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Determination of free and liposome-associated daunorubicin and daunorubicinol in plasma by capillary electrophoresis

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Abstract

Liposomal daunorubicin (DaunoXome) is a formulation of the anticancer drug daunorubicin encapsulated into vesicles of about 45 nm diameter. To understand the pharmacodynamic relationships associated with the toxicity and efficacy of liposome-encapsulated daunorubicin in vivo and in vitro, it is essential to have a rapid method of separating the free and liposomal forms of the drug. We have developed and validated a method to quantify drug concentrations of liposomal daunorubicin, free daunorubicin and its main metabolite daunorubicinol that requires only 50 μl of plasma to conduct studies in children. The method involves the use of solid-phase extraction followed by capillary electrophoresis with laser-induced fluorescence (LIF) detection. With LIF detection a limit of quantification of 1 μg/l is obtained for the free form and the metabolite. Precision and accuracy are in accordance with the generally accepted criteria for bioanalytical methods. The method is rapid and allows for multiple samples to be processed simultaneously.

Keywords: Daunorubicin; Daunorubicinol; DaunoXome; Anthracyclines; Antibiotics

1. Introduction

Anthracyclines play a very important part in many treatment protocols in paediatric oncology. Daunorubicin (Fig. 1) is mainly used for the treatment of acute lymphatic and myeloic leukaemia in combination with cytarabine [1]. Daunorubicin is necessary to achieve high response rates. The main problem is the cardiotoxicity of the anthracycline antibiotics, which can cause congestive heart failure even years after treatment [2].

Improving the therapeutic index of anthracycline antineoplastic drugs has long been considered a major aim of cancer drug delivery. One approach to reduce anthracycline-related cardiotoxicity is liposomal encapsulation. The potency of these drugs and the major problem of cardiotoxicity have fuelled a large body of drug delivery research.

DaunoXome is a liposome-encapsulated form of daunorubicin in which the anthracycline is entrapped in small unilamellar vesicles composed of a 2:1 molar ratio of distearoyl phosphatidylcholine and cholesterol [3]. In preclinical studies, DaunoXome was shown to be less cardiotoxic than the reference anthracyclines, daunorubicin and doxorubicin [4].

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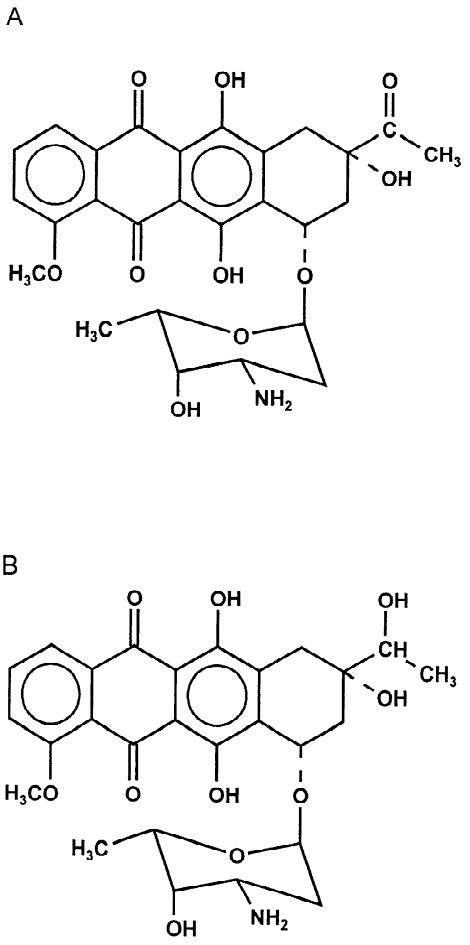

Fig. 1. Daunorubicin (A) and daunorubicinol (B).

Studies with DaunoXome in adults with aquired immunodeficiency syndrome (AIDS)-related karposi sarcoma showed no clinically manifested cardiotoxicity even at high cumulative doses [3]. DaunoXome has shown activity against solid tumours and leukaemia's with lower toxicity compared with the free drug [5–7]. Studies in animals suggest a better delivery of the active drug into tumour tissue, while the concentrations in the heart were low [3,8]. Low peak plasma concentrations of free daunorubicin after administration of liposomal daunorubicin and the reduced tendency to accumulate in the myocardium suggest that a reduction in cardiac toxicity compared with free daunorubicin may be observed.

