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PROTEIN BINDING OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS IN PLASMA AND SYNOVIAL FLUID OF ARTHRITIC PATIENTS

SOMPON WANWIMOLRUK,[1] PETER M. BROOKS,[2] & DONALD J. BIRKETT[1]

[1]Department of Clinical Pharmacology and [2]Department of Medicine, School of Medicine, The Flinders University of South Australia, Bedford Park, South Australia 5042.

1 The protein binding of seven non-steroidal anti-inflammatory drugs (indomethacin, tolmetin, salicylic acid, ibuprofen, flurbiprofen, naproxen and GP53,633) and warfarin was investigated by equilibrium dialysis in simultaneous samples of synovial fluid and plasma from 12 arthritic patients.

2 The protein binding of all drugs studied except warfarin and flurbiprofen was significantly lower in synovial fluid than in plasma.

3 The decreased protein binding of these drugs is likely to explain the lower total drug concentrations found in synovial fluid in comparison to plasma.

4 The lower albumin concentration plays an important role in determination of reduced drug binding in synovial fluid compared to plasma and the fatty acid concentration in synovial fluid may also influence the protein binding of some of these drugs.

Introduction

A number of studies have shown that non-steroidal anti-inflammatory drugs (NSAID) used in the treatment of rheumatoid arthritis readily enter the synovial fluid (SF). However, in all cases where simultaneous plasma and SF drug concentrations have been measured, the concentrations in SF are lower than those in plasma (Sholkoff *et al.*, 1967; Emori *et al.*, 1973; Thomas *et al.*, 1975; Aylward *et al.*, 1976; Jalava *et al.*, 1977; Farr & Willis, 1977; Glass & Swannell, 1978; Ray *et al.*, 1979; Soren, 1979; Chiccarelli *et al.*, 1980). The pharmacological effects of NSAID are generally considered to be related to the levels achieved in SF (Emori *et al.*, 1973). