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The influence of protein binding on the excretion of some sulphanilamidopyrimidines in man

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The metabolism and excretion of sulphadimethoxine, sulphorthodimethoxine, sulphamethomidine, sulphasomidine and sulphadimethoxypyrimidine have been investigated in man. The plasma protein binding data including the percentage bound at various concentrations of the drugs, the number of binding sites (n), the binding capacity of plasma albumin (r_{max}) and the dissociation constant of the sulphonamide-albumin complex (K) for these compounds have been measured by ultrafiltration. The short-acting sulphasomidine is bound to a much lesser extent at the concentrations obtained clinically in the plasma than the long-acting drugs. Sulphasomidine occupies only one binding site on plasma albumin compared with the other sulphonamides investigated which occupy two sites. This may partly account for the rapid excretion of this compound in man. Sulphadimethoxine has two major metabolites, the N^4-acetyl derivative and sulphadimethoxine-N^1-glucuronide. N^4-Acetyl sulphadimethoxine has similar binding constants to the parent compound. However the N^1-glucuronide forms a weak complex with plasma albumin which has a lower binding capacity for this metabolite ($r_{max} = 0{\cdot}58$) compared with sulphadimethoxine itself ($r_{max} = 1{\cdot}77$). The results are discussed in relation to the excretion of these compounds.

The metabolism and excretion of several clinically important sulphanilamidopyrimidines in man and other species have been investigated.

Sulphadimethoxine (2,4-dimethoxy-6-sulphanilamidopyrimidine, Madribon) (Bridges, Kibby & others, 1968), and sulphamethomidine (4-methoxy-2-methyl-6-sulphanilamidopyrimidine) (Bridges, Walker & Williams, 1969b) are long-acting sulphonamides in man (25% of the oral dose being excreted in the 24 h urine). Sulphorthodimethoxine (4,5-dimethoxy-6-sulphanilamidopyrimidine, Fanasil) (Bridges, Kibby & others, 1969a) and sulphadimethoxypyrimidine (4,6-dimethoxy-2-sulphanilamidopyrimidine, Sulphamoprine) (Walker & Williams, 1969) are very long-acting (6% and 10% of the dose fed excreted in 24 h respectively). Sulphasomidine (2,4-dimethyl-6-sulphanilamidopyrimidine, Elkosin) (Bridges, Walker & Williams, 1969b) is a short-acting sulphonamide in man (72% excreted in the 24 h urine).

The main excretory products of these sulphonamides are the unchanged drug, the N^4-acetyl derivative and the N^1-glucuronide. N^4-Acetyl sulphorthodimethoxine is the major metabolite of this sulphonamide in man (85% of the first 24 h urine) whereas sulphasomidine is excreted mainly unchanged (96% of the first 24 h urine). The N^1-glucuronides of sulphamethomidine and sulphadimethoxine are the major metabolites of these two sulphonamides (68% and 70% respectively of the first 24 h

urine). Sulphadimethoxypyrimidine is excreted in roughly equal amounts as N^1-glucuronide (29%) and unchanged compound (30%) and to a lesser extent as the N^4-acetyl derivative (18% of the first 24 h urine).

The duration of action of these sulphonamides may be related to a number of factors including: rate of absorption and distribution, nature and rate of metabolism, renal excretion and reabsorption, and tissue binding. The plasma protein binding data for these compounds has been measured by ultrafiltration and the results discussed in relation to the urinary excretion data. The protein most generally involved in drug interaction is albumin (Thorp, 1964). Experiments by Anton (1960) and by Newbould & Kilpatrick (1960) have demonstrated the ability of albumin to bind sulphonamides in *in vitro* experiments. Clausen (1966) has shown that although some sulphonamides may bind to other plasma proteins, quantitatively this is only of minor importance. The experiments of Jardetzky & Wade-Jardetzky (1965) using high-resolution nuclear magnetic resonance indicate that the *p*-aminobenzenesulphonamide moiety is the primary and probably the sole binding site in the simpler N^1-substituted derivatives of sulphanilamide.

EXPERIMENTAL

Materials

Sulphadimethoxine m.p. 200–201°, N^4-acetyl sulphadimethoxine m.p. 210–211°, sulphasomidine m.p. 240–241°, sulphorthodimethoxine m.p. 195–197° were gifts from Roche Products Ltd., Welwyn Garden City, Herts. Sulphadimethoxypyrimidine m.p. 179–180° was a gift from Imperial Chemical Industries Ltd., Alderley Park. Sulphamethomidine m.p. 177–178° was a gift from Warner-Lambert, Morris Plains, New Jersey. Sulphadimethoxine N^1-glucuronide (ammonium salt) m.p. 150–160° (decomp.) was synthesized (Bridges, Kibby & Williams, 1965).

Protein binding

Fresh human blood (40 ml) mixed with heparin (0·4 ml or 2000 units; Weddel Pharmaceuticals, London, E.C.1) was centrifuged at 2000 *g* for 10 min. The sulphonamide drug was dissolved in the plasma (20 ml) and 4 ml of 0·1M citrate-0·2M Na_2HPO_4 buffer, pH 7·4 added. The resulting solution (5 ml) was placed in a bag made of Cellophane tubing tied at both ends (15 cm × 3 cm flat width). The bag was placed with a flat surface against a sintered-glass disk (20 mm diam.; porosity 1, Pyrex Co., Sunderland) near the bottom of a polythene centrifuge tube (10 cm × 2·7 cm). The tube was centrifuged at 3000 *g* for 2 h at 18°. The contents of the bag were analysed for total drug (bound and unbound) by adding 20% (w/v) trichloroacetic acid (1 ml) to the plasma (1 ml), then centrifuging at 2000 *g* for 5 min and analysing the supernatant by the Bratton & Marshall (1939) method. The ultrafiltrate below the sintered-glass disk in the polythene tube was analysed for unbound drug by the Bratton & Marshall method.

