concentration (c_0) was reduced significantly by QUI from 7359 ± 506 ng/ml to 4351 ± 1682 ng/ml (−42%, p < 0.005). Furthermore, QUI caused a reduction of the serum bioavailability of EPR (expressed as AUC_{0-24}-values) from 3404 ± 1008 ng/ml × h to 2359 ± 1073 ng/ml × h (−31%, p < 0.05). Vd and Vdß were increased at about 90% and the mean total body clearance was accelerated from 45.3 to 148.7 ml/min, but due to the large standard deviations the calculated parameters for these parameters was not statistically significant. In the observed time interval of 24 h, the red blood cell coefficient of distribution k_{RBC} of EPR was lower if QUI was coadministered ($k_{RBC} = 1.25 ± 0.12$ for the control group $k_{RBC} = 1.15 ± 0.13$ under QUI; p < 0.04). The results point out that QUI induces an accelerated distribution of EPR from the blood into the tissue and that QUI additionally may have influence on the red-blood cell partitioning of EPR.

Introduction

Anthracyclines remain the most single chemotherapeutic agent in the treatment of advanced breast cancer, with response rates between 40% to 50% in previously untreated patients. Epirubicin (EPR), the 4′-epimer of doxorubicin*, represents a well established representative of this group of antineoplastic agents, which seems to have lower side effects as cardiotoxicity or myelosuppression at equal doses (Weenen *et al.*, 1986).

A common form of multidrug resistance (MDR1) in human cancer is associated with the expression of the MDR1 gene, which appears to be one of major impediment to more successful cancer chemotherapy (Pastan *et al.*, 1987). The MDR1 gene regulates the activity of P-glycoprotein (GP170), which regulates the efflux of cytotoxic compounds from the cell. A possibility to increase the response rate of the tumor is the combination of an antineoplastic agent with a chemosensitizer, which binds to GP170 and is capable of modulating the overexpression of P-glycoprotein by retarding the drug efflux from the tumor cell (Safa 1988). For EPR the coadministration of a chemosensitizer such as R-verapamil** (Mross *et al.*, 1993a, 1993b; Scheithauer *et al.*, 1993; Czejka

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* (1 *S*,3 *S*)-3-Glycoloyl-1,2,3,4,6,11-hexahydro-3,5,12-trihydroxy-10-methoxy-6,11-dioxonaphthacen-1-yl-3-amino-2,3,6-trideoxy-alpha-L-lyxopyranosid.
** 5-[N-(3,4-Dimethoxyphenethyl)-N-methylamino]-2-(3,4-dimethoxyphenyl)-2-isopropylvaleronitril.

et al., 1993) or cyclosporin A (Verweij et al., 1991; Yahanda et al., 1991) has been shown (in vitro and in vivo) to inhibit this drug efflux leading to an accumulation of the drug inside the tumor cell. But MDR1-modulation is limited by cardiotoxicity, e. g. associated with plasma verapamil concentrations that are less than those required for MDR1 reversal (Young et al., 1981).

The chemotherapeutic agent quinine* (QUI), and its optical isomer quinidine** (a class 1A antiarrhythmic agent) are considered to interact with a wide range of drugs such as propanolol or flecainide (Gibaldi, 1993) and have been shown to reverse MDR1 of EPR in different in vitro models (Dalton et al., 1993; Tsuruo et al., 1984). Both isomers appear to have equal activity in reversing resistance, but QUI is less toxic as quinidine and the therapeutic index for QUI may be superior in the clinical setting (Lehnert et al., 1991).

Pharmacokinetic data assessment represents a very useful method to demonstrate a chemosensitizing effect based on drug interaction in regard to drug metabolism and/or distribution. Therefore in the present pilot trial, the concentrations and pharmacokinetics of unbound EPR in serum and red blood cells (a subcompartment of the blood for drugs of the anthracycline family) were studied under the influence of coadministered QUI and compared (as a cross over design) with the control group without QUI.

Materials and Methods

Chemicals

All used solvents were from HPLC-grade purity (Merck, Darmstadt, Germany). Isoton-II was obtained from Hellige (Vienna, Austria), ascorbic acid from Riedel de Haen (Hannover, Germany).

Drugs and administration

EPR (Farmorubicin; Farmitalia Carlo Erba, Milano, Italy) was administered as a 2 min intravenous bolus (high dose of 90 mg/m^2 body surface). Treatment was repeated after a 4 week interval provided hematological parameters were satisfactory, whereby QUI was given three times day p.o.

in capsules which had been produced in the hospital pharmacy of the hospital Rudolfstiftung (each gelatin capsule containing 250 mg QUI; 1000 mg total individual dose, for three days prior to EPR bolus).

