

## RUBOMYCIN LOADED ERYTHROCYTES IN THE TREATMENT OF MOUSE TUMOR P388

Fazoil I. Ataulakhanov, Victor M. Vitvitsky,  
Violetta L. Kovaleva and Svetlana B. Mironova

National Scientific Centre for Hematology  
Moscow, Russia

A therapeutic effect of anthracyclin loaded erythrocytes has been shown to be very promising.<sup>1-5</sup> In our present investigation a possibility to use erythrocytes as the carriers of antileukemic agent rubomycin was studied.

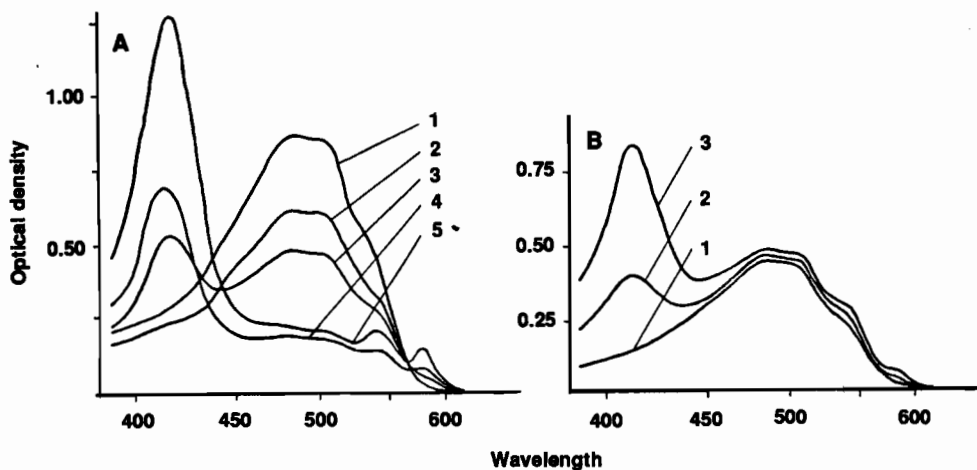
### Rubomycin Binding to Erythrocytes

Washed human or mouse erythrocytes were diluted to a hematocrit of approximately 40% with isotonic buffered solution containing rubomycin. Rubomycin concentrations from 0.3 to 5.0 mg/ml were used. The suspensions were incubated either at room temperature, 37° C or 4° C. During the incubation period several aliquotes of the suspension were centrifuged and concentrations of rubomycin and hemoglobin in supernatants were measured spectrophotometrically (Fig.1).

Intact human and mouse erythrocytes were shown to bind up to 80% of rubomycin added to the medium at room temperature and hematocrit value near 40% within 30 min. At rubomycin concentration about 5 mg/ml its binding rate was approximately 200µg/min/ml cells at room temperature and increased markedly when temperature was increased (Fig.2A, 3A). Simultaneously a significant hemoglobin leakage into the suspension medium was observed (Fig.2B, 3B). If an incubation medium was substituted for a fresh one, a parallel increase in hemoglobin and antibiotic extracellular concentrations was observed. Only less than 20% of intracellular rubomycin leaked into the medium at 37° C and at room temperature.

Rubomycin neither bound to the erythrocytes (Fig.1) nor leaked from the erythrocytes at 4° C.

The observed rubomycin binding to erythrocytes simplifies markedly a preparation of rubomycin loaded erythrocytes and gives some advantages compared with adriamycin (Adriamycin has been shown to bind to canine erythrocytes but not to human.<sup>5</sup>)



**Figure 1.** Evolution of spectrum obtained on supernatant of human erythrocyte suspension incubated with rubomycin at different temperatures. A - 37° C, 1 - initial rubomycin spectrum, 2, 3, 4 and 5 - after 2, 10, 18, and 30 min of incubation. Initial rubomycin concentration in the incubation medium - 4 mg/ml, hematocrit - 45%. B - 4° C, 1 - initial rubomycin spectrum, 2 and 3 - after 15 and 30 min of incubation. Initial rubomycin concentration in the incubation medium - 2 mg/ml, hematocrit - 40%. Concentration changes were registered at 415 nm for hemoglobin and at 500 nm for rubomycin.