A graph of r (representing mol of sulphonamide bound per mol of albumin) was plotted against X (representing mol of unbound sulphonamide). The quantity of albumin in human plasma was assumed to be 0·63 mM and the molecular weight of human albumin to be 69 000. At high concentrations of unbound drug the total number of mol of sulphonamide bound per mol of protein (r_{max}) remains constant. This constant was measured at a total sulphonamide concentration in the plasma of 1·6 mM, and it is a measure of the binding capacity of the protein.

For calculations of the binding constant, K, and the number of binding sites, n, the equation of Goldstein (1949) was used in the form:

$$\frac{1}{r} = \frac{K}{n} \cdot \frac{1}{X} + \frac{1}{n}$$

The values of K and n were obtained from the straight line plot of 1/r against 1/X. The slope of the line is K/n and the intercept on the 1/r axis is 1/n. The dissociation constant (K) of the binding reaction between drug and protein is inversely proportional to the strength of binding (Goldstein, 1949).

RESULTS AND DISCUSSION

Bridges & others (1969a, b) have shown that the physical properties of these sulphanilamidopyrimidines and their metabolism affect the rate of urinary excretion. However binding to human plasma proteins also appears to be an important factor in determining their excretion rate. The percentage bound at various concentrations and the binding constants for these sulphonamides can be seen in Table 1.

Table 1. *The binding of some sulphanilamidopyrimidines and their metabolites to human plasma proteins*

Sulphonamide	% bound* at total sulphonamide concentration 0·4 mM	0·8 mM	1·2 mM	K constant mM	r_{max}* at sulphonamide concentration 1·6 mM	Number of binding sites (n)
Sulphasomidine	67	58	44	0·20	1·02	1·1
Sulphamethomidine	90	85	79	0·10	1·73	2·1
Sulphorthodimethoxine ..	96	88	79	0·08	1·91	2·1
Sulphadimethoxypyrimidine	95	80	75	0·25	1·86	2·2
Sulphadimethoxine	92	85	80	0·10	1·77	2·0
N^4-Acetylsulphadimethoxine	90	83	78	0·11	1·80	1·9
Sulphadimethoxine N^1-glucuronide	35	29	23	1·04	0·58	1·0

* The average of three values.

The results show that the short-acting sulphasomidine in man is bound to a much smaller extent at all concentrations measured than the longer-acting sulphonamides. At a concentration of 0·4 mM, which is the plasma level obtained 4 h after an oral dose of this drug (0·05 g/kg) (Prior & Saslaw, 1951), sulphasomidine is only 67% bound, whereas sulphamethomidine, sulphadimethoxine, sulphorthodimethoxine and sulphadimethoxypyrimidine are 90%, 92%, 96% and 95% bound respectively. At concentrations obtained clinically, the long-acting sulphanilamidopyrimidines are highly bound. The percentage of bound drug decreases as the concentration of the sulphonamide increases (Table 1). The r_{max} value for sulphasomidine is less than 60% that of the other drugs having only one binding site instead of two per molecule of albumin. Sulphasomidine is very water soluble and so is rapidly excreted in the urine unchanged. The other sulphonamides being highly bound, less water soluble and more lipid soluble (Bridges & others, 1969a, b) are metabolized before being excreted. However no relation could be found between the charge distribution over the sulphonamide molecule (electronic indices) and the strength of binding of these drugs to plasma albumin.

A comparison between the protein binding data for sulphadimethoxine and the

two major metabolites N^4-acetyl sulphadimethoxine and sulphadimethoxine-N^1-glucuronide is made in Table 1. The N^4-acetyl derivative is bound to approximately the same extent as the parent compound, the K constant and r_{max} are also very similar. Each molecule of albumin can bind either two molecules of sulphadimethoxine or the N^4-acetyl derivative. However sulphadimethoxine is not excreted as rapidly as the N^4-acetyl metabolite because the latter has a much lower lipid-water partition coefficient (Bridges & others, 1969a) and is probably not reabsorbed by the kidney to the same extent. Sulphadimethoxine N^1-glucuronide is not bound to human plasma protein to the same extent as the parent compound (35% compared with 92% at a concentration of 0·4 mM). These differences are reflected in the r_{max} constant (1·77 for sulphadimethoxine and 0·58 mol/mol of albumin for the N^1-glucuronide). Not only does the protein bind less N^1-glucuronide than the parent compound, but the strength of the protein-N^1-glucuronide complex (K = 1·04) is only one tenth that of the protein-sulphadimethoxine complex (K = 0·10). Sulphadimethoxine is therefore poorly excreted as it is lipid soluble, highly and tightly bound at low concentrations (0·4 mM), whereas the N^1-glucuronide, having a low lipid solubility and being poorly and weakly bound, is rapidly excreted.

Conclusion

It is apparent that a small change in the structure of a sulphonamide may alter the binding of that sulphonamide to plasma proteins, i.e., substitution of a methoxyl group for a methyl group of sulphasomidine in position '4' and there is a large increase in the percentage bound. Certain metabolic pathways of sulphonamides alter the binding characteristics. Acetylation of sulphadimethoxine in the N^4 position alters the percentage bound to a minor extent. However the attachment of a glucuronic acid moiety in the N^1 position results in a large reduction in the binding capacity of the protein and a decrease in the strength of the sulphonamide-protein complex.

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