Anti-Cancer Drugs 2001, **12,** pp. 859–864

Report

Quantification of doxorubicin in plasma—a comparative study of capillary and venous blood sampling

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Doxorubicin, an anthraquinone glycoside, is currently one of the clinically most important antineoplastic drugs. The aim of the present study was to identify potential concentration differences of doxorubicin in plasma from capillary and venous blood samples. Sixteen patients (seven females and nine males; median age: 37 years, range: 1–77 years) were included. The quantitative analysis of doxorubicin was carried out by reversed-phase liquid chromatography with fluorometric detection. The concentration of doxorubicin in capillary and venous samples were closely correlated (r=0.98; $p<0.0001$). The median plasma concentration ratio capillary/venous was 1.13 (95% confidence interval: 1.06–1.20) and not affected either by plasma drug concentration, age or the body mass index of the patient. The concentration ratio was significantly higher in males (median: 1.18) than in females (median: 1.01). The observed concentration differences of doxorubicin in plasma from capillary and venous samples are of minor importance only. Capillary blood sampling is recommended for use in pharmacokinetic studies of doxorubicin, especially in pediatric patients, in order to avoid sometimes traumatic venous blood sampling.

Key words: Anthraquinone glycosides, doxorubicin, capillary and venous blood sampling, pharmacokinetics, statistics.

Introduction

Anthraquinone glycosides are an important class of antineoplastic drugs. Doxorubicin, the most frequently used drug within this class, has activity against a large variety of malignant neoplasms in adults and children.[1] The clinical use of doxorubicin is limited by myelosuppression and irreversible cardiac toxicity.[1,2] The plasma pharmacokinetics (area under plasma concentration time curve and maximum plasma concentration) show a more than 10-fold inter-individual variability despite dosing based on body surface area.[3] An individualized doxorubicin dose, based on determined plasma concentrations, would therefore most likely result in an improvement of treatment.[4,5] The use of a simplified sampling technique has made it possible to obtain accurate estimates of the systemic exposure of doxorubicin and 4′-epi-doxorubicin using only one blood sample from the patient.[6] Plasma levels of anthraquinone glycosides have so far been measured using blood samples obtained by puncturing of a peripheral vein. With improved technique for measuring plasma concentrations it is now possible to quantify anthraquionone glycosides in capillary samples, collected by a finger lancet puncture. The practical advantage of collecting capillary blood instead of venous blood is evident. The advantages of capillary blood sampling are most pronounced in pediatric patients, who often find venous blood sampling very traumatic.

The aim of the present study was to identify potential concentration differences of doxorubicin in plasma from capillary and venous blood samples for use in our limited sampling procedure for pharmacokinetic studies.

Material and methods

The present study was approved by the Local Ethics Committee at Karolinska Hospital, Stockholm, Sweden

Financial support from the Swedish Cancer Society is gratefully acknowledged.

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(Dnr-93-068). Oral informed consent was obtained from all patients and/or, when appropriate, their parents.

Patients

Sixteen patients (seven females and nine males) with a median age of 37 years (range: 1–77 years) participated in the study. Their diagnoses included acute lymphocytic leukemia, chronic lymphocytic leukemia, lymphoma, neuroblastoma, multiple myeloma and Ewing's sarcoma.

Drug administration

Doxorubicin was prepared by dilution of a commercially available stock solution (2 mg/ml; Pharmacia Upjohn, Stockholm, Sweden) with 5% glucose solution to a final volume of 250–1000 ml, depending on the treatment schedule. The preparations were made at the Compounding Unit at Karolinska Pharmacy, Stockholm, Sweden according to standard procedures. The administered dose of doxorubicin was 33.6 mg/m^2 (median value; range: 14.0–50.1 mg/m^2) given through a central venous access as an i.v. infusion (2–24 h) using a IVAC model 561 infusion pump (Medical Instrument Systems Scandinavia, Täby, Sweden).

