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Liquid Chromatographic Monitoring of Daunorubicin and Daunorubicinol in Plasma from Leukemic Patients Treated with Daunorubicin or the Daunorubicin-DNA Complex

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Summary. *Fifteen patients with acute nonlymphocytic leukemia have been randomized for treatment with daunorubicin (1.0–1.5 mg/kg) either as the free drug (for 45 min or 4 h) or as the drug bound to a DNA carrier (for 5–6 h). The correlation between plasma kinetics of daunorubicin and its main metabolite daunorubicinol and the different administration schedules of daunorubicin has been studied by reversed-phase liquid chromatography.*

Plasma concentration kinetics of daunorubicin as well as the daunorubicin-DNA complex was biphasic in character. Maximum plasma level of daunorubicin was found during the infusion period. Its concentration decreased rapidly when the infusion stopped and was below the detection limit of the analytical method 2–4 h later. The data suggests a slower disposition of the daunorubicin-DNA complex compared with the free drug.

Introduction

Daunorubicin is an anthraquinone glycoside widely used in the treatment of acute leukemia. Generally, it is given intravenously as free drug, but recently the drug has also been infused as a complex with herring sperm DNA (Sokal et al., 1973). This technique was introduced by Trouet et al. (1972), who suggested that the drug complex would accumulate within cells of high endocytic activity, e.g., cancer cells and human leukemic cells. Compared with results obtained for the free drug, a higher therapeutic index has been found for the daunorubicin-DNA complex in some animal models (Seeber et al., 1977). In other studies, however, no differences were found (Ohnuma et al., 1975).

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The scope of the present investigation was to study the correlation between kinetics of the plasma concentrations and different administration schedules of daunorubicin and the daunorubicin-DNA complex in man. Daunorubicin is extensively metabolized to daunorubicinol, which, at least in vitro, also is reported to have cytostatic activity (Asbell et al., 1972). For this reason we have analyzed plasma levels for both compounds simultaneously. The preliminary results of the study are presented in this report.

Materials and Methods

Herring sperm DNA (Sigma, type VII) was dissolved in 0.9% NaCl to a concentration of 2.34 mg/ml, autoclaved for 15 min at 120° C, and allowed to stand overnight at room temperature. Daunorubicin (Pharma Rhodia, Stockholm, Sweden) was dissolved in 0.9% NaCl to a concentration of 10 mg/ml and added to the DNA solution to a final drug concentration of 0.20 mg/ml.

Fifteen patients with acute nonlymphocytic leukemia were included in this study. At the time of the study, patients were at different stages of the disease. At the first presentation, all patients were randomized for treatment with daunorubicin according to one of the following schedules (Gahrton et al., 1978):

A. Infusion for 45 min (1.0–1.5 mg/kg, the daunorubicin dissolved in 200 ml 0.9% NaCl).

B. Infusion for 4 h (1.0–1.5 mg/kg, the daunorubicin dissolved in 500 ml 0.9% NaCl).

C. Infusion as DNA complex, 20 mg of daunorubicin in 100 ml 0.9% NaCl per hour. Dose of daunorubicin: 1.0–1.5 mg/kg.

Infusions were controlled by the use of a Tekmar® T92 infusion pump.

Blood samples (5–7 ml) were collected in 10-ml glass test tubes (Vacutainer®) containing 250 IU heparin (freeze dried). Blood samples were taken immediately before, during, and at appropriate time intervals after administration of the drug. The samples were immediately cooled on ice and centrifuged at 4080 *g* for 10 min at 2–4° C. The plasma fraction was carefully removed and frozen at −20° C until assay. The plasma levels of daunorubicin and daunorubicinol were determined by reversed-phase liquid chromatography (Eksborg et al., 1978).

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Results and Discussion

Examples of plasma concentration curves of daunorubicin and daunorubicinol obtained by analysis of samples from patients treated according to the three regimens are presented in Fig. 1a–c. During the latter part of the infusion period, the plasma level of daunorubicin was almost constant when infused as the free drug for 4 h, while it increased with time during the administration of the daunorubicin-DNA complex using an infusion time of 5–6 h. The concentration of daunorubicin in plasma decreased rapidly when the infusion stopped and was below the detection limit of the analytical method (5–10 ng/ml) 2–4 h later.

