In Vitro Uptake of Salicylate by Human Red Blood Cells

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(Received 3 April 1979)

Abstract Distribution and binding properties of sodium salicylate to human red blood cells were studied under various experimental conditions. The effect of tonicity and hemolysis on the steady state level of the drug within the human red blood cells were accounted for in this study. When the washed cells were suspended in normal saline solution, the drug was so rapidly permeated into red cells. Since the pH of the system forces nearly complete ionization of the drug, ionic diffusion through aqueous pores is thought to be the mode of salicylate transport. Human red cell binding capacity and association constant for salicylate were estimated. This work supports the view that the red cells act as an important reservoir of salicylate.

Keyphrases

Salicylate-distribution to human red cells; Salicylate-binding to human red cells; Salicylate-fluorescence measurement; Binding-salicylate; Distribution-salicylate; Hemolysis effect to the binding of sodium salicylate; Crenated human cells-salicylate binding.

The red blood cell compartment of the blood is often dismissed as an insignificant consideration in pharmacokinetics. Blood and plasma salicylate levels are often merely assumed to be equivalent terms. The possible influence of red blood cells and other cellular components on salicylate binding in blood has been largely neglected. Only recently has binding for salicylate in blood other than plasma received scant attention. The concentration of salicylate within red cells is in excess of the free drug concentration in the plasma over the certain concentration range. The nature of the interaction and accumulation is unknown and probably varies, and the significance of these interactions in the overall pharmacokinetics of the drugs has not been determined. The extent and strength of binding of salicylate in red cells have not been determined. In vivo, in the blood circulation, a single event such as red cell uptake is difficult to isolate from the multiplicity of kinetic events occurring. Therefore, to determine the distribution characteristics of salicylate into red cells, and the strength and capacity of salicylate binding, salicylate uptake by human red cell was studied under various experimental conditions in vitro in a closed system.

EXPERIMENTAL

Materials

Sodium salicylate (J.T.Baker), potassium bisulfate (J.T.Baker), and spectro grade chloroform (Matheson Coleman & Bell) were obtained from commercial sources. Human red blood cells (HRBC) were obtained from blood bank
stored for 3 weeks at 4°C in acid-citrate-dextrose solution. The whole blood was centrifuged at 1200g for 10 minutes. Plasma and buffy coat were removed. The packed HRBC were washed three times with three volumes of 0.9% normal saline solution. The packed HRBC were collected following a final centrifugation at 1200g for 10 minutes and removal of saline solution.

Uptake of Salicylate by HRBC

The distribution of sodium salicylate into HRBC was determined in plasma, 0.9%, 2.4% saline solution, 4.2% human serum albumin (HSA), and isotonic phosphate buffer with pH 7.4. Five milliliters of washed HRBC was resuspended in 5ml of each suspending medium containing the drug at the concentration of 10, 20, 40, 60, 80, 100, 1200, 400, 600, 800, 1000 µg/ml, respectively. The suspensions were then incubated for 15 minutes at room temperature. After incubation, the samples were immediately centrifuged at 1200g for 10 minutes. Percentage of hemolysis and the concentration of sodium salicylate in the supernatant were measured. The distribution ratio of salicylate between HRBC and suspending medium was calculated. To study the effect of hemolysis and alteration in the nature of the HRBC during the distribution and binding studies, 5 ml of washed HRBC was resuspended in 5 ml of 0.9% saline solution containing various concentrations of sodium salicylate. Each suspension were tumbled up-side down ten times and then incubated for two hours. This procedure was repeated twice. After incubation, the samples were centrifuged at 1200g for 10 minutes. Supernatant was separated and the degree of hemolysis occurring during incubation period was determined. The concentration of salicylate in supernatant was measured with blank.

Analytical Method

One ml of each samples was acidified with 1 ml of 10% potassium bisulfate solution in 15 ml glass-stoppered centrifuge tubes. Five ml of chloroform were added to each tubes and these were then agitated on a mechanical shaker (Thermolyne Sybron Co.) for two minutes and situated for minimum 30 minutes. Aqueous and buffy layer were pipetted off. The concentration of salicylate in chloroform was measured by spectrophotofluorometer (Perkin-Elmer model 204). The fluorescence of salicylate was determined by activating at a wave-

Overall Concentration of Sodium Salicylate in HRBC

Fig. 1: Distribution of salicylate in HRBC suspending medium. ● = 0.9% Saline Solution ○ = 2.4% Saline Solution ▲ = Plasma △ = 4.2% HSA Solution