Sampling procedure

Capillary and venous blood samples were collected simultaneously, i.e. with a time difference of less than 30 s (11 patients) or less than 5 min (five patients). All samples were drawn at the end, but before completion of the infusion. Venous blood samples (5–7 ml) were drawn from a peripheral vein and collected in Vacutainer® tubes (Becton Dickinson, Franklin Lakes, NJ) containing 250 IU sodium heparin (freeze-dried). Capillary blood (400–500 μl) was collected, from a finger tip using a Minilancet (CCS Clean Chemical Sweden, Borlänge, Sweden), into micro hematocrit capillary tubes (internal diameter 1.2 mm; length 75 mm) treated with ammonium heparin (Kebo Lab, Stockholm, Sweden). The venous blood samples were centrifuged (800 *g*) for 10 min to separate the plasma fractions using a Technospin® centrifuge (Sorvall Instruments; Du Pont, Wilmington, DE). The capillary plasma fractions were separated by centrifugation for 5 min in an Adams Autocrit Centrifuge (Clay Adams, Parsippany, NJ). All plasma fractions were transferred into capped glass vials and stored at −80°C until time of analysis.

Standard curves

Standard solutions of doxorubicin, its corresponding metabolite doxorubicinol, and the internal standards daunorubicin and idarubicin were prepared in 0.1 M phosphoric acid. Standard curves in plasma were obtained by adding 50 μl standard solution to 450 μl drug-free plasma derived from the Department of Clinical Immunology and Tranfusion Medicine, Karolinska Hospital, Stockholm, Sweden.

Two standard curves were prepared for doxorubicin, one with a plasma concentration range of 2–200 ng/ml using daunorubicin (30 ng/ml) as internal standard (IS) and one with a plasma concentration range of 200–1400 ng/ml using idarubicin (400 ng/ml) as IS. A standard curve for doxorubicinol, 2–45 ng/ml, was prepared with daunorubicin (30 ng/ml) as IS. Daunorubicin and idarubicin were added as ISs to all plasma samples from patients giving concentrations of 30 and 400 ng/ml, respectively.

Standard curves were evaluated by extended least-squares regression.[7] Intra-day precision was determined by analysing 10 samples containing standard solution in drug-free plasma on the same day. Inter-day precision was determined by analysis of samples containing standard solution on 10 different days.

Analytical procedure

Plasma levels of doxorubicin and doxorubicinol were assayed by a modified analytical method based on extraction and reversed-phase liquid chromatography.[8] An aliquot of 100 μl plasma sample was mixed with 100 μl daunorubicin (IS) and 100 μl idarubicin (IS), and then transferred into a pre-activated (methanol and 0.1 M phosphate buffer) SepPak C18 extraction column (Waters, Milford, MA). After rinsing with 5 ml of 0.1 M phosphate buffer (pH 7.0), the analytes and the internal standards were eluted with 4 ml methanol (Merck, Darmstadt, Germany). The elute was evaporated under a stream of nitrogen to 1 ml, 400 μl 0.1 M phosphoric acid was added and the elute was further evaporated to approximately 200 μl. The elute was analyzed by liquid chromatography carried out with an LC pump model 10-AVVP and a fluorometric detector model RF-551 (Shimadzu, Kyoto, Japan) operating at 501/600 nm. The mobile phase consisted of 32% acetonitrile (Merck, Darmstadt, Germany) in 0.01 M phosphoric acid. Samples were introduced by a Valco model C6W injector (Valco, Houston, TX) with a fixed loop of 50 μl into a Nova-Pak® Phenyl Radial-Pak cartridge with a Nova-Pak® Phenyl precolumn (Waters). Chromatographic data was collected and

processed using Datamonitor version 3.0 extra (Watrex, Prague, Czechoslovakia) integration system. All plasma concentration data used are mean values of duplicate analysis.

Statistics

Calculations of median values and their approximate 95% confidence intervals (CIs) were based on the Wilcoxon signed-ranks test. Correlation between capillary and venous samples was established by linear regression. All other correlations were established by the Spearman rank correlation test. The Mann–Whitney *U*-test was used to compare the capillary/venous concentration ratio in males and females. Factors influencing the capillary/venous concentration ratio were evaluated by multiple regression with stepwise variable selection using Minitab version 10*Xtra* (Minitab, State College, PA). Age, gender, infusion time, body surface area, administered dose in mg/m^2 and drug concentrations in venous plasma were used as independent variables; p values of <0.05 were considered statistically significant.

