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INFLUENCE OF ACETYLSALICYLIC ACID, BUTAZOLIDINE, COLCHICINE, HYDROCORTISONE, CHLORPROMAZINE AND IMIPRAMINE ON THE PHAGOCYTOSIS OF POLYSTYRENE LATEX PARTICLES BY HUMAN LEUCOCYTES

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Abstract—It has been demonstrated that acetylsalicylic acid, butazolidine, colchicine, hydrocortisone, chlorpromazine and imipramine inhibit the uptake of polystyrene latex particles and oxygen consumption during phagocytosis by human leucocytes.

ACETYLSALICYLIC acid is one of the most used drugs in medicine, and in connection with rheumatic diseases both salicylates, butazolidine, colchicine and hydrocortisone are the main pharmaca. These drugs may act therapeutically by stabilizing the lysosomal or plasma membrane, or by other mechanisms, thus enabling them to change the function of lysosomes or the release of agents that provoke inflammation.[1,2] Chlorpromazine and imipramine which belong to the tranquilizer group of drugs inhibit certain enzymes, but also reduce membrane permeability, and inhibit release from platelets.[3,4]

As lysosomes and membranes are involved in phagocytosis it was thought of interest to determine the effect of these drugs on this process using two standardized methods.

MATERIALS AND METHODS

Chemicals

Anticoagulants. Heparin (Nyegaard & Co., Oslo, Norway) 5000 I.U. per ml was diluted 1/50 with 0·154 M NaCl. Chlorpromazini chloridum and imipramini chloridum (Dumex, Copenhagen, Denmark). Colchicine (Rikshospitalets Apotek, Oslo, Norway). Fresh solutions of colchicine were prepared for each experiment. Solu-Cortef and hydrocortisone, hydrogen succinate, sodium salt (The Upjohn Company, Kalamazoo, Mich., U.S.A.) were dissolved in 0·154 M NaCl. Butazolidini-reinsubstanz (J. R. Geigy A.G., Basel, Schweitz) and acetylsalicylic acid (Rikshospitalets Apotek, Oslo, Norway) were dissolved in 0·154 M NaCl and NaOH, and adjusted to pH 7·4. The drugs were incubated in leucocyte suspensions for 5 min at 37°. All concentrations stated are final concentrations.

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Polystyrene latex particles (PLx). (1·10 μm in diameter, sp.wt. 1·05 g/ml) from the Dow Chemical Co., Midland, Michigan, U.S.A. were available as 10 per cent aqueous suspensions.

Biological materials

Normal human blood was freshly collected each day into siliconized glass tubes, containing 0·1 vol. of anticoagulant. The blood samples were from blood donors at the Red Cross Blood Bank, Oslo, Norway or from soldiers, usually 20–22 years old.

Isolation of leucocytes was according to the method of Böyum.[5] The plasma containing leucocytes and platelets is termed leucocyte suspension.

Leucocyte counting was performed using an electronic particle counter (Celloscope 101, AB Lars Ljungberg & Co., Stockholm, Sweden).[6]

Quantitation of phagocytosis of PLx was performed as described by Kvarstein.[7]

Measurement of oxygen consumption during phagocytosis with the Clark oxygen electrode was performed as previously described.[8]

RESULTS

All concentrations of acetylsalicylic acid, except 12·7 mM where there was a slight stimulation, decreased the uptake of polystyrene latex particles (Table 1). Oxygen consumption during phagocytosis was also decreased with increasing concentrations of acetylsalicylic acid (Table 2).

Butazolidine in increasing concentrations initially decreased both the uptake of polystyrene latex particles and the oxygen consumption during phagocytosis (Tables 1 and 2). The inhibition leveled off at higher concentrations. Increasing concentrations of colchicine decreased both the uptake of polystyrene latex particles and oxygen consumption during phagocytosis (Tables 1 and 2).

Hydrocortisone showed the same pattern as colchicine (Tables 1 and 2). It was examined using both the drug as "Solu-Cortef" and in its pure form. No difference was observed.

Increasing concentrations of both chlorpromazine and imipramine inhibited the uptake of polystyrene latex particles and the oxygen consumption during phagocytosis (Tables 1 and 2).

