INFLUENCE OF α-6-DEOXY-5-OXYTETRACYCLINE ON SOME PHARMACOLOGICAL CHARACTERISTICS OF DAUNOMYCIN*

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(1) Treatment with doxycyline substantially reduces the acute toxicity of daunomycin to the mouse. Treatment with doxycycline alters the distribution of daunomycin amongst the body tissues of the mouse. The ability of the isolated kidney to bind the daunomycin is enhanced by pretreatment with doxycycline. This observation is in agreement with the phenomenon noted *in vivo* with the same organ.

(2) The antineoplastic activity of daunomycin, tested *in vivo* in mice bearing Sarcoma 180 is not modified by treatment with doxycycline, nor does doxycycline modify the inhibition of DNA synthesis in isolated Sarcoma 180 cells by daunomycin.

(3) The experiments carried out on isolated cell, namely: (a) lack of interactions between daunomycin and doxycycline on the incorporation of thymidine into DNA, (b) no modifications in the uptake and retention of daunomycin by cells pretreated with doxycyline, suggest the hypothesis that the similar pharmacological behavior of the two molecules with regard to the tumor is due to 2 kinds of receptors that are affected differently by the two drugs.

(4) The decrease in the acute toxicity of the antibiastic drug might be due to an interaction between doxycycline and those receptors of the toxic effect which are normally affected by daunomycin in the animals not treated with doxycycline.

In a previous paper[1] it was demonstrated that the acute toxicity of daunomycin in the rat can be significantly affected by pretreating the animals with α-6-deoxy-5-oxytetracyline (doxycycline) or acetylsalicylic acid within a short time before the administration of daunomycin: in fact, these two compounds have proven able to substantially modify the distribution of the antitumor drug in the body.

In the present paper, the results of experiments carried out to explore the relationship between the changes induced in the pharmacokinetic by doxycycline and the antineoplastic effects of daunomycin are reported.

Materials and Methods

In Vivo Experiments

Influence of Treatment with Doxycycline on the Lethality of Daunomycin

A group of 20 COBS mice was treated intravenously with 18 mg/kg of the antiblastic drug. A second group was treated according to the following schedule: 60 mg/kg of doxycycline, *per os*, 2 hours before the administration of the antitumor drug; 40 mg/kg on the 2nd day and 30 mg/kg on the 3rd day. The animals were kept under observation for a period of 40 days.

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Pharmacokinetic Studies

The pharmacokinetics of daunomycin was studied in COBS mice into which Sarcoma 180 had been transplanted 9 days before. The animals were divided into two groups of 10 animals each. Daunomycin was administered at the dose of 5 mg/kg, by intravenous route; the second group also received 60 mg/kg of doxycycline *per os* 2 hours before the treatment with the antiblastic drug. The animals were sacrificed 4 hours after administration of the antiblastic drug, and the concentration of daunomycin in the tissues was measured.

Study of the Antitumor Activity

COBS mice into which Sarcoma 180 had been transplanted 24 hours before, were divided into groups of 10 animals each and treated according to the following scheme:

Group 1 : 5 mg/kg of daunomycin for 8 days, by subcutaneous route;

Group 2 : 40 mg/kg of doxycycline on the 1st days and 30 mg/kg for the following 7 days, by intramuscular route;

Group 3 : Doxycycline (as in group 2) followed by 5 mg/kg of daunomycin two hours later, for 8 days, by subcutaneous route;

Group 4 : saline solution by subcutaneous route (controls).

The animals were sacrificed 24 hours after the last treatment, and the weights of their spleen and tumor were recorded.

In Vitro Trials

Experiments on Isolated Cells

(a) Incorporation of ^{3}H-6-thymidine by isolated cells of Sarcoma 180: These experiments were carried out on cells of Sarcoma 180 isolated from the solid tumor by trypsinization.[2] In each experiment, 25 mg of cells (wet weight) suspended in 1.5 ml of HANKS medium containing 10 μCi of the tracer (^{3}H-6-thymidine 2 Ci/mM) were mixed with 18 μg/ml of doxycycline, or with 10 μg/ml of daunomycin, or with doxycycline+daunomycin. Incorporation of ^{3}H-6-thymidine into DNA was measured in a liquid scintillation spectrometer[3].