Results

Chromatograms of a capillary and a venous plasma sample from a male patient with Hodgkin's disease are shown in Figures 1 and 2. The method used in the present study enabled doxorubicin concentration in plasma to be determined within the range 2–1400 ng/ml with intra- and inter-day precision of 2.2 % (CV) and 3.4% (CV), respectively. The limit of detection was not established since all sample concentrations were within the range of the standard curves.

The median plasma concentration of doxorubicin found in capillary samples was 268 ng/ml (range: 35.3–815 ng/ml) and in venous samples 248 ng/ml (range: 34.8–748 ng/ml).

The concentrations of doxorubicin in capillary and venous samples were closely correlated (r=0.98;

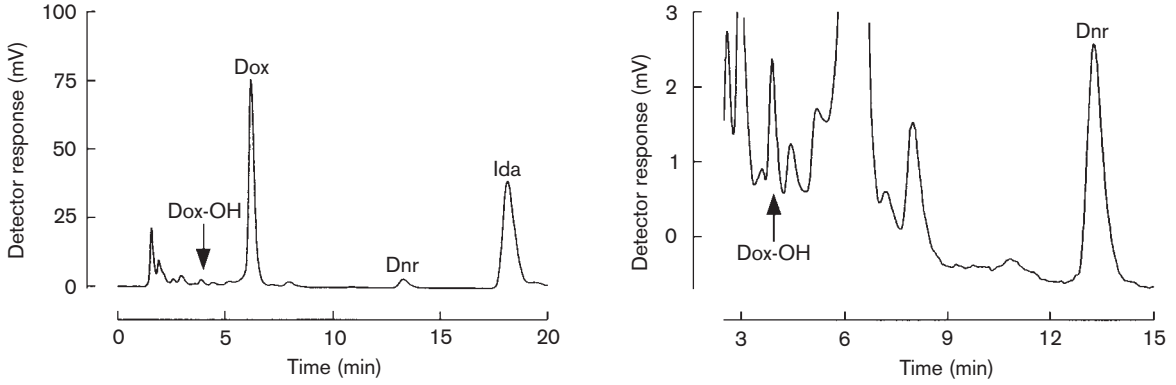

Figure 1. Chromatogram of a capillary plasma sample. Capillary plasma sample (100 μl) from a male patient with Hodgkin's disease, daunorubicin (Dnr, 30 ng/ml) and idarubicin (Ida, 400 ng/ml) added as ISs. Dox=doxorubicin; Dox-OH=doxorubicinol.

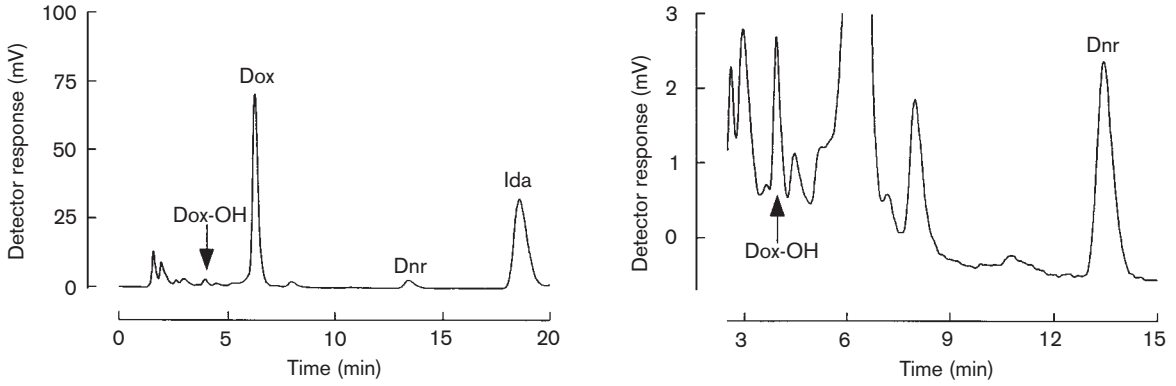

Figure 2. Chromatogram of a venous plasma sample. Venous plasma sample (100 μl) from the same patient as in Figure 1.