### The In Vivo Experiments

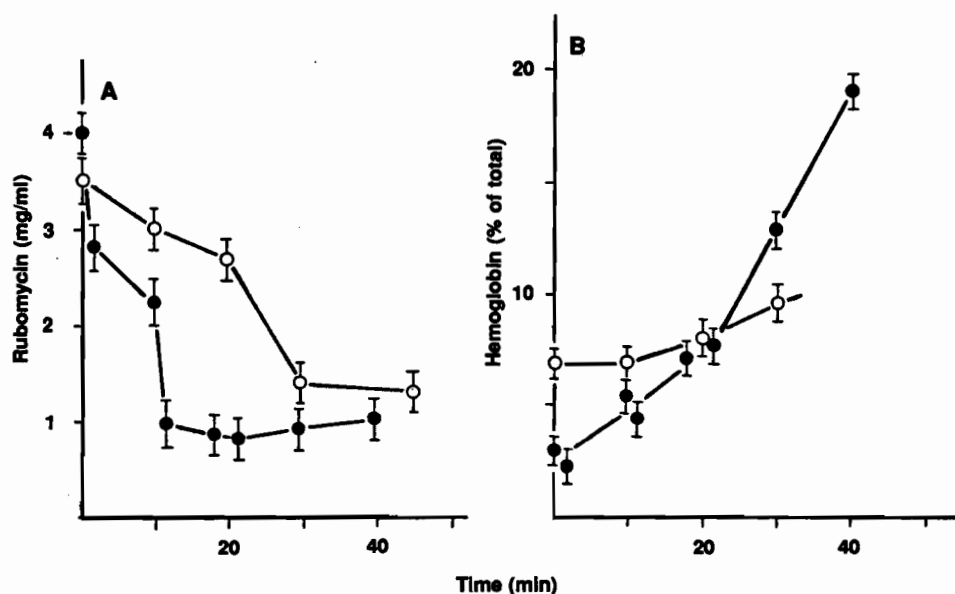
P388 cells were maintained within DBA female mice. In the experiments BDF-1 female mice weighting 20 - 25 g were used. The tumor cells were inoculated by i.p. injection of  $1-2 \times 10^6$  cells per mouse. During the next five days after the inoculation the 200  $\mu$ l i.p. injections were made daily. Several groups containing ten mice each were formed. The control group received 0.9% NaCl solution in i.p. injections. Other groups received rubomycin solution or suspension of rubomycin loaded erythrocytes.

Rubomycin was dissolved in PBS, 3 mg/kg weight of rubomycin were found to be an optimal therapeutic dose. To prepare rubomycin loaded erythrocytes fresh washed erythrocytes were resuspended in the rubomycin solution up to a hematocrit 30% and incubated for 30 min at room temperature. Then the cells were sedimented and the amount of erythrocyte bound rubomycin was measured quantitatively. Rubomycin loaded erythrocytes were resuspended in PBS to obtain an appropriate dose of rubomycin in 200  $\mu$ l of the suspension (hematocrit of approximately 50%) and used for the injections. All preparations of rubomycin were made daily just before the injection.

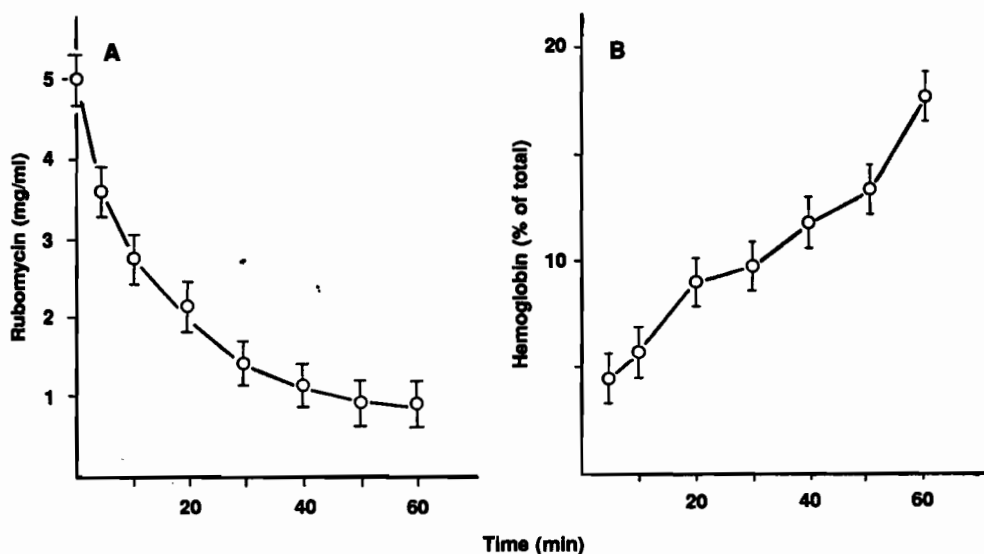
The results obtained are shown in Fig. 4. The therapeutic effect of rubomycin loaded erythrocytes on the survival of the mice inoculated with tumor P388 is similar to that of the rubomycin solution.

