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QUANTITATIVE ASSESSMENT OF THE POLYSOME PROFILE OF THE LIVERS OF MICE TREATED WITH TETRACYCLINE OR DOXYCYCLINE

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Abstract—The influence of tetracycline and doxycycline (10–100 μg/g i.v.) on the aggregational state of ribosomes from mouse liver was tested. Both drugs caused a disaggregation of the ribosomes as evidenced by a rise of the monosomes + disomes/polysomes ratio.

Tetracycline was much more potent than doxycycline, the minimum effective doses for tetracycline being 10 μg/g i.v. as compared to 100 μg/g for doxycycline. The results show that tetracycline but not doxycycline at therapeutic dose range may interfere with the protein synthesis of the liver.

The antibacterial effects of the tetracyclines are due to an inhibition of protein synthesis. By binding of these antibiotics to the 30 S subunits of the ribosomes [1, 2] they inhibit the binding of the aminoacyl-*t*-RNA to the ribosome–messenger–RNA-complex [3, 4] and thus prevent the elongation of the nascent peptide chains [5, 6].

Because of structural and functional differences between ribosomes from prokaryotic and eukaryotic cells the affinity of the tetracyclines to the ribosomes from animal cells is much lower than the affinity to ribosomes from bacterial cells [6, 7].

Nevertheless, inhibition of protein synthesis in cell free systems from rat liver [6, 8] and in isolated cells of rat small intestine [9] by several tetracyclines has been reported. *In vivo* studies with various species of experimental animals gave contradictory results: inhibition of the protein synthesis [10–13], no effect on the rate of incorporation of amino acids [14] or even a stimulation [15] have been observed (for review see [16, 17]).

As the results of these studies are equivocal we have made another approach to evaluate the effect of tetracyclines on protein synthesis by trying to analyse quantitatively the polysome profile of liver cells of female mice after application of tetracycline, the prototype drug of this group of antibiotics, and doxycycline, the derivative most often used in clinical practice.

METHODS

All experiments were performed on female NMRI mice kept on soft wood bedding in plastic cages and fed *ad libitum* with standard diet (Herilan®) and tap water. A constant room temperature (25°) and a 12/12 hr dark/light rhythm were maintained.

Tetracycline and doxycycline were dissolved in 1.5 mmoles $MgSO_4$-solution and injected i.v. at doses of 10, 25, 50 and 100 μg/g. Control animals received the same volume of vehicle (1.5 mmoles $MgSO_4$ solution). At various times (0.5, 1, 2, 4, 6, 8, 16 and 24 hr) after treatment animals were killed by decapitation. Their livers were rapidly dissected and cooled on ice.

Some of the doxycycline treated animals were pretreated with phenobarbital (35 μg/g i.p. twice daily for 3 days). This treatment is known from prior experiments from our laboratory to induce the hepatic monooxygenase system [18].

Samples of liver tissue were cautiously homogenized with the four fold volume of AMT-buffer according to Falvey and Staehelin [19] (10^{-1} moles/l NH_4Cl, 5×10^{-3} moles/l $MgCl_2$, 2×10^{-2} moles/l Tris–HCl, 6×10^{-5} moles/l acetic acid) adjusted to pH 7.5 with m-HCl and containing 0.2 mol/l sucrose and 10^{-3} mol/l dithiothreitol. The homogenates were centrifuged for 10 minutes at 30,000 *g* using a J21B Beckman centrifuge. For disaggregation of ribosomes from membranes 3 ml of the supernatant were mixed with sodium desoxycholate to a final concentration of 1% and left for 5 min at 0°.

The above mixture was used for the analysis of the polysome profile according to Falvey and Staehelin [19]: 100 μl were given on 16 ml 10–50% sucrose gradient and centrifuged for 180 min at 100,000 *g* using a Beckman L5-65 ultracentrifuge with a SW27 rotor. The polysome profile was continuously recorded at 254 nm by using an Isco Type 6 ultraviolet absorbance monitor. A quantitative assessment was made by planimetry measuring the area under the curve obtained and comparing the ratio/monosomes + disomes/polysomes.

The validity of the method was checked by means of pactamycine, a drug known to inhibit translation by binding to the 30S or 40S ribosomes of prokaryotic or eukaryotic cells, respectively [20]. Pactamycine was given to the animals at a dose of 1 μg/g i.p. 30 min before sacrifice.

Mean values were calculated from 8 to 12 single determinations. The results were statistically evaluated by means of two factorial analysis of variance. Differences between values from drug treated and control animals were regarded significant if $P \leq 0.05$.

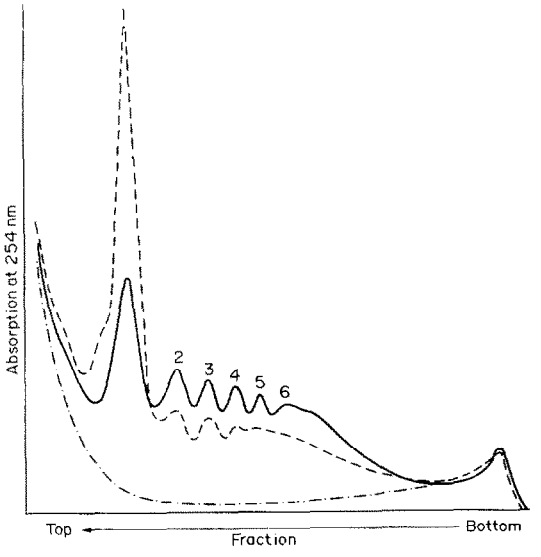

Fig. 1. Polysome profile of the liver of normal and tetracycline treated mice: ————, control; – – – –, tetracycline (100 μg/g); –·–·–·–, base line; 1, 2, 3 . . ., mono-, di-, trisomes etc.

