



Doxorubicin Binding by Human Erythrocytes

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ABSTRACT

Native human erythrocytes can bind doxorubicin (adriablastin) during incubation in doxorubicin containing isotonic medium. The kinetics of doxorubicin binding were found to be independent on the initial doxorubicin concentration in the incubation medium in the range from 0.07 to 0.4 mg/ml (from 0.4 to 2.5 mg/ml of adriablastin). At pH 7.0, 37°C and 50% hematocrit the initial rate of doxorubicin binding to erythrocytes is about 15% per minute and maximal binding achieved within an hour is approximately 80-85% of the initial doxorubicin content in the medium. Temperature decrease from 37°C to 5°C as well as the decrease of pH from 7.5 to 6.5 lead to the decrease of initial rate of doxorubicin binding and diminish the level of maximal binding of doxorubicin. At pH 7.5 and temperature less than 30°C doxorubicin precipitates due to the decrease of its solubility and this results in erythrocyte aggregation. More full doxorubicin binding is achieved at higher hematocrit value. On the other hand, at low hematocrit values much more rapid accumulation and significantly greater concentration of doxorubicin in erythrocytes may be obtained at the same initial doxorubicin concentration in the medium.

KEYWORDS

Binding; doxorubicin; erythrocyte; human; kinetics.

INTRODUCTION

Erythrocytes loaded with doxorubicin seem to be a very perspective preparation. It was shown that administration of doxorubicin loaded erythrocytes might improve

the therapeutic efficacy of doxorubicin due to specific targeting and diminishing toxicity of the drug (Gasparini *et al.*, 1992; Zocchi *et al.*, 1988, 1989). First results of clinical trial of doxorubicin carrier erythrocytes have been reported recently (Tonetti *et al.*, 1992). In this connection the problem of simplification and optimization of doxorubicin carrier erythrocytes preparation procedure appears to be rather important. Earlier it was shown that human erythrocytes are able to bind anthracycline antibiotics during incubation in antibiotic containing medium (Ataullakhanov *et al.*, 1992; Tonetti *et al.*, 1992). This work is intended to study the influence of some physico-chemical factors on doxorubicin binding by human erythrocytes. This information is of importance for the optimization of procedure for erythrocyte loading with doxorubicin. It also may be useful for understanding of the mode of action of doxorubicin carrier erythrocytes *in vivo*.

MATERIALS AND METHODS

Erythrocytes were separated from donor blood and washed two times with double volume of glucose containing phosphate buffered saline (GPBS). As erythrocytes were incubated at different pH values the pH of washing solution was adjusted to that of the incubation solution. Washed erythrocytes were brought to the desirable temperature and mixed with doxorubicin solution in GPBS maintained at the desirable temperature and pH. Doxorubicin was used as adriablastin, commercially available from Farnitalia Carlo Erba Montedison Group, Italy. Erythrocyte suspension was incubated at constant temperature and periodical stirring. Aliquots of erythrocyte suspension were taken during incubation for the determination of doxorubicin. To do it, chloroform extracts were prepared from the whole suspension samples and from supernatants obtained after centrifugation of suspension samples. To 1 ml of the sample (either of erythrocyte suspension or supernatant) 0.1 ml of 1.6 M potassium bicarbonate solution and 3 ml of chloroform were added. The mixture was vigorously shaken and centrifuged for 3 min at 1000 g. After centrifugation 2 ml of the lower layer of liquid (chloroform extract of doxorubicin) were aspirated with precautions by syringe and used for spectrophotometrical measurements of doxorubicin concentration. It was determined by comparison of the extract visible spectra with the spectra of calibrating solutions of doxorubicin in chloroform. Hemoglobin concentration in the incubation medium was measured spectrophotometrically at 415 nm.

RESULTS

During incubation of human erythrocytes in the doxorubicin containing medium the decrease of doxorubicin concentration in the medium was observed while the total doxorubicin concentration in erythrocyte suspension remained unchanged. The difference between total and medium doxorubicin concentrations allow estimation of the amount of the drug bound to erythrocytes. Doxorubicin binding to human erythrocytes was