Human subjects

Six female patients with histologically confirmed advanced mamma carcinoma entered the study. All patients had a WHO performance status of 1 with no renal or hepatic impairment as judged by standard biochemical parameters. The age of the patients ranged from 50 to 56 years (x = 52.6 ± 2.4 y) and their body weight was from 51 to 68 kg (x = 63.2 ± 6.7 kg). All patients had given informed consent before entering the study, in accordance with the guidelines of the ethics committee of the University of Vienna.

Analytical procedure

Blood samples of 5 ml were collected in EDTA-impregnated vacutubes at the following times: 0, 3, 5, 10, 15 and 30 min and 1.0, 2.0, 4.0, 6.0 and 24 h after administration. An aliquot of 2.7 ml was mixed with 0.3 ml of ascorbic acid (1 mg/ml Isoton) for stabilization of EPR and vortexed for 2 min under protection from light to avoid photodegradation of the drug. Separation of red blood cells from serum was performed as described (Czejka et al., 1992a, 1992b) and EPR was quantified in the samples by high performance liquid chromatography using fluorimetric detection (Czejka et al., 1988). Analysis of blood samples (serum, RBCs) was performed within one week after collection in order to prevent loss of EPR during frozen storage.

Biometric data assessment

Pharmacokinetic calculations were performed by a PC 486 DX40 using the kinetic program PCNonlin V 4.0 with Nelder Mead and Gauss Newton iterative curve-fitting algorithm (SCI Software, Clin Trials, Lexington KY 40504, U. S. A.). Statistical calculations (F-test and paired Student's t-test) were performed with the scientific statistic package WiStat on an Atari MegaST computer.

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* (8S,9R)-6'-methoxycinchonan-9-ol).
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The following parameters were calculated and examined for significancy:

A [ng/ml]	intercept of y-axis of the semilog plot for the α-phase of the concentration-time curve (time = 0 min);
B [ng/ml]	intercept of y-axis of the semilog plot for the β-phase of the concentration-time curve (time = 0 min);
C_0 [ng/ml]	intercept of the concentration-time curve with y-axis (time = 0 min);
$t_{1/2}$cp [h]	half-life of distribution from the central to a deep compartment;
$t_{1/2}$el [h]	half-life of the terminal elimination;
AUC_{last}	[ng/ml $\times$ h] area under the concentration-time curve from 0 to 24 hours;
$_{v}$d [ml]	volume of distribution;
$V_{d\beta}$ [ml]	volume of distribution for the β-phase;
Cl_{tot} [ml/h]	total clearance of the blood.

The red blood cell coefficient of partition (k_{RBC}) was calculated as given in Eqn. (1) whereby C is the concentration (corrected for the hematocrit) of EPR in serum or RBCs [ng/ml].

$$k_{RBC} = \frac{C_{RBC}}{C_{serum}} . \qquad (1)$$

The percental amount of EPR which is present in serum and RBCs, respectively, was obtained according to Eqns (2) or (3):

$$\%_{serum} = \frac{C_{serum} \times 100}{(C_{serum} + C_{RBCs})} \qquad (2)$$

$$\%_{RBCs} = \frac{C_{RBCs} \times 100}{(C_{serum} + C_{RBCs})} \qquad (3)$$

Results

Serum and RBC concentrations

After bolus of EPR without QUI, the mean concentration time curve declined in a biexponential manner with a rapid phase of initial distribution followed by a phase of terminal elimination. (A third intermediate phase of drug-redistribution from the deep to the central compartment could be observed, too, but not in all individuals; so this intermediate phase had to be rejected for further pharmacokinetic calculations.) Preadministration of p.o. QUI caused a similar concentration-time profile of EPR in the serum but with distinctly lower concentrations within the first half hour after administration. Statistical analysis of these EPR serum concentrations without versus with QUI revealed a significancy with a level of probability of p < 0.05 (for the time points 0, 3, 5, 15 and 30 min, compare with Fig. 1). The difference of EPR serum concentrations collected at 10 min was not significant due to the high standard deviation of the data sets. In the following observed time interval from 1 to 24 h, the mean EPR concentration declined slowly in both groups without
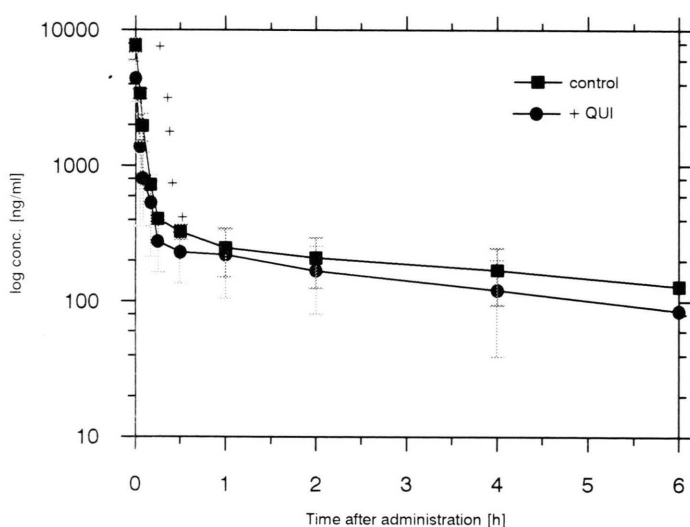