In this context, pharmacokinetic studies, especially in children, are necessary to investigate the role of the pharmacokinetics of the free and the liposomal forms and the importance of the main metabolite, daunorubicinol (Fig. 1), for both the cytotoxic effect and adverse events. Several studies have demonstrated a significant alteration of the pharmacokinetic profile of the total amount of drug compared with free drug. The pharmacokinetics of total liposome-encapsulated daunorubicin are characterised by a greater than 35-fold increase in the area under the curve (AUC), slow plasma clearance and a reduced volume of distribution [5]. Only one study has evaluated the plasma concentration of free and liposomal daunorubicin [9]. Several methods have been proposed to estimate the free and liposomal concentrations, including centrifugation, size-exclusion chromatography, ultrafiltration, ion-exchange chromatography and solid-phase extraction [10,11]. Most of these methods have important limitations. Because of the small size of the liposomes of DaunoXome (~45 nm), centrifugation is not possible. Dialysis was found to be unsuitable due to slow equilibration. Gel chromatography results in high dilution of the sample and is too slow [10]. For ultrafiltration, problems arise due to adsorption of the anthracyclines to the ultrafiltration device [12]. Solid-phase extraction (SPE) has been used for several liposomal compositions to separate the free and liposomal fractions [11,13–15]. The liposomal form passes reversed-phase C_{18} silica gel cartridges, while the free form is retained on the stationary phase. Bellott et al. [15] developed a method using SPE for the separation of liposomal and free daunorubicin. Methods for separating the free and liposomal forms of the drug have the problem of liposome release, therefore the SPE was optimised to avoid artificially high free drug concentrations. Total plasma daunorubicin concentrations are measured using the method of Hempel et al. [16] and free daunorubicin and daunorubicinol are measured after SPE with capillary electrophoresis and laser-induced fluorescence (LIF) detection. The liposomal fraction is quantified by subtraction of the free drug from total daunorubicin.

To conduct studies in children, analytical methods requiring only small sample volumes are necessary. The method of Bellott et al. requires a sample volume of 1 ml plasma, which is too much for our purposes [15]. To reduce the sample volume, car-

tridges with a smaller amount of sorbent and capillary electrophoresis with LIF detection were used. Capillary electrophoresis (CE) is an attractive technique for this purpose, because, theoretically, the method requires only a sample volume of a few nanoliters. With UV detection, the sensitivity of CE is often insufficient to quantify drugs in biological fluids in low mg amounts [17]. Laser-induced fluorescence detection can be used for the detection of anthracyclines with a high sensitivity [18]. Recently, we developed methods for the quantification of daunorubicin, idarubicin and doxorubicin and their main metabolites in plasma [16,19,20]. On the basis of this work, we have developed and validated an assay for the quantification of free and liposomal daunorubicin and daunorubicinol in biological fluids.

In cell culture systems, liposomal encapsulation of daunorubicin showed similar, higher or lower levels of in-vitro cytotoxicity [21–24]. Up to now no adequate attention has been paid to the potential problems with leakage of the free drug from the liposomes into the culture medium. The chemical stability of the free and encapsulated forms under in-vitro culture conditions is important for the interpretation of the effects of liposome-encapsulated daunorubicin and free daunorubicin in cell lines. For this purpose, the method was successfully applied to the investigation of the stability of liposomal daunorubicin in cell culture media.

2. Materials and methods

2.1. Drugs and reagents

DaunoXome was obtained from Gilead Pharmaceuticals (Boulder, CO, USA) in ready-for-use vials containing 50 mg liposomal daunorubicin at a concentration of 2 mg/ml. Daunorubicin, daunorubicinol and doxorubicinol were kindly supplied by Farmacia (Erlangen, Germany). All chemicals used were of analytical grade. Acetonitrile, methanol and sodium hydroxide solution (0.1 *M*) were purchased from Malinckrodt Baker (Deventer, Netherlands), spermine tetrahydrochloride from Aldrich (Steinheim, Germany), Triton X-100 from Sigma (Steinheim, Germany), and glycerol, sodium chloride, and sodium dihydrogenphosphate from Merck (Darmstadt, Germany). Purified water was prepared using a Millipore-Q-UF system. Sep-Pak cartridges containing 50 mg reversed-phase C_{18} silica gel were obtained from Waters (Eschborn, Germany). RPMI 1640 medium was obtained from Life Technologies (Karlsruhe, Germany)

2.2. Sample preparation

Total plasma daunorubicin concentrations were measured by the method of Hempel et al. with capillary electrophoresis and LIF detection [16]. Free daunorubicin and daunorubicinol were measured after SPE. The liposomal fraction was quantified by subtraction of the free drug from the total daunorubicin.