$p < 0.0001$; $n=16$) (Figure 3). The median capillary/venous plasma concentration ratio was 1.13 (95% CI: 1.06–1.20) and independent of drug concentration (Figure 4). The plasma concentration ratio did not correlate with age (Figure 5) or with body mass index (data not shown). The concentration ratio was significantly higher ($p=0.03$) in males (median value: 1.18; $n=9$) than in females (median value: 1.01; $n=7$). Multiple regression with step-wise variable selection revealed that gender was the only tested variable affecting the capillary/venous plasma concentration ratio.

The median plasma concentration of the metabolite doxorubicinol found in capillary samples was 8.8 ng/ml (range: 2.3–18.3 ng/ml) and in venous samples 8.3 ng/ml (range: 2.5–25.1 ng/ml). Due to the low concentration and minor clinical importance of doxorubicinol as compared to doxorubicin, these results were not further evaluated.

Discussion

The pharmacokinetics of the anthraquinone glycosides show very large inter-individual variability.[3] Therapeutic drug monitoring has been suggested as a proper way to overcome the pharmacokinetic variability, thereby improving therapeutic efficacy and/or reducing side effects.[5] We have previously shown that the drug concentration in one single venous plasma sample drawn at the end of constant infusions gives highly accurate estimates of the systemic exposure of the anthraquinone glycoside doxorubicin and 4′-epidoxorubicin.[6] This approach may substitute a complete pharmacokinetic evaluation requiring at least 12 blood samples collected over a 24-h period.[3] The previously established clinical applicability of this method[9] would further be improved by the use of capillary blood sampling, especially in pediatric patients.[10]

The chromatographic system used in this study is highly selective and it is possible to separate a number of closely related anthraquinone glycosides in a single chromatographic run. The high sensitivity of the used fluorometric detector has made it possible to quantify doxorubicin and doxorubicinol in the low ng/ml range using only 100 μl plasma. Hence it is now feasible to use plasma from capillary blood in our simplified

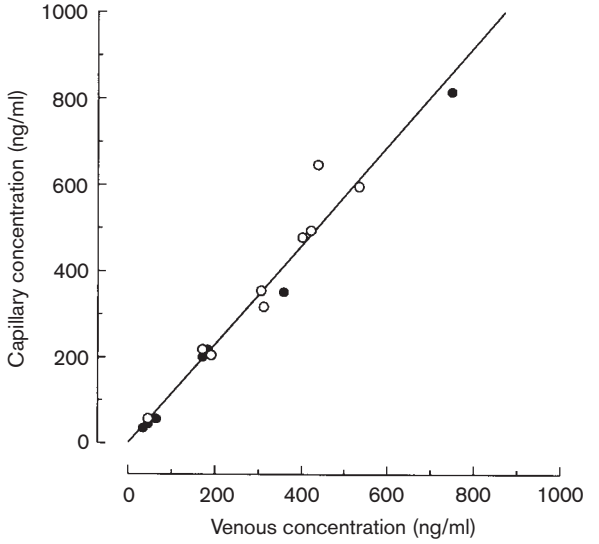

Figure 3. Capillary versus venous plasma concentration of doxorubicin: (●) females and (○) males. The line is obtained by linear regression analysis ($r=0.98$; $p<0.0001$).

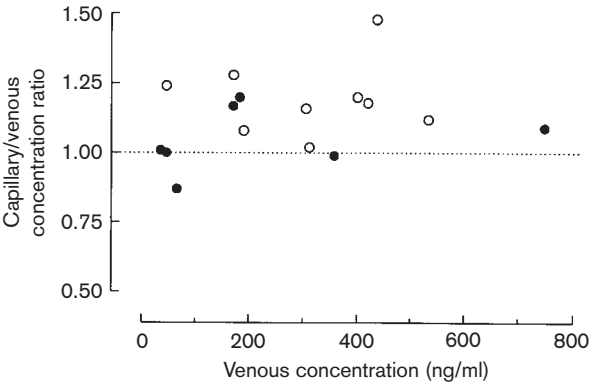

Figure 4. Capillary/venous plasma concentration ratio of doxorubicin versus venous plasma concentrations of doxorubicin: (●) females and (○) males. The dashed line represents the capillary/venous ratio when capillary concentrations are equal to venous concentrations.