Fig. 1. Mean concentration-time curve of EPR in serum for the first 6 hours after administration (+ ... EPR concentrations which differ significantly (p < 0.05) between control and QUI-group).

any significant difference. The mean concentration-time curve of EPR for the RBCs declined very similar compared to serum; for a better overview the RBC concentration-time curves are not depicted in Fig. 1 (but data are presented in Table I).

The percent ratio of EPR between serum and RBCs with and without QUI is summarized in Table I. For the control group EPR can be found in its unbound form at about 44.6% (range 40.4–48.7%) in serum and with a mean of 55.4% in RBCs (range 51.3–59.8%).

No clue to a concentration dependent partitioning of EPR between serum and RBCs could be found; in such a case the percental amount which is bound to RBCs must increase with increasing time. As listed in Table I, the mean red-blood cell coefficient of partition k_{RBC} was 1.25 ± 0.12 (range 1.05–1.48) for the control. If QUI is preadministered, the distribution of EPR between serum and RBCs seems to be diminished whereby the steady state shifts from serum to RBCs ($k_{RBC} = 1.15 \pm 0.13$).

Although this reduction of k_{RBC} is of rather little order of magnitude (–6.2%), this change was statistically significant with a level of probability of $p < 0.05$ for serum and $p < 0.04$ for k_{RBC}.

Pharmacokinetics

The basic pharmacokinetic parameters of EPR in serum (using an open two-compartment model for calculation) are presented in Table II. As described above, QUI causes an accelerated distribution of EPR during the α-phase. The mean initial serum concentration (c_0) is reduced at 41.2% from 7085 ng/ml to 4816 ng/ml with a level of probability of $p < 0.005$. The intercepts of the hybrid constants (A, B) with the y-axis are influenced by QUI in a similar order of magnitude: "A" (representing the α-phase) decreases at 32% ($p < 0.05$) and "B" (representing the β-phase) at 56.3% ($p < 0.02$). These two parameters indicate an accelerated drug distribution, if QUI is coadministered. The half-life of distribution was 3.1 ± 2.8 min for the QUI group, this was an acceleration of about 50% in compare to the control (6.6 ± 3.8 min).

The total body clearance was accelerated by QUI from 45.3 to 148.7 ml/h; this is an increase of more than 220%, but due to the high standard deviation of the data set especially in the QUI-group, this increase was not statistically significant. The volume of distribution (V_d) and the V_d in the β-phase were increased by QUI at about 90%, too, but as in the case of clearance, significancy was very weak.

The most important pharmacokinetic parameter, representing the availability of a drug in the central compartment, is the area under the concentration-time curve (AUC).

The mean AUC_{last} of EPR was diminished under QUI coadministration from 3404 ± 1008 to

Table I. Mean percental ratio of epirubicin in serum and red blood cells with/without quinine.

Time [h][a]	Control %$_{Serum}$	%$_{RBCs}$	k_{RBC}	+ QUI %$_{Serum}$	%$_{RBCs}$	k_{RBC}
0.0	42.9	57.1	1.33	47.4	52.6	1.11
0.05	45.6	54.4	1.19	45.7	54.3	1.19
0.08	46.7	53.5	1.15	46.1	53.9	1.17
0.17	40.4	59.6	1.48	43.8	56.2	1.28
0.25	43.2	56.8	1.31	47.1	52.9	1.12
0.5	42.4	57.8	1.36	49.6	50.4	1.02
1	45.2	54.8	1.21	50.1	49.9	1.00
2	45.0	55.0	1.22	44.1	55.9	1.27
4	46.6	53.4	1.15	45.3	54.7	1.21
6	48.7	51.3	1.05	51.7	48.3	0.93
24	43.6	56.4	1.29	42.5	57.5	1.35
Minimum	40.4	51.3	1.05	42.5	48.3	0.93
Maximum	48.7	59.6	1.48	51.7	57.5	1.35
Mean	44.6[b]	55.4	1.25[c]	46.7	53.3	1.15
S.D.	2.3	2.3	0.12	2.9	2.9	0.13

[a] Time after administration of EPR.
[b] Level of probability $p < 0.05$ between %$_{serum}$control <> %$_{serum}$QUI.
[c] Level of probability $p < 0.04$ between k_{RBC}control <> k_{RBC}QUI.
S.D., standard deviation.