Two different schemes for sample preparation were used depending on the expected concentration:

Total plasma daunorubicin:

1. between 50 and 1000 μg/l daunorubicin: 10 μl of plasma (patient plasma, calibration plasma or quality control samples) was mixed with 90 μl of a solution of idarubicin in acetonitrile (50 μg/l) using a vortex mixer and ultrasound, both for 60 s. Subsequently, the mixture was centrifuged at 1500 *g* for 3 min.
2. daunorubicin >1 mg/l: 20 μl of plasma was diluted to 100 μl with water. To 10 μl of this dilution, 90 μl of a solution of the internal standard (I.S., idarubicin, 50 μg/l) in acetonitrile was added and vortex-mixed for 60 s followed by ultrasound treatment for 60 s. 35 μl of the supernatant was transferred into a polycarbonate vial for CE analysis.

Free daunorubicin and daunorubicinol:

1. 50 μl of plasma (patient plasma, calibration plasma or quality control samples) was mixed with 50 μl of a solution of 2 ml plasma with 10 μl of the internal standard, doxorubicinol (10 μg/l).

2.3. Solid-phase extraction

Free daunorubicin and daunorubicinol were separated from liposomal daunorubicin in plasma using Sep-Pak C_{18} cartridges attached to a vacuum manifold apparatus. The separation procedure is summarised in Table 1. Plasma samples containing the

Table 1
Separation procedure for free and liposomal daunorubicin in plasma

1. *Column preparation*
(i) Methanol washing (1×300 μl)
(ii) Buffer washing (1×750 μl)
(iii) Apply blank plasma onto the column (1×50 μl)

2. *Separation*
(i) Apply sample with I.S. onto column (1×100 μl) without vacuum

3. *Column washing*
(i) Layer blank plasma onto the column (1×50 μl) without vacuum
(ii) Buffer washing (1×500 μl) without vacuum
(iii) Water washing (1×100 μl) [vacuum (15 mmHg) at the end for 5 min]

4. *Elute free daunorubicin and daunorubicinol*
(i) Apply 400 μl methanol onto the column and collect the eluate

internal standard were applied and passed through the conditioned cartridges without applying a vacuum. After plasma had settled into the sorbent, 50 μl plasma was added. Phosphate buffer (500 μl, pH 7.4) was also passed through the cartridges without applying a vacuum. The columns were further washed with 100 μl water. Free daunorubicin and daunorubicinol were then eluted into a 1.5 ml Eppendorf vial with 400 μl methanol. The eluates were evaporated to dryness under a stream of nitrogen.

The phosphate buffer was prepared by adjusting a solution of 10 mmol sodium dihydrogenphosphate and 150 mmol sodium chloride with 500 mmol sodium hydroxide to a pH value of 7.4.

The residues were reconstituted with 50 μl acetonitrile containing 5% phosphate buffer, pH 7.4. When the concentrations of anthracyclines were >50 μg/l, the solutions were diluted 1:10 with acetonitrile containing 5% phosphate buffer, pH 7.4, and were measured again. At least 15 μl of the reconstituted residue was transferred into a polypropylene vial for CE analysis.

2.4. Capillary electrophoresis

A Model 5510 P/ACE (Beckman Coulter, Munich, Germany) equipped with an air-cooled argon ion laser operating at 488 nm was used (5 mW, Beckman Coulter). Detection was carried out with a Beckman LIF detector equipped with a 520 nm band pass filter. Fused-silica capillaries of 40 cm effective length and an inner diameter of 50 μm were used (O.D. 375 μm, Beckman Coulter). Separation was performed at 532 V/cm (25 kV) applied voltage with the cathode at the detection end of the capillary. The running buffer was prepared by adjusting a solution of 100 m*M* sodium dihydrogenphosphate with 100 m*M* sodium hydroxide to a pH value of 5.0. Subsequently, spermine was added to obtain a concentration of 19.8 μ*M* and the solution was mixed with acetonitrile to a final concentration of 70% (v/v). All solutions for CE were filtered through a 0.45 μm filter.