RESULTS

Tetracycline

Figure 1 shows the polysome profile of liver cells of control animals in which monosomes and polysomes can be clearly distinguished. One hour after the application of 100 μg/g tetracycline the polysomes are diminished and the monosome peak is greatly enlarged, showing that disaggregation of the ribosomes has taken place. This effect was clearly dose and time dependent. Maximum effects were seen after 1 hr (Table 1A).

Doxycycline

Doxycycline had an essentially similar effect on the polysome profile but was far less effective. Only the 100 μg/g dose increased the monosomes + disomes/polysomes ratio significantly with a maximum effect after 2 hr (Table 1B).

Doxycycline in phenobarbital-pretreated mice

Pretreatment of the animals with phenobarbital enhanced the effect of 50 μg/g doxycycline. Significant changes of the polysome profile occurred as early as 1 hr after injection of the drug and lasted for at least 3 hr (Table 1B).

DISCUSSION

Disaggregation of polyribosomes and an accumulation of monomers and dimers has been observed under various experimental conditions going along with a decreased rate of protein synthesis [21–23]. According to Pronczuk *et al.* [24] and Sidransky *et al.* [25] the monosomes + disomes/total polysomes ratio is a measure of the aggregational status of the polysomes. An increase of the monomers + dimers in relation to total polysomes gives evidence for a

Table 1. Changes of the hepatic monosomes + disomes/polysomes ratio produced by tetracyclines (deviations from control values ± S.D.)

Dose (μg/g)	Time (hr): 1/2	1	2	4	6	8	16	24	
A. Tetracycline									
10	− 1.2 ± 1.5	+ 8.6 ± 3.3*	+ 5.7 ± 3.3*	− 0.2 ± 3.2	− 0.8 ± 3.2	−0.3 ± 3.3	−6.9 ± 3.4	n.m.	% monosomes + disomes
25	+ 2.2 ± 2.9	+11.9 ± 4.7*	+ 9.0 ± 4.7*	+ 3.2 ± 4.6	+ 2.6 ± 4.6	+ 3.0 ± 4.7	−3.6 ± 4.8	n.m.	
50	+ 5.4 ± 2.9*	+15.1 ± 4.7*	+12.2 ± 4.7*	+ 6.4 ± 4.6*	+ 5.8 ± 4.6*	+ 6.2 ± 4.7*	−0.4 ± 4.8	−7.0 ± 5.5	
100	+13.0 ± 2.9*	+22.8 ± 4.6*	+19.9 ± 4.7*	+14.4 ± 4.6*	+13.4 ± 4.6*	+13.8 ± 4.7*	+7.2 ± 4.8*	+0.6 ± 5.5	
B. Doxycycline									
50	−2.3 ± 7.5	−0.1 ± 5.1	−1.6 ± 2.5	− 0.9 ± 4.4	−3.8 ± 2.8	+2.0 ± 4.0	−1.4 ± 4.6	+0.6 ± 5.7	% monosomes + disomes
100	−0.2 ± 5.5	+2.8 ± 4.6	+7.9 ± 6.9*	+ 3.7 ± 2.5*	+2.4 ± 7.3	+2.4 ± 3.3	+2.9 ± 6.2	−0.8 ± 6.9	
50 after treatment with phenobarbital	+0.8 ± 5.8	+9.9 ± 5.7*	+6.5 ± 4.7*	+10.7 ± 4.7*	−0.6 ± 4.0	n.m.	n.m.	n.m.	

Average of control values: 35.7 ± 6.7% monosomes + disomes.
* P vs corresponding control ≤ 0.05. n.m.: not measured.

deterioration of the status of the polyribosomes, i.e. a reduced protein synthesis.

Our experiments show that tetracycline at a dose of 100 μg/g causes a very long lasting change of the polysome profile by reducing the relative amount of polyribosomes. Even with the lowest dose (10 μg/g), which is well within the therapeutic dose range for men, a significant effect can be obtained. These results are in good agreement with earlier findings from this laboratory [26] that showed that under identical experimental conditions tetracycline at doses of 50–100 μg/g produced an increase of the serum urea level which was maximum after about 4 hr. In this way the results are suited to explain the disturbance in the nitrogen balance and protein metabolism observed by many clinical investigators [27–30].

Doxycycline has a smaller affinity to ribosomes from eukaryotic cells [7] than tetracycline. This becomes also apparent from our experiments. Only the highest dose of doxycycline, which is by far above the therapeutical dose range, raises the monosomes + disomes/polysomes ratio. This is also in good agreement with our earlier observation that doxycycline raises the serum urea level far less than tetracycline [26].

Experimental and clinical investigations have given indirect evidence that doxycycline is metabolized to a considerable extent but apart from one minor metabolite isolated recently by Böcker and Estler [18] no metabolites of doxycycline have been detected. Therefore, it is unknown whether these hypothetic metabolites are pharmacologically active. Our present observation that pretreatment of the animals with phenobarbital enhances the effect of doxycycline on the polysome profile suggests that at least part of the effects of doxycycline are caused by an active metabolite.

CONCLUSION

Our data show that tetracycline even in the therapeutic dose range significantly inhibits formation of higher aggregates of ribosomes and, thus, may interfere with the synthesis of proteins. This is in good agreement with the hypothesis that part of its hepatotoxic effects (i.e. statosis) is caused by an inhibition of protein synthesis. Doxycycline has this effect, too, but only at excessive doses. This is in line with its much smaller hepatotoxicity [26].

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