Interestingly, we did not observe an increase in therapeutic effect of rubomycin loaded erythrocytes compared to the rubomycin solution, as one would expect from the similar studies on daunomycin treatment of mice ascite tumor L 1210.<sup>1,2</sup>

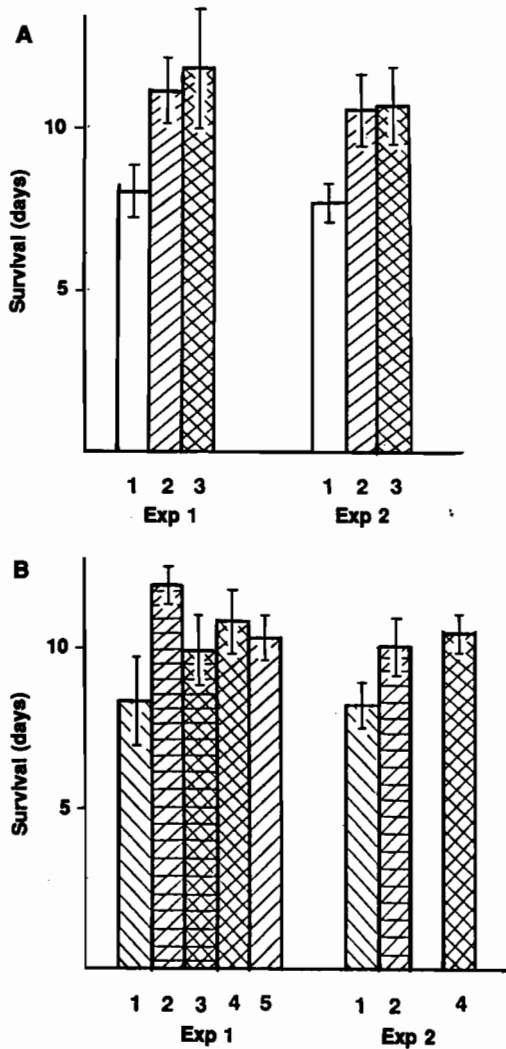
However, it is possible that rubomycin loaded erythrocytes could become more therapeutically effective after glutaraldehyde treatment, as was shown for adriamycin loaded erythrocytes.<sup>3,4</sup> It would facilitate rubomycin targeting and treatment of tumor formations, especially if simplicity of preparation is taken into account.



**Figure 2.** Decrease of the rubomycin concentration (A) and increase of the hemoglobin concentration (B) in the medium during incubation of human erythrocytes with rubomycin. Open symbols 22° C, hematocrit 39%, black symbols 37° C, hematocrit 45%.



**Figure 3.** Decrease of the rubomycin concentration (A) and increase of the hemoglobin concentration (B) in the medium during incubation of mouse erythrocytes with rubomycin. 22° C, hematocrit 35%.



**Figure 4.** Effect of rubomycin treatment on survival of mice with tumor P388. A: 1 - control, 2 - rubomycin (3 mg/kg of weight) as a solution, 3 - rubomycin (3 mg/kg of weight) as rubomycin loaded mouse erythrocytes. B: 1 - control, 2 - rubomycin (3 mg/kg of weight) as a solution, 3 - rubomycin (6 mg/kg of weight) as a solution, 4 - rubomycin (3 mg/kg of weight) as rubomycin loaded human erythrocytes, 5 - rubomycin (6 mg/kg of weight) as loaded human erythrocytes.

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