Samples were applied to the capillary by electrokinetic injection at 10 kV for 10 s. Between runs, the capillary was rinsed with sodium hydroxide (100 m*M*) for 1 min and the running buffer for 1 min. Each day before analysing the first sample, the capillary was rinsed with sodium hydroxide (100 m*M*) for 20 min and the running buffer for 10 min.

2.5. Stability study in RPMI 1640 medium

To assess the degradation of liposomal daunorubicin, 10 mg/l DaunoXome was incubated in RPMI 1640 medium supplemented with 10% FCS in a humidified atmosphere with 5% CO_2 at 37 °C for up to 72 h. At the start of the incubation, three times within the first 8 h and after 24, 48 and 72 h, aliquots of the cell culture medium were analyzed for total daunorubicin by capillary electrophoresis [16]. The release of entrapped daunorubicin was studied using a fluorescence-dequenching assay [25]. The self-association of daunorubicin induces an almost total quenching of its fluorescence [26]. Inside the vesi-

cles, the encapsulated drug is totally self-associated because of the high concentration [8] and the fluorescence is negligible. If daunorubicin leaks out of the liposomes it is highly diluted in the external medium, and it recovers its monomeric form and its fluorescence.

The in-vitro degradation of liposomes was analysed by comparing the fluorescence of the cell culture medium supplemented with the liposome-encapsulated anthracyclines prior to and after cracking of liposomes by Triton-X 100 treatment on a Shimadzu RF-540 spectrofluorophotometer. A 1-ml aliquot of the cell culture medium was well mixed with 1 ml of 5% Triton X-100 solution. Release (%) of daunorubicin from liposomes was calculated from the fluorescence intensities (excitation 510 nm, emission 555 nm) with and without Triton-X treatment according to the equation

$$\text{Release (\%)} = (F_T / 2F_0) \cdot 100$$

where F_T and F_0 are the fluorescence intensities with and without 5% Triton X-100 treatment.

Free daunorubicin was also separated from liposome-encapsulated anthracyclines by SPE. With this method it was also possible to assess the degradation of 1 mg/l DaunoXome. All experiments were carried out in triplicate

2.6. Preparation of standard solutions

Stock solutions containing 200 to 500 mg/l daunorubicin, daunorubicinol and doxorubicinol were prepared in acetonitrile. The stock solutions were stored at −20 °C and could be used for up to 12 weeks. These solutions were diluted with water before preparation of standards. Blank plasma for the preparation of calibration solutions was taken from healthy donors from the Department of Transfusion Medicine, University of Münster. Standards were prepared by serial dilution of the stock solutions with blank plasma to obtain the desired concentrations of both daunorubicin and daunorubicinol. Quality control samples were prepared in the same manner.

2.7. Quantification

All calculations were performed using the corrected peak area (peak area/migration time). Calibration graphs were calculated by analysing six different standard solutions from 1 to 50 μg/l and six different standard solutions in the higher concentration range from 50 to 1000 μg/l for daunorubicin and daunorubicinol using the internal standard method and weighted linear regression ($1/x$). Within every series, two to three quality control samples were analysed (concentrations 1, 20, 50, 750 μg/l).

Precision and accuracy were assessed by repeated analysis of quality control samples in one series (intra-day) or on subsequent days (inter-day).

3. Results

3.1. Separation

The separation of daunorubicin, daunorubicinol and the I.S. in spiked plasma after solid-phase extraction is shown in Fig. 2A. The conditions for the separation are the same as developed by our group for the separation of idarubicin, daunorubicin and doxorubicin [16,19,20]. As anthracyclines have the tendency to interact with the silanol groups of glass surfaces [1], the addition of acetonitrile and spermine is necessary to reduce the adsorption of the analytes to the capillary wall.

In blank plasma, no peaks interfering with the analytes were observed (Fig. 2B). This is due to the selective detection of the analytes based on their fluorescence when excited at 488 nm.

Doxorubicinol was chosen as the internal standard because of its structural similarity to the analytes. The addition of an internal standard is necessary to adjust for deviations during electrokinetic injection, deviations due to the SPE and due to evaporation of the sample.