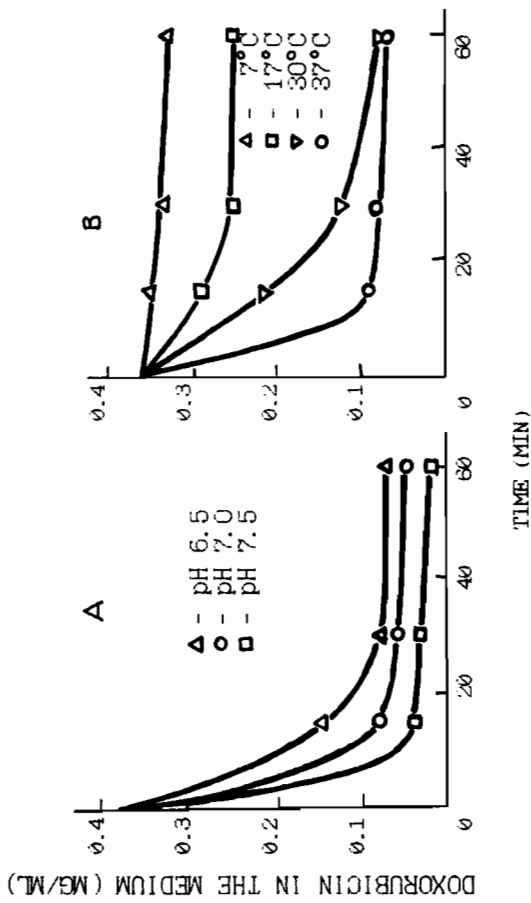


Fig. 1. The dependence of kinetics of doxorubicin binding by human erythrocytes on pH and temperature.

(A) - 37°C; hematocrit - 50%
(B) - pH 7.0; hematocrit - 50%

found to depend strongly on temperature and pH (Fig. 1). In general, temperature decrease as well as the decrease of pH lead to the decrease of initial rate of doxorubicin binding and diminish the level of maximal binding of doxorubicin. However, in the range of temperature from 30°C to 37°C the maximal binding level remains almost unchanged. The dependence of the kinetics of doxorubicin binding on temperature at pH 6.5 was qualitatively similar to that at pH 7.0. In our preliminary experiments it was found that at pH 7.5 and temperatures less than 30°C doxorubicin may precipitate due to decrease of its solubility. It results in erythrocyte aggregation. For this reason the dependence of doxorubicin binding kinetics on temperature was not studied at pH 7.5.

The kinetics of doxorubicin binding by human erythrocytes were found to be independent on the initial doxorubicin concentration in the incubation medium in the concentration range from 0.07 to 0.4 mg/ml (from 0.4 to 2.5 mg/ml of adriablastin) (Fig. 2). At pH 7.0, 37°C and

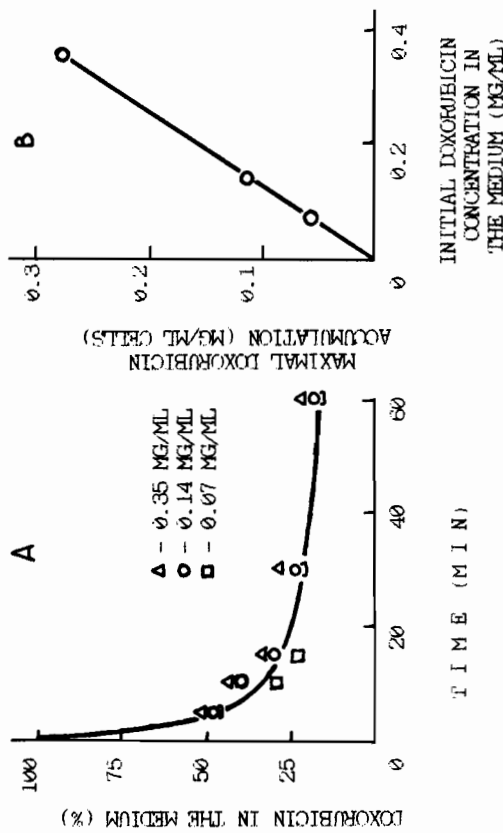


Fig. 2. Binding of doxorubicin by human erythrocytes at different initial doxorubicin concentrations in the incubation medium. 37° C; pH 7.0; hematocrit - 50%. (A) - Kinetics of doxorubicin binding. Different symbols were used for points obtained at different initial doxorubicin concentrations as indicated. (B) - The dependence of maximal erythrocyte doxorubicin concentration on the initial doxorubicin concentration in the medium. Points are calculated from the data presented in Fig. 2A.

50% hematocrit value the initial rate of doxorubicin binding to erythrocytes was about 15% per minute and maximal binding achieved within an hour was approximately 80-85% of the initial doxorubicin content in the medium. Hence, the maximal quantity of doxorubicin which may be bound by erythrocytes is proportional to the initial doxorubicin concentration in the medium (Fig. 2B).