(b) Uptake of daunomycin by isolated cells of Sarcoma 180: Cells isolated by trypsinization of the solid tumor[2] were washed and centrifuged at low speed; 500 mg of cells were mixed with 0.5 ml of HANKS medium containing 500 μg of daunomycin. After mixing, the suspension was centrifuged and the concentration of daunomycin was measured in the supernatant and in the cells. At the end of each experiment, the morphological state of cells was checked microscopically and the samples showing coarse morphological modifications were discarded. In order to determine the amount of daunomycin retained by cells, 10 mg of cells (wet weight) were washed by suspending in one ml of HANKS medium, in order to release the antiblastic drug not tightly bound. Free intracellular water, available for equilibrating the concentrations against extracellular compartments, was measured using ^{14}C-urea tracer.

Isolated Kidneys

The kidneys were removed from adult rabbits, pretreated with heparin. The organs, without the capsules, were perfused in a Spadolini apparatus. A first series of organs was perfused with LOCKE's fluid, into which 20 μg/ml of daunomycin had been solubilized. A second series of kidneys was perfused with LOCKE's fluid, into which 100 μg/ml of doxycycline had been dissolved; after short washing, they were perfused with the medium containing 20 μg/ml of daunomycin. Perfusion time was regulated in order to perfuse each organ with an amount of fluid proportional to its weight. At the end of each treatment, the organs were washed in LOCKE's fluid. The amount of daunomycin in the kidneys was subsequently determined after drying the tissues on filter paper.

Identification and Quantitation of Daunomycin

Daunomycin was quantitated in the tissues and in the cells using the spectrofluorimetric tecnique already described.[4]

Experimental Results

Fig. 1 summarizes the acute toxicity of daunomycin alone and in combination with doxycycline as determined in the COBS mouse. No deaths were observed in untreated controls.

Table 1 and Fig. 2 give the concentrations of daunomycin found in various tissues, when administered alone and with doxycycline to mice bearing Sarcoma 180. As a consequence of the pre-treatment of the mice with doxycycline, the concentration of the antitumor drug markedly increased in the blood and in the kidneys, possibly increased in the lungs and decreased in the all other tissues.

Fig. 3 illustrates the effect of daunomycin alone and in association with doxycycline on the development of Sarcoma 180 in the mouse. Based on a comparison of the weights of tumors and of spleens of control and experimental groups of animals, one concludes that doxycycline had no effect on the antineoplastic activity of daunomycin in this model.

In Fig. 4 the incorporation of labelled thymidine into isolated cells of Sarcoma 180 is reported.

Fig. 1. Mortality curve in animals treated with daunomycin and daunomycin + doxycycline.
Daunomycin: 18 mg/kg i.v.
Doxycycline: 60 mg/kg on the 1st day, 40 mg/kg on the 2nd day, 30 mg/kg on the 3rd day, *per os*, 2 hours before daunomycin.

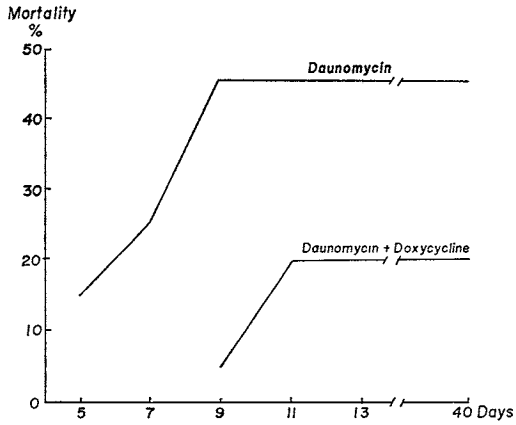


Table 1. Influence of doxycycline on the distribution of daunomycin.

Tissues	Daunomycin	Daunomycin +Doxycycline
Blood	0.01 mcg/100 mg w.w.	0.026 mcg/100 mg w.w.
Liver	1.75 〃	1.74 〃
Kidney	3.02 〃	13.2 〃
Spleen	2.75 〃	1.24 〃
Tumor	0.1 〃	0.043 〃
Heart	1.65 〃	0.76 〃
Intestine	2.63 〃	0.51 〃
Lung	1.02 〃	1.19 〃
Brain	0.36 〃	0.02 〃

Daunomycin: 5 mg/kg i.v.; Doxycycline: 60 mg/kg, *per os*, 2 hours before the administration of daunomycin.

Fig. 2. Variation % of daunomycin concentration in tissues of COBS mice bearing Sarcoma 180 treated with doxycycline.
Doxycycline (60 mg/kg *per os*) was administered 2 hours before daunomycin (5 mg/kg iv).