The separation of daunorubicin, daunorubicinol and the I.S. in spiked RPMI 1640 medium is shown in Fig. 3A. In blank RPMI 1640 medium, no peak interfering with the analytes was observed (Fig. 3B).

3.2. Extraction procedure and method optimisation

Methods for separating the free and liposomal forms of the drug have the problem of liposome

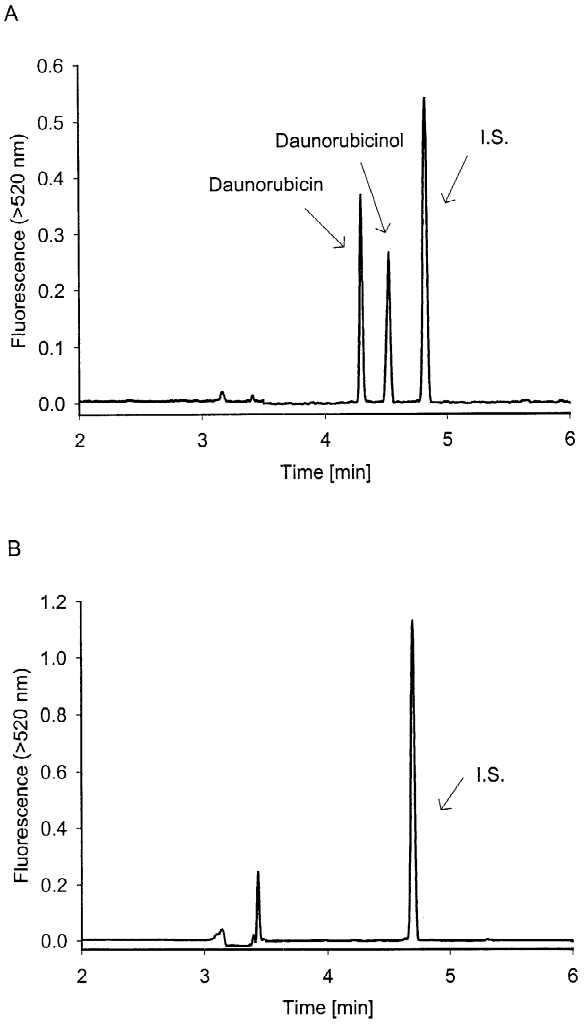

Fig. 2. (A) Electropherogram of a spiked plasma sample containing 50 μg/l daunorubicin and daunorubicinol. (B) Blank plasma with I.S. prepared in the same manner.

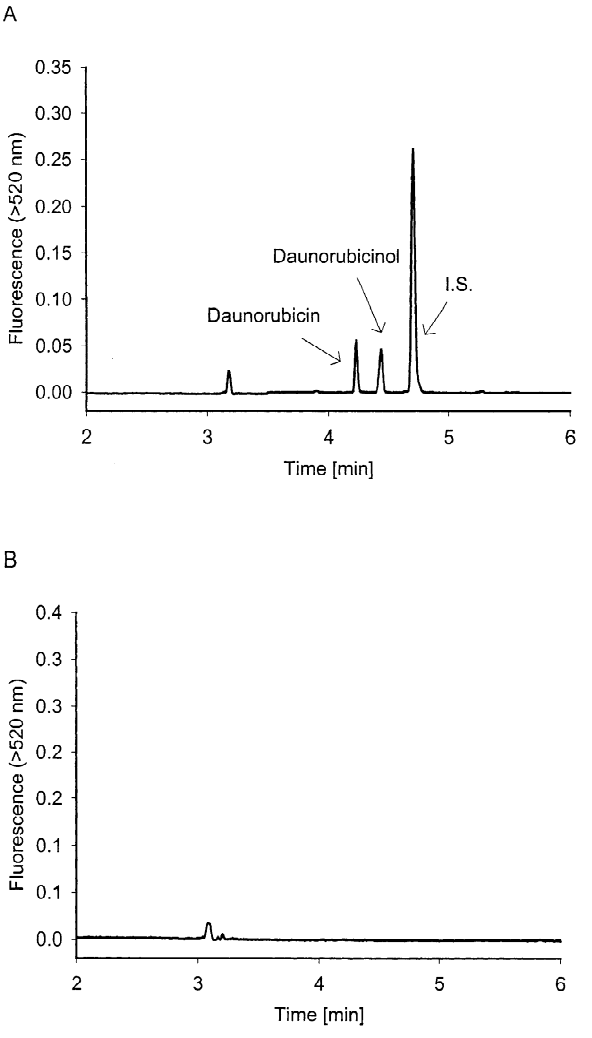

Fig. 3. (A) Electropherogram of a spiked RPMI medium containing 20 μg/l daunorubicin and daunorubicinol. (B) Blank RPMI medium prepared in the same manner.