DISCUSSION

Salicylates interfere with carbohydrate metabolism in several tissues by uncoupling oxidative phosphorylation[9] and inhibiting erythrocyte glycolysis.[10] Recently, Doery, Hirsch and de Gruchy[11] demonstrated that acetylsalicylic acid inhibits glycolysis and produces a fall in the concentration of adenosine triphosphate by human platelets. Evans, Packham, Nishizawa, Mustard and Murphy[12] reported that acetylsalicylic acid inhibits the platelet aggregation induced by collagen, antigen–antibody complexes, gamma globulin-coated particles or thrombin, but does not affect the ADP-induced platelet aggregation. The mechanism by which acetylsalicylic acid produces its effects on platelets is uncertain. It is postulated that it acetylates numerous human proteins,[13,14] and it is possible that it inhibits ADP release and glucose transport by acetylation of membrane proteins. Miller and Smith[15] found that acetylsalicylic

TABLE 1. THE EFFECT OF INCREASING CONCENTRATIONS OF ACETYLSALICYLIC ACID, BUTAZOLIDINE COLCHICINE, HYDROCORTISONE, CHLORPROMAZINE AND IMIPRAMINE ON THE UPTAKE OF POLYSTYRENE LATEX PARTICLES

Acetylsalicylic acid					
Control Mean ± 1 SD (*n*)	12·7 mM Mean ± 1 SD (*n*)	25·2 mM Mean ± 1 SD (*n*)	37·4 mM Mean ± 1 SD (*n*)	49·4 mM Mean ± 1 SD (*n*)	72·6 mM Mean ± 1 SD (*n*)
100 (36·9 ± 7·0) (8)	107 ± 5·8 (8)	93 ± 12·5 (8)	55 ± 18·7 (8)	42 ± 16·7 (8)	29 ± 9·8 (8)
Butazolidine					
Control	1·4 mM	2·8 mM	5·5 mM	10·9 mM	20·9 mM
100 (36·5 ± 8·7) (5)	96 ± 6·8 (5)	84 ± 12·3 (5)	61 ± 6·2 (5)	48 ± 13·9 (5)	41 ± 17·5 (5)
Colchicine					
Control	1·5 mM	2·9 mM	5·8 mM	8·5 mM	11·1 mM
100 (32·3 ± 3·9) (4)	90 ± 24·6 (4)	62 ± 23 (4)	28 ± 9·6 (4)	24 ± 9·8 (4)	36 ± 10·7 (4)
Hydrocortisone					
Control	1·23 mg/ml	2·45 mg/ml	4·81 mg/ml	7·08 mg/ml	9·26 mg/ml
100 (30·7 ± 4·5) (4)	76 ± 9·3 (4)	42 ± 11 (4)	25 ± 7·4 (4)	24 ± 8 (4)	39 ± 15·1 (4)
Chlorpromazine					
Control	0·14 mM	0·28 mM	0·55 mM	1·1 mM	
100 (28·6 ± 12·7) (4)	89 ± 8·5 (4)	65 ± 7·0 (4)	30 ± 13·3 (4)	9 ± 3·5 (4)	
Imipramine					
Control	0·06 mM	0·16 mM	0·31 mM	0·62 mM	
100 (33·7 ± 12·8) (4)	76 ± 1·9 (4)	57 ± 2·4 (4)	25 ± 8·4 (4)	7 ± 1·7 (4)	

The uptake of PLx is expressed as per cent of the control sample. The uptake of PLx in μg PLx per 10^6 leucocytes in the control samples is given in parenthesis.

TABLE 2. THE EFFECT OF INCREASING CONCENTRATIONS OF ACETYLSALICYLIC ACID, BUTAZOLIDINE, COLCHICINE, HYDROCORTISONE, CHLORPROMAZINE AND IMIPRAMINE ON THE OXYGEN CONSUMPTION DURING PHAGOCYTOSIS OF POLYSTYRENE LATEX PARTICLES