Table II. Pharmacokinetic parameters of epirubicin with/without quinine.

Parameter	Control Mean	S.D.	+QUI Mean	S.D.	+/– %	p
A [ng/ml]	7085	436	4816	2413	–32.0	0.05
B [ng/ml]	309	96	135	114	–56.3	0.02
C_0 [ng/ml]	7395	506	4351	1682	–41.2	0.005
$t_{1/2}\alpha$ [min]	6.6	3.8	3.1	2.8	–46.9	0.05
$t_{1/2}el$ [h]	9.45	0.25	8.56	4.13	–9.4	NS
AUC_{0-24} [ng/ml×h]	3404	1008	2359	1073	–30.7	0.05
V_d [ml]	26.5	2.5	51.0	37.9	+92.5	NS
$V_{d\beta}$ [ml]	615	221	1147	851	+86.5	NS
Cl_{tot} [ml/h]	45.3	15.1	148.7	162.5	+228	NS

+/– %, percental change of the parameter under QUI in compare to the control-group; p, level of probability; NS, not significant.

2359 ± 1073 ng/ml × h (-31%, $p < 0.05$). This change of the *AUC* is in accordance with the results obtained from distribution half-life. The half-life of the terminal elimination of EPR did not seem to be affected by QUI as the change is rather little (9.45 ± 0.25 h for control, 8.56 ± 4.13 h for QUI group (-10%).

Discussion

According to our pharmacokinetic results the preadministration of QUI might accelerate the initial distribution of EPR from the blood into the tissue. At the present it is not clear whether this QUI-effect on EPR pharmacokinetics is caused by the inhibition of drug efflux from the tumor cell (as proposed by Bisset *et al.*, 1994) or not. Acceleration of the total body clearance as well as the increase of the volume of distribution suggest a change of drug distribution from the central to the deep compartment.

Another explanation for this drug interaction might base on a changed hepatic metabolism of EPR as QUI is known to inhibit a certain range of drugs. This is a result of the high affinity between QUI and cytochrome P_2D_6, an isoenzyme of cytochrome P-450, which mediates the oxidative metabolism of drugs in hepatocytes (Gibaldi *et al.*, 1993). Cytostatics from the anthracycline family undergo extensive hepatic metabolism for phase-I and especially for phase-II reactions. So it would be possible, theoretically, that QUI induces the hepatic enzymes which are responsible for EPR-metabolism leading to lower serum concentrations of EPR. In such a case the determination of the metabolites of EPR in faeces (glucuroni-dation) could verify this theory.

As cytochrome activity (cytochrome P-420) can be observed at a certain extent in RBCs, too, (Cossum *et al.*, 1988) and antineoplastic agents from the anthracycline-type own a high affinity towards RBCs (Boroujerdi *et al.*, 1990a, 1990b; De Flora *et al.*, 1986), the decrease of EPR concentrations inside the blood cells could amplify the observed QUI-effect. Increased metabolism of EPR inside the RBCs caused by QUI can be excluded after evaluation of the chromatograms.

Further it has to be considered that drug interactions also may base on competitive protein binding or red-blood cell partitioning in those cases, when a drug owns a high affinity to serum proteins and/or RBCs.

The study had to be finished after six patients because no tumor response could be stated by QUI (this observation is in accordance with the findings of Bisset *et al.* (1994). Additionally it must be pointed out that the high dose of QUI, which is necessary to obtain a definitive effect for such a drug interaction, leads to high serum concentrations ($1-5$ μg/ml) with particular severe side effects. In this pilot we observed typical "cinchonism" with vomiting, dizziness and tinnitus. For two patients it was necessary to perform audiometric measurements due to QUI toxicity.

The present results demonstrate, that the observed *in vitro* effect of an increased cell-uptake of EPR when combined with QUI takes place *in vivo*, too, but only at a small extent. If drug efflux from the cell is inhibited continuously, the serum concentrations of EPR must be lower significantly for the whole observed period (24 hours) and not only for the first 30 min after administration.

In compare to the control group, the slightly diminished concentrations of EPR in RBCs under QUI indicate a certain change in RBC-partitioning, but absolute no significant difference in the metabolic pathway of EPR (reduction of the keto-function in position 13 by aldo-ketoreductase activity) could be observed.

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