release, therefore the SPE was optimised to avoid an artificially high free drug concentration. The percentage of free drug found in a sample spiked with liposomal daunorubicin could be due to free drug present in the solution of DaunoXome in 5% glucose, release of the drug during the mixing procedure with plasma, a time-dependent release and/or release during SPE.

Blank plasma was spiked with known amounts of DaunoXome diluted with phosphate buffer, pH 7.4, or with 5% glucose in the concentration range from 1 to 15 mg/l. After 5 min incubation, extraction was performed according to the described method either without plasma or using plasma for column preparation and washing (Fig. 3). Incompatibility with saline solutions was observed. Because of this incompatibility the cartridges were prepared and washed with blank plasma. Due to this preparation step the free amount could be reduced from 7.5±0.7 to 6.6±0.1% (Fig. 4). The percentage 7.5±0.7% of

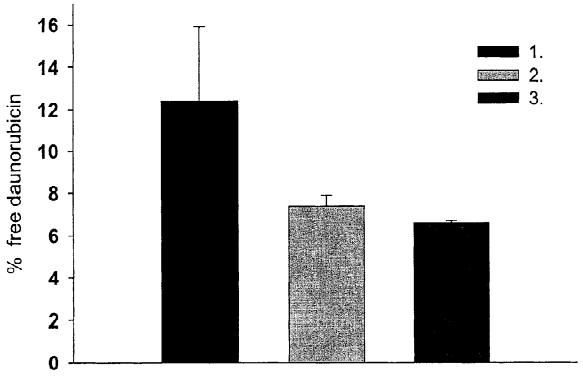

Fig. 4. Percentage of free daunorubicin after spiking plasma with daunoxome and the first SPE. 1=DaunoXome diluted with phosphate buffer, pH 7.4, before spiking plasma, without blank plasma for column preparation. 2=DaunoXome diluted with 5% glucose before spiking plasma, without blank plasma for column preparation. 3=Like 2 with blank plasma for column preparation.

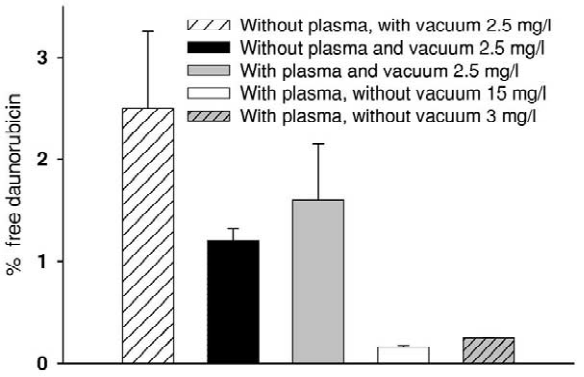

Fig. 5. Percentage of free daunorubicin after spiking plasma with DaunoXome and the second SPE.

free daunorubicin is comparable to the amount 7.4±1.56% found by Bellott et al. without blank plasma for preparation of the columns [15].

One approach to determine the amount of drug released from the liposomes during SPE is to first remove the free drug from the spiked plasma sample by SPE. The following determination of free drug by SPE would represent the amount of liposomal drug which crosses over into the free drug during SPE and that due to drug release over time. After the first SPE of different amounts of liposomal daunorubicin in plasma through a prepared, blank plasma wetted cartridge to avoid changing the plasma protein concentration, the liposomal fraction was collected. An aliquot of this processed plasma sample was used for the following, second SPE. This second SPE was performed with and without blank plasma for column preparation and column washing and with and without applying a vacuum (Fig. 5).

With vacuum, the percentage of free daunorubicin and the standard deviation were higher in comparison with the procedure without vacuum. With blank plasma for column preparation the percentage of free daunorubicin and the standard deviation were lower in comparison with the procedure without blank plasma. The lowest percentage of free daunorubicin was found without applying a vacuum and with blank plasma for column preparation. Higher concentrations of DaunoXome showed a lower percentage of free daunorubicin (0.15±0.02%) than lower concentrations (0.24±0.01%) (Fig. 5).