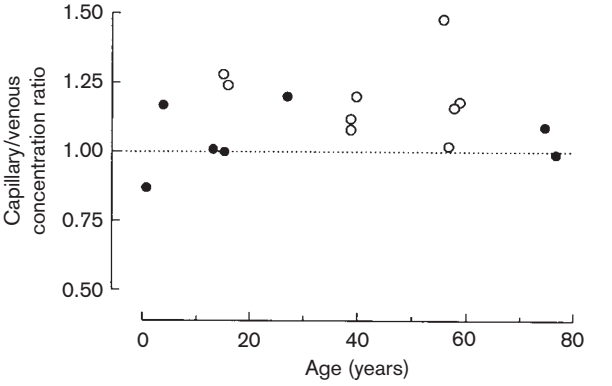

Figure 5. Capillary/venous plasma concentration ratio of doxorubicin versus age: (●) females and (○) males. The dashed line represents the capillary/venous ratio when capillary concentrations are equal to venous concentrations.

procedure for pharmacokinetic monitoring of doxorubicin. To cover the wide concentration ranges of doxorubicin and doxorubicinol expected in plasma samples from cancer patients, standard curves were constructed using two ISs at different concentrations.

Comparative studies of capillary and venous plasma concentration of anthraquinone glycosides have not previously been published. The very high correlation between concentrations of doxorubicin in capillary and venous plasma samples might falsely give the impression that capillary and venous sampling sites can be used interchangeable (Figure 3). In contrast, the ratio plots[11] clearly demonstrate minor but significantly higher capillary plasma concentrations (Figures 4 and 5). The wide plasma concentration range of doxorubicin, obtained by inclusion of samples from patients treated with large variations in dose and infusion times, did not affect the relative concentrations of doxorubicin in capillary and venous samples.

The capillary/venous plasma concentration ratio of doxorubicin was significantly higher in males than in females. Multiple regression with step-wise variable selection revealed that gender was the only tested variable affecting the capillary/venous concentration ratio. Gender differences in plasma protein binding, body composition or blood flow might influence the amount of doxorubicin diffusing into the tissue, thereby affecting the capillary/venous concentration ratio. Gender specific sampling site differences of drug concentrations has to our knowledge not previously been reported.

The phenomenon and rationale of blood sampling site dependence on drug concentrations have been reviewed.[12,13] Capillary blood is a mixture of arterial blood, venous blood and interstitial fluid.[14] To minimize diluting of the blood with the interstitial fluid, the capillary blood sampling should, as done in the present study, be conducted with free-flowing blood with minimum squeezing of the finger. A careful examination of drug concentration differences in capillary and venous blood samples is necessary prior to change of sampling site. In fact, concentrations of a large numbers of drugs in capillary and venous blood samples have been compared.[15–21] However, the results are in general difficult to interpret due to unsuitable treatment of data, i.e. the use of scatter diagrams. A high correlation coefficient and/or $p < 0.05$ is often considered sufficient for conclusions of interchangeable sampling sites. Hence it was concluded that methotrexate venous blood sampling can be substituted by capillary blood sampling, since a scatter plot of the concentration data showed a correlation coefficient of 0.934.[21] A closer examination of the data showed that the capillary/venous concentrations ratio ranged from 0.2 to 3.1, a fact that may have serious consequences when basing the leucovorin rescue on measured methotrexate concentrations.

Conclusions

- Capillary sampling, being faster, cheaper and less traumatic than venous sampling, is convenient for use in pharmacokinetic studies, especially in pediatric patients.
- Scatter plots can falsely give the impression that capillary and venous sampling sites can be used interchangeable even if highly correlated.
- The concentration differences of doxorubicin in plasma from capillary and venous sampling are of minor importance.

Acknowledgments

All the nurses at the Department of Pediatric Oncology and the Division of Hematology, especially Ms Mia Gustavsson, are gratefully acknowledged for professional help with sampling the patients. Annika Lindberg is acknowledged for skilful technical assistance. We also thank our patients for their kind cooperation.

