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Effects of Carbenicillin and Phosphomycin on ADP Induced Platelet Aggregation

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The effects of carbenicillin and phosphomycin separately or simultaneously, on ADP induced platelet aggregation have been studied *in vivo*. Platelet aggregation, ADP induced, was inhibited by carbenicillin and phosphomycin. The inhibition was proportional to the concentration of antibiotic. A slight inhibition was observed when platelet rich plasma was incubated simultaneously with both antibiotics, but synergy on the ADP-induced platelet aggregation was absent.

There is a large number of drugs which inhibit ADP induced platelet aggregation (18, 19). It is known that many antibiotics inhibit ADP induced platelet aggregation like penicillin G (7, 17) and carbenicillin (1, 6, 14, 17).

Nevertheless a few papers have been published which show that the combination of drugs which inhibit platelet aggregation alter de platelets at very low concentration which individually, as much *in vitro:* prostaglandins E_1 and dipiridamol (2, 16), as *in vivo* acetylsalicilic acid and dipiridamol (11, 21), penicillin and non steroidal antiinflammatories (12).

In this paper the effects of carbenicillin and phosphomycin on platelet aggregation induced by ADP are investigated.

Materials and Methods

Blood was taken from normal healthy donors and mixed with one-ninth volume of 3.8 per cent (w/v) sodium citrate in centrifuge tubes. All equipment used throughout the experiments was of plastic (15).

After centrifugation at 500 g for 15 minutes, platelet rich plasma (PRP) was removed, and 0.9 ml samples were placed in tubes for aggregation studies. Platelet aggregation was studied by the turbidimetric technique of BORN (3-5). The PRP contained from 3 to 5×10^9 platelets per millilitre. After equilibration to 37° C, saline solutions on ADP were added to initiate aggregation. The aggregation was monitored by measuring the change in

optical density with a colorimeter connected to a strip chart recording system. The maximum deflection was measured in millimeters.

Platelet aggregation was induced by ADP 2.34×10^{-6} M. All solutions of the disodium salt of ADP were freshly prepared in ice-cold 0.9 NaCl and stored at 2° C.

The carbenicillin and the phosphomycin were incubated at 37° C with PRP previous to the aggregation induced by ADP. The incubation time was 15 minutes for carbenicillin and 5 minutes for phosphomycin.

Results

ADP (2.34×10^{-6} M) induced platelet aggregation. The maximal deflection was considered to be 100 per cent.

When ADP was incubated at 37° C with carbenicillin for 15 minutes, or with phosphomycin alone 5 minutes, these antibiotics inhibited the effect of ADP. The inhibition of platelet aggregation was proportional to the concentration of the antibiotic (table I).

Table I. *Effect of carbenicillin and phosphomycin on ADP induced platelet aggregation.* The numbers indicate aggregation response as % of the control.

		Effect %
ADP (control)	2.34×10^{-6} M	100
Carbenicillin	23.6×10^{-3} M	83.7±4.74
	47.3×10^{-3} M	65.2±4.91
Phosphomycin	13.7×10^{-3} M	94.06±2.37
	34.3×10^{-3} M	62.9±3.28
	13.7×10^{-2} M	14.2±5.07
Carbenicillin	*Phosphomycin*	
	13.7×10^{-3} M . .	79.9±3.37
23.6×10^{-3} M	34.3×10^{-3} M . .	62.5±5.62
	13.7×10^{-2} M . .	14.2±4.98
	13.7×10^{-3} M . .	62.1±3.08
47.3×10^{-3} M	34.3×10^{-3} M . .	42.0±4.41
	13.7×10^{-2} M . .	10.9±4.66

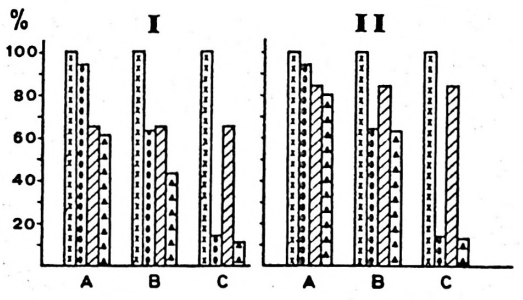

Fig. 1.. *Effect of carbenicillin and phosphomycin on ADP induced platelet aggregation.* I. A: Carbenicillin (23.6×10^{-3} M), Phosphomycin (13.7×10^{-3} M). B: Carbenicillin (23.6×10^{-3} M), Phosphomycin (62.5×10^{-3} M). C: Carbenicillin (23.6×10^{-3} M), Phosphomycin (13.7×10^{-2} M).
II. A: Carbenicillin (47.3×10^{-3} M), Phosphomycin (13.7×10^{-3} M). B: Carbenicillin (47.3×10^{-3} M), Phosphomycin (62.5×10^{-3} M). C: Carbenicillin (47.3×10^{-3} M), Phosphomycin (13.7×10^{-2} M).
[X] = control; [●] = phosphon; [▨] = carbenic; [▲] = phospon + carbenic.

The simultaneous incubation of carbenicillin (15 min) and phosphomycin (5 min) with PRP also inhibited the effect of ADP. The inhibition was larger than that produced by carbenicillin or phosphomycin alone at the same concentration, but there was not statistical difference ($p > 0.05$) between the inhibition produced by both antibiotics simultaneously and the maximum effect produced by only one of the antibiotics (fig. 1).

Discussion

It has been observed that administration of large doses of carbenicillin occasionally produced alterations in the coagulation (1, 14, 20). For LURIE *et al.* (13) this antibiotic inhibits the transformation from fibrinogen to fibrin. MCCLURE *et al.* (14) consider that the effect on platelet aggregation, is mainly due to the changing morphology of the platelet.

The same author consider that it was necessary to incubate PRP with carbenicillin at 37° C for two hours, and to use doses in the range of 700 mcg/ml. This dose is approximately ten times less than that used in the present experiment. The way in which carbenicillin inhibits platelet aggregation is not well known (17).

Phosphomycin also inhibits ADP induced platelet aggregation. the inhibition being proportional to the concentration of the antibiotic. Phosphomycin is an antibiotic with a phosphoric group in its molecule and moves freely in plasma unattached to plasmatic proteins (10).

It acts as antibiotics inhibiting the incorporation of the pyruvate — from the phosphoenolpyruvic — with the UDP-N-acetylglucosamine and with that the formation of muramic acetyl acid (8, 9).

Penicillin and carbenicillin also act as antibiotics inhibiting the formation of wall cell. It is possible that phosphomycin, on platelet aggregation, acts as those antibiotics changing platelet morphology. The same mechanism of action could explain why no synergy exists between carbenicillin and phosphomycin.

The simultaneous incubation of PRP with both antibiotics at the same time inhibits platelet aggregation induced by ADP, although no synergy exists which could be used positively in the treatment of patients with subsidiary infections of these antibiotics, and which at the same time present a thrombotic state.

Resumen

Se estudian los efectos de la fosfomicina y carbenicilina solos y simultáneamente sobre la agregación plaquetaria producida por ADP *in vitro*. Ambos antibióticos inhiben la agregación plaquetaria proporcionalmente a la concentración de antibiótico. La incubación simultánea de plasma rico en plaquetas con ambos antibióticos no presenta una sinergia sobre la inhibición de la agregación plaquetaria inducida por ADP.

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