The method was successfully applied to the investigation of the stability of liposomal daunorubicin in cell culture media. SPE was performed in the same manner as described for plasma. The free amount after spiking the RPMI 1640 medium after 5 min of incubation in the concentration range from 1 to 13 mg/l was 6.7±0.1%, comparable to the 6.6±0.1% after spiking blank plasma.

3.3. Stability of DaunoXome in plasma

To determine whether the free amount found after the second SPE is due to time-dependent release from the liposomes, the stability at room temperature over time was measured after spiking blank plasma with 1 and 10 mg/l liposomal daunorubicin. At the start of the incubation and after 45, 90 and 180 min, aliquots of the plasma were analyzed for free daunorubicin and total daunorubicin.

A concentration-dependent release was found (Fig. 6). High liposomal concentrations are more stable than lower concentrations. A release of 0.21% of daunorubicin every 10 min was found at a concentration of 1 mg/l and of 0.07% at a concentration of 10 mg/l. This could explain the higher percentage of free daunorubicin at lower concentrations of DaunoXome after the second SPE.

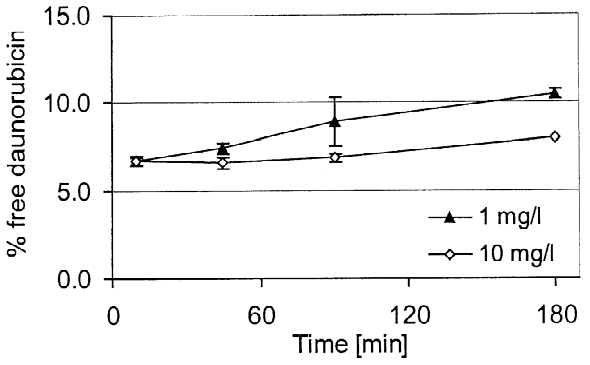

Fig. 6. Stability of liposomal-encapsulated daunorubicin in plasma.

3.4. Stability study in RPMI 1640 medium

As the fluorescence of liposomes is quenched by liposome encapsulation, the in-vitro degradation of liposomes can be analysed by comparing the fluorescence prior to and after cracking of liposomes by Triton-X treatment [27]. Both methods, fluorescence measurement and SPE, showed comparable results with regard to the free amount (Fig. 7). With fluorescence measurement, a percentage of 6.4 and with SPE 6.7±0.1% free daunorubicin was found 5 min after incubation. The proportion of daunorubicin released from DaunoXome increased rapidly in a concentration-dependent manner. At a concentration of 10 mg/l DaunoXome, about 30% of daunorubicin was released within 24 h and about 30% was released at the end of the incubation (Fig. 7). With 1 mg/l DaunoXome, almost all daunorubicin was released from the liposomes within 48 h.

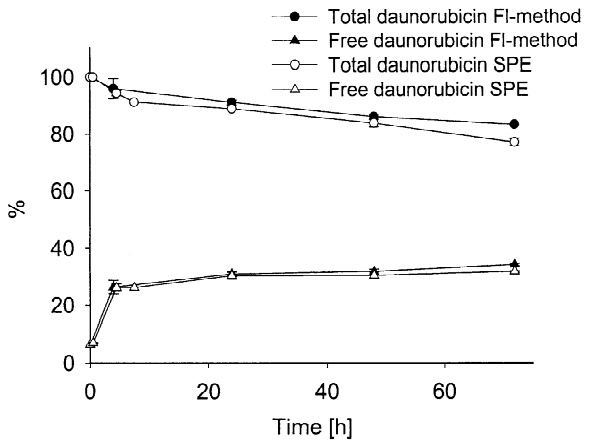

Fig. 7. In-vitro stability of liposomal-encapsulated daunorubicin (10 mg/l) in RPMI 1640 medium. Comparison of fluorescence and SPE methods.