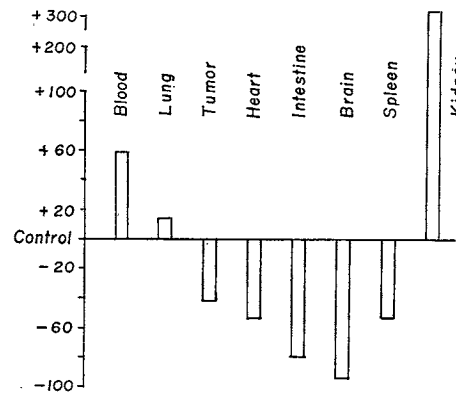


Fig. 3. Antineoplastic activity of daunomycin on COBS mice bearing Sarcoma 180 treated with doxycycline.
Treatment for 8 days: Daunomycin 5 mg/kg s.c. Doxycycline 1 hour before daunomycin, 40 mg/kg i.m. on the first day, 30 mg/kg the other days.

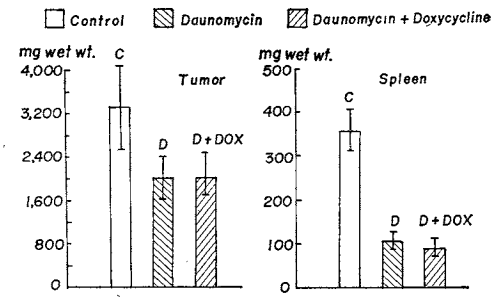


Fig. 4. Incorporation of ^{3}H-6-thymidine by isolated Sacroma 180 cells.
1: Control and doxycycline (8 μg/ml)
2: Daunomycin (10 μg/ml)+doxycycline (8 μg/ml)
3: Daunomycin (10 μg/ml)

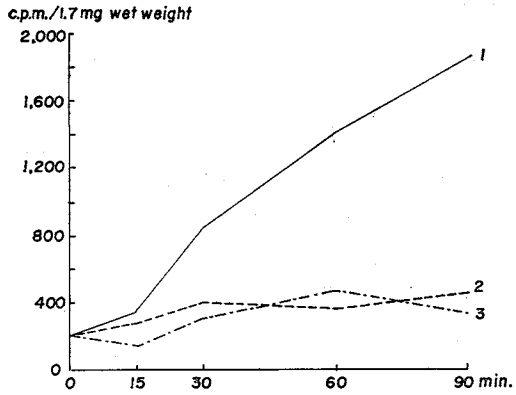


Fig. 5. Isolated Sacroma 180 cells. Percentage of intake and retention of the drugs dissolved in the medium.
1) Daunomycin: 1 mg/ml
2) Doxycycline: 849 μg/ml
3) Doxycycline: 849 μg/ml
Daunomycin: 1 mg/ml
Continuous line indicates the intake of ^{14}C-urea, a molecule which is not retained by cells.

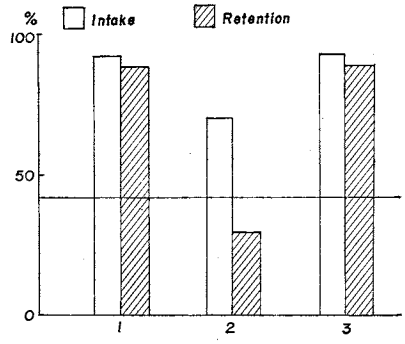
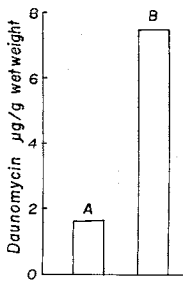


Fig. 6. Isolated rabbit kidneys.
A: Kindney perfused with daunomycin.
B: Kindney perfused before with doxycycline and then with daunomycin.



Again in this experimental system, it was noted that doxycycline did not modify the inhibition exerted by daunomycin on the incorporation of the nucleoside into DNA. Doxycycline alone does not affect the incorporation of ^{3}H-6-thymidine showed by untreated cells.

Fig. 5 shows the almost quantitative uptake of daunomycin by isolated cells of Sarcoma 180, after contact with the antitumor drug. The figure also shows that daunomycin taken up by the cells was not removed by washing performed with the suspension medium as the one used for suspension. In fact, when the Sarcoma 180 cells are suspended at a concentration of 1 g in 1 ml of medium the uptake of the antiblastic drug cease when its concentration in the medium reaches the value of 1 mg/ml. The treatment with doxycycline, which is apparently taken up by cells at slightly lower concentrations, does not modify the uptake of the drug, nor the ability of cells to retain the drug.

In contrast to the observations with the isolated Sarcoma 180 cells doxycycline has a significant effect on uptake of daunomycin by isolated kidneys of the rabbit. As can be seen in Fig. 6, pretreatment with doxycycline remarkably increased binding of the antiblastic drug by the isolated organ.

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