Naturally, the kinetics of doxorubicin binding by erythrocytes depend on the ratio of erythrocyte and medium volumes. As Fig. 3 shows the more full doxorubicin binding is achieved at higher hematocrit value. On the other hand, at low hematocrit values much more rapid accumulation and significantly greater

concentration of doxorubicin in erythrocytes may be obtained at the same initial doxorubicin concentration in the medium.

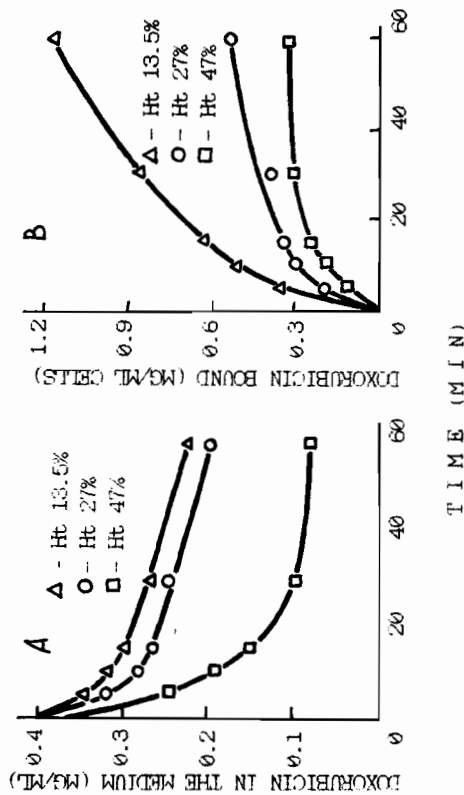


Fig. 3. Kinetics of doxorubicin concentration changes in the medium (A) and in erythrocytes (B) during incubation of human erythrocytes in doxorubicin containing medium at different hematocrit values. 37° C; pH 7.0.

Hemoglobin outflux observed during incubation of erythrocytes with doxorubicin was negligible and reached 0.5-1.0% of the total suspension hemoglobin per hour.

DISCUSSION

The pH values equal to 7.5 or higher do not seem to be desirable for doxorubicin binding by erythrocytes. Neutral or slightly acid conditions are preferable because the doxorubicin precipitation is excluded while doxorubicin binding is only slightly lower than at pH 7.5. Using neutral or slightly acid medium is not harmful for erythrocytes. Moreover, stored blood or erythrocytes may show slightly acid reaction. So, pH values 6.5-7.0 may be recommended for loading erythrocytes with doxorubicin. Optimal temperature for erythrocyte loading with doxorubicin is 37° C, but the satisfactory doxorubicin binding may be achieved in

a wide range of temperatures, not lower than room temperature. Since the kinetics of doxorubicin binding do not depend on initial doxorubicin concentration the final drug content in erythrocytes may be easily adjusted by varying its initial concentration. It should be noted that by varying hematocrit value one can obtain either the more complete binding of doxorubicin that means more cost saving loading at high hematocrit values or faster achievement of desirable drug concentration in cells at low hematocrit values.

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Organ Distribution of Glutaraldehyde Treated Erythrocytes in Patients with Hepatic Metastases

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ABSTRACT

Organ distribution of autologous glutaraldehyde treated erythrocytes was determined in ten patients bearing hepatic metastases from carcinomas of various origin. Glutaraldehyde treated erythrocytes were labelled with ^{99m}Tc and their fate after reinfusion was monitored by dynamic and static scintigraphic analyses. The profiles of label uptake in the different organs varied among patients. Three patterns of distribution could be identified: 1) uptake mainly in lungs and spleen, 2) uptake in both liver and spleen and c) uptake in spleen only. Unlike what is observed in experimental animals, liver targeting of treated erythrocytes could be achieved in a fraction of cases only. Because of the great interindividual variability in organ distribution observed in this study, glutaraldehyde treatment does not seem an effective method to achieve liver targeting for antineoplastic therapy.

KEYWORDS

Carrier erythrocytes, targeting, glutaraldehyde, liver metastases, scintigraphic studies.

INTRODUCTION

The rationale of targeting of antineoplastic drugs to tumoral lesions is that drug delivery to the site of disease would be increased and the amount of drug escaping into the systemic circulation reduced. As a result, tumor kill will be enhanced and toxicity will be minimized.

In this perspective, erythrocytes may represent effective vehicles to achieve organ targeting of encapsulated drugs. Many procedures have been proposed to induce specific organ or tissue targeting of loaded erythrocytes. They include heat and oxidative stresses (Zoc-