Table 2
Precision and accuracy of the assay in one series ($n=7$, intra-day)

Conc. added (μg/l)	Mean conc. found (μg/l)	Accuracy (%)	Precision (%)
Daunorubicin			
750	746.4	−0.5	1.5
50	53.4	6.9	2.1
20	20.6	3.0	3.9
1	1.1	6.0	8.6
Daunorubicinol			
750	742.6	−1.0	0.8
50	52.6	5.1	2.7
20	20.5	2.5	2.6
1	1.1	9.1	6.6

3.5. Reproducibility

With a sample volume of 50 μl, the limit of quantification was found to be 1 μg/l. The accuracy and precision of the method, determined in one series, are shown in Table 2. It is apparent that the method meets the generally accepted requirements for bioanalytical methods [28]. Table 3 shows the results for the precision and accuracy determined on consecutive days. The method was robust over a long time period.

Table 3
Precision and accuracy of the assay on consecutive days ($n=6$, inter-day)

Conc. added (μg/l)	Mean conc. found (μg/l)	Accuracy (%)	Precision (%)
Daunorubicin			
750	744.9	−0.7	2.2
50	51.8	3.6	7.5
20	19.4	−3.1	4.2
1	1.1	9.5	11.4
Daunorubicinol			
750	743.3	−0.9	0.8
50	52.2	4.5	2.7
20	19.7	−1.7	4.6
1	1.1	5.9	11.2

4. Discussion

To understand and predict the efficacy and/or toxicity of liposomal drugs in vitro and in vivo, determining the kinetics of encapsulated drugs as well as free drugs is essential. The kinetics of free and liposomal drug are complex, because the observed data result from convolution of the disposition of the liposomes, the rate of release of encapsulated drugs and the disposition of free drug [29].

A satisfactory method for separating free and liposomal drug from biological fluids should be fast and simple, and avoid sample dilution and an artificially high free drug concentration. Several methods have been described for the determination of free and liposomal anthracyclines in biological fluids [10–12,15]. Most of these methods have important limitations. Due to the possibility of concentration of the analyte and the fast separation with SPE, which occurs within 60 s, we developed an optimised method which accurately and reproducibly separates free daunorubicin from liposomal daunorubicin in plasma and cell culture medium. The HPLC method described by Bellott et al. [15] requires plasma volumes of 1000 μl. For pharmacokinetic studies in children, these amounts of plasma are not acceptable, especially with infants. Therefore, we investigated the potential of cartridges with a smaller amount of sorbent for SPE and capillary electrophoresis to reduce the required sample volume. For the anthracyclines, a very sensitive detection system is available, since a LIF detector with an Ar ion laser emitting at 488 nm can be used to excite the analytes. With SPE and capillary electrophoresis the limit of quantification can be reduced from 2 to 1 μg/l in comparison with liquid–liquid extraction [16].

The main advantages of this method are the small plasma volume of 50 μl with a low limit of quantification, the rapid procedure and, especially, the small amount of artificial free daunorubicin. The free percentage after the first and second SPE can be reduced and the results can be confirmed by fluorescence measurements. At most, $0.21 \pm 0.01\%$ of liposome-encapsulated anthracyclines was liberated by the SPE treatment. This is important considering the high c_{max} of liposomal daunorubicin. Bellott et al. found $0.58 \pm 0.45\%$ of artificial free daunorubicin [15]. Determination of plasma stability showed a release of 0.21% of daunorubicin every 10 min for lower, and of 0.07% for higher, concentrations. This time-dependent release can explain the free amount found after the second SPE. With fluorescence measurement we could confirm the free amount measured after spiking with DaunoXome and separation with SPE. This shows that most of the non-liposomal daunorubicin found in plasma spiked with DaunoXome originates from the preparation procedure and only a very small amount originates artificially from the extraction procedure. To avoid free daunorubicin originating artificially from the time-dependent release, the extraction should be performed immediately.

To understand the pharmacodynamic relationships associated with the toxicity and efficacy of liposome-encapsulated daunorubicin in vitro it is also important to have a rapid method of separating the free and liposomal forms of the drug. Therefore, the method was applied successfully to the investigation of the stability of liposomal daunorubicin in cell culture media.

To date, there have only been a few reported studies describing the pharmacokinetics of daunorubicin after application of DaunoXome [5,30–34]. In children, we are only aware of one study with a pharmacokinetic investigation of liposomal daunorubicin [9]. Therefore, several questions need to be answered about the pharmacokinetics of liposomal daunorubicin, especially in children. For this purpose, this method offers several advantages, i.e. the smaller sample volume required and a smaller amount of solvent.

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