REVERSIBLE INHIBITION OF PLATELET FUNCTION BY TETRACYCLINE DERIVATIVES

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Activation of platelets by various stimuli leads to the following series of successive reactions: changes in shape of the cell; aggregation and adhesion; synthesis of prostaglandin endoperoxides and thromboxanes, and secretion of substances contained in dense granules (liberation reaction I); secretion of the contents of α-granules (liberation reaction II). This sequence of basic reactions is the same for all inducers (thrombin, collagen, ionophores, ADP, adrenalin, serotonin, and so on), but depending on the strength of action of a given inducer, either all the above-mentioned reactions are exhibited or only some of them. This uniformity of functional manifestations led Holmsen [7, 8] to postulate a "basic platelet reaction," in which interaction of each inducer with the specific receptor on the platelet surface leads to liberation of a mediator substance into the cytoplasm, initiating all five basic reactions. There is now much evidence to show that this mediator may be calcium ions [2, 5, 10]. Binding of extracellular Ca^{++} by means of EDTA or EGTA is known to inhibit ADP aggregation without affecting changes in shape due to ADP. Extracellular calcium chelating agents have almost no effect on interaction of platelets with thrombin, collagen, ionophores, and arachidonic acid [9]. At the same time it has been shown that chlortetracycline (CTC), which binds intracellular calcium, inhibits both aggregation and changes of shape induced by ADP; this inhibition is partly preserved after addition of exogenous calcium to the medium [3, 4]. It has recently been shown that CTC also inhibits secretory activity of platelets [11].

The aim of the present investigation was to study the effect of tetracycline hydrochloride (TC) and its derivatives – oxytetracycline (OTC) and CTC – on the basic functions of platelets.

EXPERIMENTAL METHOD

Platelet-enriched plasma (PEP) was obtained by centrifugation of donor's blood prepared in preservative TsOLIPK 7b for 15 min at 450g.

TC, OTC and CTC were dissolved in physiological saline (3 mg/ml) and added to an equal volume of 0.067M Na_2HPO_4 solution. The resulting solution of TC or its derivatives (1.5 mg/ml, pH 7.2) was added to the PEP in different proportions.

Aggregation of platelets and their reaction to hypotonic shock (RHS) were recorded by a nephelometric method at 37°C.

The intracellular ATP concentration was determined by a bioluminescence method.

A suspension of washed platelets was prepared by adding EDTA to PEP in a final concentration of 5 mM, sedimenting the cells by centrifugation, washing them with modified Tyrode solution (without Ca^{++} and Mg^{++} with EDTA (4 mM) and, finally, resuspending them in modified Tyrode solution. To record the thrombin-induced liberation reaction, 0.5 ml of platelet suspension (5×10^5 to 8×10^5 cells/μl) was added to a measuring cuvette containing 0.5 ml of a preparation of luciferin–luciferase (from Sigma, USA), and 50 μl of thrombin solution was injected. The intensity of the luminescence which developed was recorded and was a measure of the quantity of liberated ATP [1].

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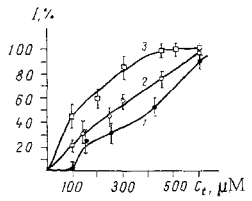

Fig. 1

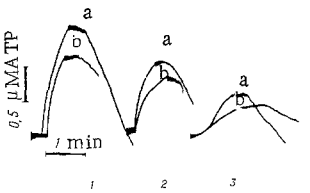

Fig. 2

Fig. 1. Dependence of inhibitory action of TC on ADP-induced platelet aggregation on concentration (M ± m). Abscissa, concentration of tetracycline-HCl (μM) in PEP; ordinate, inhibitory effect: $I = 100(1 - (A_t/Ac)\%$, where A_t is aggregation in the presence of TC and A_c is aggregation in the control. Duration of incubation with tetracycline before addition of ADP 30 min. ADP concentration: 1) 200 μM, 2) 20 μM, 3) 2μM.

Fig. 2. Inhibition by TC of thrombin-induced ATP liberation reaction in suspension of washed platelets (suspension made up from blood kept for 2 days at 4°C. a) Control; b) TC-HCl added to suspension in final concentration of 300 μM. Duration of preincubation with TC 10 min. Thrombin concentration: 1) 0.8 unit/ml; 2) 0.4 unit/ml; 3) 0.16 unit/ml.

EXPERIMENTAL RESULTS

The inhibitory effect of TC on ADP-induced aggregation reached a maximum 20-40 min after the beginning of incubation with the antibiotics. Data on dependence of the maximal inhibitory action of TC on platelet aggregation induced by different concentrations of ADP on concentration are shown in Fig. 1. As Fig. 1 shows, depending on the dose of ADP, 50% inhibition of aggregation was observed between TC concentrations of 150 and 400 μM. OTC had rather weaker, and CTC rather stronger inhibitory action. TC and its derivatives also inhibited platelet aggregation under the influence of arachidonic acid and aggregation of platelets suspended in salt medium by the action of thrombin.

TC depressed not only the aggregating power of the platelets, but also their adhesiveness, clot retraction, RHS, and the adenine-nucleotide liberation reaction under the influence of thrombin (Fig. 2).

Within the concentration range 50-1000 μM (much higher than therapeutic blood levels – 2-4 μM), TC, OTC and CTC inhibited to some degree all the basic reactions connected with activation of platelets.* This inhibition evidently was not due to the harmful action of antibiotics on the cell, for by resuspending the platelets in medium not containing TC their functional activity could be partly or completely restored (Table 1).

Partial (50%) recovery of aggregating power on replacement of the plasma was observed even after incubation of PEP with TC (300 μM) for 48 h. Removal of the inhibitor from PEP was accompanied by recovery of the remaining functions of the platelets; retractility, RHF, thrombin-induced liberation reaction. The absence of a harmful action of short (under 4 h) incubation with TC also was reflected by the significant (using Wilcoxon's two-sample test) independence of the intracellular ATP concentration in the platelets of the presence of TC.

The reversible inhibitory action of TC on phagocytosis and migration of polymorphonuclear leukocytes has recently been reported [6]. Evidently the inhibitory action of TC on functional activity of both types of cells is based on its ability to penetrate easily through cell membranes and to bind intracellular calcium reversibly, thus not giving rise to any serious irreversible damage. Under these circumstances inhibition of all the basic reactions which normally accompany activation of the cell was observed in the platelets. When platelets were transferred to medium not containing TC its intracellular concentration fell sharply and their functional activity, in all its diversity, was restored.

*In [11] no inhibitory action of TC on platelets was found up to a concentration of 2 mM. The reason for this disagreement is not yet clear.

TABLE 1. Reversibility of Inhibitory Action of TC-HCl and CTC-HCl on ADP-Induced Platelet Aggregation

Inhibitor	Index	Functional activity of washed platelets, % of control	
		$M \pm m$	number of determinations
TC	ADP-induced aggregation, ADP concentration, μM:		
	200	93±4	11
	20	92±4	10
	2	70±10	7
	RHS	72±7	6
CTC	ADP-induced aggregation, ADP concentration, μM:		
	200	77±5	8
	20	70±10	8
	2	33±10	6

Legend. PEP incubated with TC (200-500 μM) and with CTC (50-200 μM) for 1 h at 37°C, after which cells were sedimented and resuspended in autologous cell-free plasma. ADP-induced aggregation and RHS recorded 1-2 h after washing and expressed in percent of corresponding values in control (before addition of TC and CTC).

The results of the present investigation are thus further evidence in support of a single system of activation of platelets and of the leading role of Ca^{++} in this process.

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