Haemolysis of human erythrocytes by pentachlorophenol and its suppression by albumin

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Pentachlorophenol (PCP), a potent and inexpensive biocide, has been used widely, especially for wood preservation. Because of its extensive use, there is concern about the possible health hazards caused by PCP. Cases of severe PCP intoxication have been described, some fatal. At least one case of haemolytic anaemia associated with PCP intoxication has been found¹ but little is known about the effects of PCP on erythrocytes.

Methods

Pentachlorophenol and γ -globulin (from human serum) were purchased from Sigma (St Louis, MO) and human serum albumin from Chemical Dynamic Corporation (South Plainfield, NJ). Blood was obtained from healthy males. The erythrocyte suspension (packed cell volume 4.5%) and the membranes were prepared as described.²

The PCP was first dissolved in ethanol. The reaction mixture for the determination of haemolysis contained 0.68 ml isotonic buffer (30 mM Tris HCl, pH 7.4, with 120 mM NaCl, 5 mM KCl, and 2 mM $MgCl_2$) and 0.02 ml ethanol with or without PCP. The incubation and the calculation of haemolysis were as reported previously.³

To examine binding of PCP, the concentrations in solutions were estimated spectrophotometrically.⁴ The membrane suspension (in 10 mM Tris HCl pH 7.4, 4 mg protein/ml, 0.25 ml) was mixed with 0.25 ml 0.1 M potassium phosphate buffer pH 7.4 and 0.01 ml ethanol with or without PCP. After incubation at 37°C for 30 minutes, the mixture was centrifuged at 15 000 g for 30 minutes. The supernatant was diluted five times with the phosphate buffer and the absorbance at 320 nm was measured (free PCP). The PCP solution (in ethanol) was diluted in the same way and the absorbance was measured (total PCP).

In the ultrafiltration study, the isotonic buffer

Figure 1 (A) Haemolysis caused by pentachlorophenol (PCP). Washed erythrocytes were incubated at 37°C for two hours with PCP (0.08–0.8 mM) in the presence (○) and absence (●) of 5 mM glucose. (B) Effects of albumin (□) and γ -globulin (■) on PCP induced haemolysis. Washed erythrocytes were incubated with 0.8 mM PCP and various amounts of the protein (0.05–0.5%). The incubation was done at 37°C for one hour. Each line was examined with a logistic regression analysis; $y = 1/(1 + \exp(-a - bx))$ where x was the dose and $a = -12.5$ (○), -13.0 (●), 11.5 (□), and 3.7 (■), $b = 1.2$ (○), 1.3 (●), -0.2 (□), and 0.0 (■). In every case the results fitted well with the estimation ($p < 0.01$, F test).

(1.96 ml) containing albumin or γ -globulin was mixed with 0.04 ml ethanol containing 10 mM PCP, and transferred to Ultracent-10 (Tosoh, Tokyo). After centrifugation at 1000 g for 40 minutes, PCP in the filtrate was measured. All measurements on haemolysis and protein binding were done in duplicate, and each experiment was carried out at least twice.

Results

When PCP was added to the reaction mixture, haemolysis was seen (fig 1A). Presence of glucose (5 mM) did not affect haemolysis. On the other hand, haemolysis (by 0.8 mM PCP) was completely abolished by albumin (fig 1B). γ -Globulin, however, did not suppress the haemolysis. (γ -Globulin itself did not haemolyse the cells.)

The concentration of PCP in the supernatant after sedimentation of the erythrocyte membrane decreased as the amount of membrane increased. When the concentration of PCP was varied, a clear

Figure 2 Absorbance (320 nm) of the PCP solution (●) (total PCP) and that of the supernatant after sedimentation of the membrane (■) (free PCP). Solutions were diluted five times before spectrophotometric measurement. The calculated difference (○) (bound PCP) is also shown. The Scatchard plot for the binding of PCP to the membrane is shown in the inset. Coefficients in logistic regression analysis (see legend for fig 1) were: $a = -3.01$ (■), -2.02 (○), $b = 3.57$ (■), and 1.78 (○). The results fitted well with the estimation ($p < 0.01$, F test). The Scatchard plot fitted well ($r = 0.983$) with a second order model on a polynomial regression analysis consistent with a curved line.

linear relation was seen between the concentration and the absorbance at 320 nm (fig 2). Taking the difference of the total and free PCP as the bound PCP, Scatchard analysis produced a curve (fig 2).

In the ultrafiltration study, the concentration of PCP in the filtrate decreased as the concentration of albumin in the mixture increased. The presence of γ -globulin did not alter the PCP concentration in the filtrate (data not shown).

Discussion

The results show that PCP can haemolyse human erythrocytes and erythrocyte membranes can bind PCP. Moreover, PCP decreased the fluidity of purified plasma membrane.⁵ Thus it seems that PCP haemolyses human erythrocytes through its interaction with the membrane.

A curved plot was obtained from Scatchard analysis, suggesting that the erythrocyte membrane has multiple binding sites for PCP with different affinities. Hence, it is possible that PCP attacks more than one component of the membrane.

Albumin could completely suppress the PCP induced haemolysis, whereas γ -globulin could not. The results of the ultrafiltration study indicated that albumin (but not γ -globulin) bound PCP. Weinbach *et al*⁶ reported that albumin could restore mitochondrial function impaired by PCP. Thus albumin can potentially counteract the effects of PCP and this seems due to its capacity to bind PCP.

Because human serum contains more than 3% of albumin, it seems reasonable that haemolytic anaemia has only rarely been seen in PCP intoxication¹ and that exchange blood transfusion showed a dramatic effect in patients severely intoxicated with PCP.⁷ It should be noted, however, that haemolytic anaemia might be induced if the equilibrium of PCP between albumin and erythrocytes should be altered.

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