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# ANTILEUKEMIC ACTIVITY OF EPIRUBICIN CONJUGATED WITH BIOPOLYMER DEXTRAN AGAINST LYMPHOID LEUKEMIA L1210 AS TUMOR MODEL

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**ABSTRACT**
*The antileukemic activity of the original conjugate of anthracycline antitumor antibiotic epirubicin, covalently bound to the biopolymer dextran was studied. The ascitic lymphoid leukemia L 1210 (transplantation dose 1x10$^5$ tumor cells/mouse, i. p.), in hybrid mice BDF$_1$, was used as tumor model. An antileukemic activity of the studied original conjugate was found. The criterion "increase of life span" (ILS%) reached maximally 136,6% for the conjugate. The studied conjugate of biopolymer dextran with epirubicin showed lower toxicity but also lower antileukemic activity in comparison with free epirubicin. The further experiments in this field are in progress, aiming to design better conjugates, with potential clinical use.*

## Introduction

The anthracycline antibiotics are widely used in cancer chemotherapy as very strong acting and with wide spectrum of antitumor activity. Their clinical effectiveness is limited by the development of multidrug resistance (MDR) (1, 2). This problem is crucial for patients, because the tumor cells become resistant not only to drug used during cancer treatment, but also to many other drugs that are structurally and functionally unrelated (3, 4, 5).

The anthracycline derivative epirubicin is one of the most effective antitumor drugs in the treatment of various types of solid tumors. The main disadvantages of anthracyclines are their high acute toxicity, cardiotoxicity and myelotoxicity, leading to limited dose application in cancer patients. Consequently, any effort to develop new anthracycline analogs are aimed at diminishing the toxicity without altering their antitumor effect.

One putative approach to modifying anthracyclines is the synthesis of original antibiotic conjugates with biopolymers, that generally have the ability to prolong the antibiotic action and to decrease its toxicity (6, 7, 8).

The purpose of the present experimental investigations was to assess the antileukemic activity of an original conjugate of anthracycline antibiotic epirubicin, covalently boun d to dextran, in comparison with free epirubicin.

## Materials and Methods

Our in vivo studies were performed in male hybrid BDF$_1$ mice. The antileukemic activity was studied on ascitic lymphoid L1210 leukemia, with transplantation dose of 1x10$^5$

tumor cells/mouse, on day 0, intraperitoneally (i. p.). Epirubicin (commercial antitumor drug Farmorubicin, Farmacia) and its original conjugate with dextran were introduced intraperitoneally, once a day, on day 1, day 4 and day 8 after the tumor transplant.

The antileukemic activity was assessed by use of the criterion T/C %, where T was the mean survival time (MST, days) of the drug-treated mice, bearing L1210 lymphoid leukemia and C – the mean survival time (MST, days) of untreated control animals, bearing the same leukaemia.

## Results and Discussion

The results obtained by our studies on the effect of anthracycline antitumor antibiotic epirubicin and its original dextran conjugate on BDF1 hybrid mice-bearing L1210 leukemia are shown on the Table.

According to these results, the free epirubicin exhibited a pronounced and dose-related antileukemic activity on mice-bearing L1210 leukemia. An increase of the free epirubicin dose over 10,7 mg/kg x 3, i. p., caused an increase in its acute toxicity. This fact was registered by the progressive decrease in the ratio T/C (treated/control). The dose of the free epirubicin of 25 mg/kg x 3, i. p., was toxic (T/C% < 125%).

he conjugate of epirubicin exhibited an antileukemic activity against ascitic lymphoid leukaemia L1210 in $BDF_1$ mice, in four of the used doses – from 10,7 mg/kg x 3 to 25 mg/kg x 3, i. p., with T/C% varying between 121.5% and 136.%. The dose of 7,1 mg/kg

TABLE 1

**Antileukemic activity of epirubicin and its conjugate with biopolymer dextran on L1210 leukemia**

| Agent | Dose (mg/kg) x 3, i.p. | MST (days) | T/C (%) |
|---|---|---|---|
| | 7.1 | 21.1 | 226.9 |
| | 10.7 | 27.9 | 300.0 |
| Epirubicin | 16.0 | 21.0 | 225.8 |
| | 20.0 | 12.6 | 135.5 |
| | 25.0* | 7.4* | 79.6* |
| | 7.1 | 11.1 | 119.4 |
| Epirubicin-dextran | 10.7 | 11.3 | 121.5 |
| | 16.0 | 12.1 | 130.1 |
| | 20.0 | 12.3 | 132.3 |
| | 25.0 | 12.7 | 136.6 |
| Untreated control | 0 | 9.3 | – |

MST – mean survival time (days).

T – survival time of treated mice (days).

C – survival time of control mice (days).

Significant antileukemic effect at T/C% > 125% was accepted.

* Toxic dose at T/C% < 125%.

x 3, i. p., was inactive (T/C% < 125%).
Our experimental results on activity of the original conjugate showed that an increase in dose levels of conjugated epirubicin equivalent to the free drug led to an increase in the ratio T/C, indicating lower toxicity. The dose of 25 mg/kg x 3, i. p., was not toxic (T/C% = 136.6%). The antileukemic activity of the conjugate was also lower than the activity of free epirubicin, that was unfavourable by clinical point of view. Our chemical and pharmacological investigations in this field are in progress, aiming to analyse the results and trying to design better conjugates of selected antitumor drugs with biopolymers, for potential clinical use.
Recently it was found in other our experiments that the binding of epirubicin to other biopolymer – chitosan, led to a significant decrease in the toxicity of the conjugate, a prolongation of its action and improved antitumor effect against other murine tumor model – ascitic lymphocytic leukemia P388 (9).

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## REFERENCES

1. **Acton E. M.** (1981) J. Med. Chem., **24**, 669-674.
2. **Fiallo M., Borrel M., Garnier A**.(1993) Biochem. Pharmac., **45**, 3, 659-663.
3. **Todorov D., Dudov A.** (2000), Oncologos, **4**, 3-19 (In Bulgarian).
4. **Dudov A., Ilarionova M., Horvath L., Todorov D. K.** (2002), Biotechnol. Biotechnol. Eq., **16**, 2, 164-167.
5. **Ilarionova M., Todorov D. K., Timcheva K., Dudov A.** (2002), Biotechnol. Biotechnol. Eq., **16,** 1, 138-141.
6. **Zanino F., Pezzoni G.** (1997) Cancer Tret. Rep.,**71**, 367-373.
7. **Tanaka H., Kominto K., Yomamoto R. et al**. (1994), J. Antibiotics, **14,** 1025-1029.
8. **Hyun O., Won-Gil S., Na-Young K. et al.** (2001), Leukemia Res.,**25**, 339-346.
9. **Todorova N., Maneva K., Ilarionova M. et al.** (2003) Biotechnol. Biotechnol. Eq., **17** (2), 151-153.