References

1. Speth PAJ, van Hoesel QGCM, Haanen C. Clinical pharmacokinetics of doxorubicin. *Clin Pharmacokinet* 1988; **15**: 15–31.
2. Lipshultz S, Colan S, Gelber R, Perez-Atayde A, Sallan S, Sanders S. Late cardiac effects of doxorubicin therapy for acute lymphoblastic leukemia in childhood. *N Engl J Med* 1991; **324**: 808–15.
3. Eksborg S, Strandler HS, Edsmyr F, Näslund I, Tahvanainen P. Pharmacokinetic study of IV infusions of adriamycin. *Eur J Clin Pharmacol* 1985; **28**: 205–12.
4. de Valeriola D. Dose optimization of anthracyclines. *Anticancer Res* 1994; **14**: 2307–13.
5. Desoize B, Robert J. Individual dose adaptation of anticancer drugs. *Eur J Cancer* 1994; **30A**: 844–51.
6. Eksborg S. Anthracycline pharmacokinetics. Limited sampling model for plasma level monitoring with special reference to epirubicin (Farmorubicin). *Acta Oncol* 1990; **29**: 339–42.
7. Eksborg S, Ehrsson H. Calibration curves: calculation and evaluation of accuracy. *Ther Drug Monit* 1994; **16**: 629–30.
8. Eksborg S, Ehrsson H, Andersson I. Reversed-phase liquid chromatographic determination of plasma levels of adriamycin and adriamycinol. *J Chromatogr* 1979; **164**: 479–86.

9. Eksborg S, Hardell L, Bengtsson N-O, Sjödin M, Elfsson B. Epirubicin as a single agent therapy for the treatment of breast cancer—a pharmacokinetic and clinical study. *Med Oncol Tumor Pharmacotherapy* 1992; **9**: 75–80.
10. Eksborg S, Björk O, Palm C. A comparative pharmacokinetic study of doxorubicin and 4′-epi-doxorubicin in children with acute lymphocytic leukemia using a limited sampling procedure. *Anti-Cancer Drugs* 2000; **11**: 129–36.
11. Eksborg S. Evaluation of method-comparison data. *Clin Chem* 1981; **27**: 1311–12.
12. Chio WL. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site. Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (part I). *Clin Pharmcokinet* 1989; **17**: 175–99.
13. Chio WL. The phenomenon and rationale of marked dependence of drug concentration on blood sampling site. Implications in pharmacokinetics, pharmacodynamics, toxicology and therapeutics (part II). *Clin Pharmcokinet* 1989; **17**: 275–90.
14. Blumenfield TA, Hertelendy WG, Ford AH. Simultaneously obtained skin-puncture serum, skin-puncture plasma, and venous serum compared, and effects of warming the skin before puncture. *Clin Chem* 1977; **23**: 1705–10.
15. Frazer III JF, Stasiowski P, Boyd GK. A clinically useful capillary blood-sampling technique for rapid determination of therapeutic levels of theophylline. *Ther Drug Monit* 1983; **5**: 109–12.
16. Profumo RJ, Foy TM, Kane RE. Correlation between venous and capillary blood samples for cyclosporine monitoring in pediatric liver transplant patients. *Clin Transplant* 1995; **9**: 424–26.
17. Ericsson O, Fridén M, Hellgren U, Gustafsson L. Reversed-phase high-performance liquid chromatography determination of quinine in plasma, whole blood, urine, and samples dried on filter paper. *Ther Drug Monit* 1993; **15**: 334–37.
18. Ter Kuile FO, Teja-Isavatharm P, Edstein MD, *et al*. Comparison of capillary whole blood, venous whole blood, and plasma concentrations of mefloquine, halofantrine, and desbutyl-halofantrine measured by high-performance liquid chromatography. *Am J Trop Med Hyg* 1994; **51**: 778–84.
19. Gordi T, Hai TN, Hoai NM, Thyberg M, Ashton M. Use of saliva and capillary blood samples as substitutes for venous blood sampling in pharmacokinetic investigations of artemisinin. *Eur J Clin Pharmacol* 2000; **56**: 561–6.
20. Lewis AS, Taylor G, Williams HO, Lewis MH. Comparison of venous and capillary blood sampling for the clinical determination of tobramycin serum concentrations. *Br J Clin Pharmacol* 1985; **20**: 597–601.
21. Bomelburg T, Ritter J, Schellong G. Bestimmung der Methotrexatkonzentration im Serum: Vergleich zwischen Kapillar- und Venenblut. *Klin Pädiat* 1987; **199**: 230–2.

(Received 20 August 2001; accepted 4 September 2001)