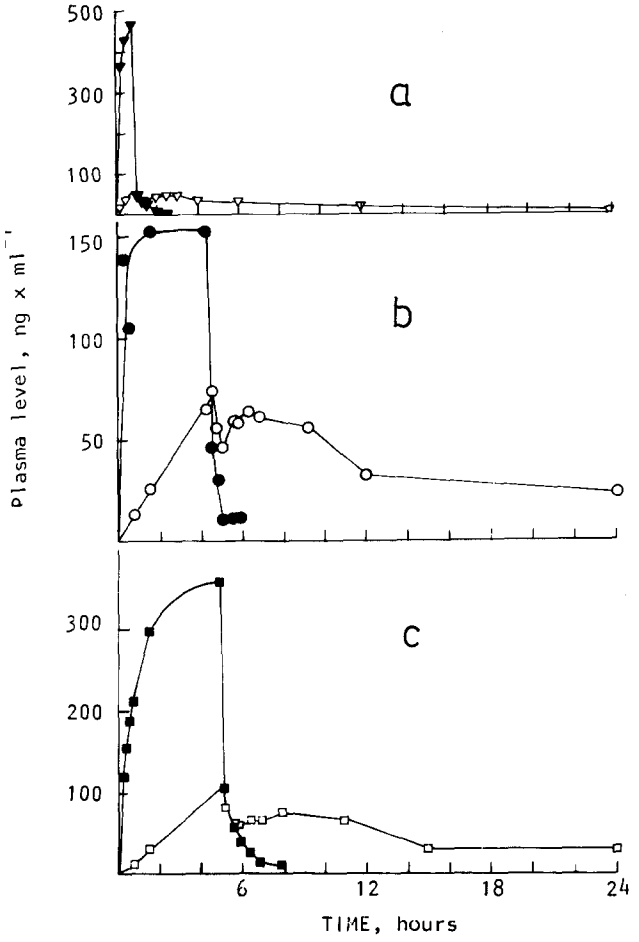

Fig. 1. Plasma levels of daunorubicin and daunorubicinol

Infusion as	Infusion time	Given dose mg/kg	Fig.
Free drug	45 min	0.93	a
Free drug	4 h	1.52	b
DNA complex	5 h	1.50	c

Filled symbols: daunorubicin
Open symbols: daunorubicinol

Maximum concentration of daunorubicinol was always found in the plasma samples taken immediately before the end of the infusions. Its concentration decreased considerably slower than the concentration of daunorubicin (cf., Huffman et al., 1972; Benjamin, 1977; Eksborg et al., 1978), and it was possible to determine the daunorubicinol levels for more than 24 h.

Plasma concentration kinetics of daunorubicin as well as the daunorubicin-DNA complex was biphasic in character. This is in accordance with earlier findings (Huffman et al., 1972) obtained by fluorescence measurements complemented by thin-layer chromatography. The disappearance rate was unaffected by the drug administration time. It was, however, somewhat slower for the DNA-complexed drug compared with the free drug.

Linear relationships were found for the different regimens when plotting the area under the plasma curves versus the maximum plasma concentration for daunorubicin (Fig. 2a). Infusion of the free drug for 45 min and 4 h, respectively, gave almost the same areas under the plasma curves. The maximum plasma level of daunorubicin was considerably higher during the shorter infusion period. The area under the plasma curves was about three times larger in patients treated with the daunorubicin-DNA complex compared with patients treated with the free drug. This data indicates differences in metabolism and/or in the distribution of daunorubicin within the body when administered as free drug and as the DNA complex. Differences in distribution of ^{3}H-labeled daunorubicin and its DNA complex in mice have been reported as detected by whole-body autoradiographic technique (Andersson et al., 1977).

Figure 2b shows a fairly good correlation between the area under the plasma concentration curves and the maximum plasma level of daunorubicinol. There were no differences in daunorubicinol plasma pattern related to either the length of administration (45 min vs. 4 h) or to the preparation (free drug vs. the DNA complex) of daunorubicin.

The importance of plasma peak levels and plasma concentration kinetics of daunorubicin and/or its complex with DNA for the accumulation of the drug in various target tissues is worth further elucidation. Such data might be related both to the therapeutic effect and to the serious adverse effects of the daunorubicin and therefore of importance for the planning and control of the therapy.

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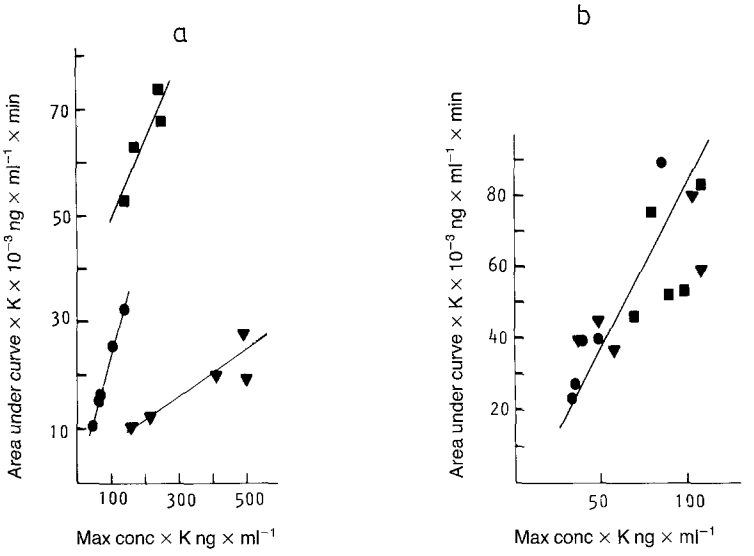

Fig. 2. Area under plasma curve and maximum plasma level: daunorubicin (**a**), daunorubicinol (**b**)

Infusion of daunorubicin as	Symbol
Free drug (45 min)	▼
Free drug (4 h)	●
DNA complex (5–6 h)	■

The value in the figure have been corrected for differences in given doses of daunorubicin by the factor K defined by:

$$K = \frac{1 \text{ mg} \times \text{kg}^{-1}}{\text{given dose in mg} \times \text{kg}^{-1}}.$$

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