Acetylsalicylic acid			
Control Mean ± 1 SD (*n*)	41·4 mM Mean ± 1 SD (*n*)	61·1 mM Mean ± 1 SD (*n*)	80·2 mM Mean ± 1 SD (*n*)
100 (5·7 ± 0·5) (3)	56 ± 30·9 (3)	19 ± 22·6 (3)	2 ± 2·7 (3)
Butazolidine			
Control	17·7 mM	25·7 mM	33·2 mM
100 (5·3 ± 0·2) (3)	61 ± 6·7 (3)	52 ± 26·3 (3)	41 ± 27·1 (3)
Colchicine			
Control	1·0 mM	2·5 mM	4·8 mM
100 (5·8 ± 1·3) (4)	75 ± 6·5 (4)	25 ± 13·1 (4)	5 ± 3·5 (4)
Hydrocortisone			
Control	4·03 mg/ml	5·95 mg/ml	7·81 mg/ml
100 (4·9 ± 1·4) (4)	50 ± 12·5 (4)	18 ± 5·5 (4)	3 ± 2·5 (4)
Chlorpromazine			
Control	0·11 mM	0·28 mM	0·55 mM
100 (5·5 ± 0·9) (4)	78 ± 9·2 (4)	41 ± 19·2 (4)	2 ± 3·5 (5)
Imipramine			
Control	0·13 mM	0·31 mM	0·62 mM
100 (6·0 ± 1·6) (4)	47 ± 18·7 (4)	4 ± 4·1 (4)	0 (4)

The increase in oxygen consumption is expressed as per cent of the control sample. The increase in oxygen consumption in n atoms/min/10^6 leucocytes in the control samples is in parenthesis.

acid stabilizes rat liver lysosomal membranes. These effects of acetylsalicylic acid may explain the results obtained in the present study.

Butazolidine inhibits the release of lysosomal enzymes.[1] Packham, Warrior, Glynn, Senyi and Mustard[16] showed that it blocks the platelet aggregating action of collagen, antigen–antibody complexes, and gamma globulin-coated surfaces, but does not block the action of ADP or thrombin. Strauss, Paul and Sbarra[17] demonstrated that it inhibits both engulfment and intracellular destruction of *E. coli* by guinea pig peritoneal polymorphonuclear leucocytes. His findings indicated that it acts upon the hexose monophosphate shunt and H_2O_2 formation, and are thus in accordance with the present study.

Colchicine interferes with some biochemical functions associated with phagocytosis.[18] It has been shown to diminish the increased oxygen uptake associated with phagocytosis without interfering with the engulfment process.[19] This finding is not in accordance with the results in the present study in which there was a good correlation between the decreased uptake of polystyrene latex particles and oxygen consumption during colchicine inhibited phagocytosis. The discrepancy is probably because Malawista and Bodel used bacteria and determined oxygen consumption with a Warburg respirometer. This clearly shows the necessity of using different stimuli and cells in such studies. The conclusions are only valid for each system.

Corticoids stabilize lysosomes against a wide variety of injurious agents and do so both *in vitro* and *in vivo*.[1] Seneca and Peer[20] using human leucocytes found that prednisolone depresses phagocytosis of *Streptococcus hemolyticus*, but stimulates that of *Proteus vulgaris*, again indicating that both the phagocytic stimuli and cells may be of importance for the result. Hydrocortisone which was used in the present study, has an inhibitory effect on both engulfment of polystyrene latex particles and oxygen consumption during phagocytosis. An explanation, or a contributing factor, for the increased susceptibility to infectious diseases during steroid treatment[21] may be due to blocking of phagocytosis. In addition to this, it may perhaps cause an inhibition of the production of antibodies. But the corticosteroids appear neither to inhibit antibody production to any great extent in man nor to prevent the union of antigen with antibody.[22]

It is postulated by several workers that *phenothiazines* produce their effects by acting on membranes and in particular, permeability of membranes.[23] It is thus shown that the uptake of water by frog gastrocnemius muscle is blocked by chlorpromazine.[24] Further, chlorpromazine inhibits certain purified enzymes. It is well established that chlorpromazine inhibits a flavin intermediate step in oxidative phosphorylation.[23] It seems therefore reasonable to conclude that the effect of chlorpromazine and imipramine on phagocytosis demonstrated in the present study occurs at the enzymatic level. The influence of these drugs on phagocytosis is of interest from a clinical point of view. Some have reported increased susceptibility to infectious diseases during chlorpromazine treatment in man[25–27] and also in animals.[28]

All the drugs which were tested in the present study, inhibit both the uptake of polystyrene latex particles and the oxygen consumption during phagocytosis. The explanation of this phenomenon may be due to their influence on lysosomal membranes In addition, they may exert their effect on the plasma membrane.[2] The experiments with the inhibited uptake of polystyrene latex particles may support such an explanation. But it should be emphasized that the mechanism certainly is complex, and the

use of advanced biochemical and pharmacological methods is necessary for the clarification of these problems.

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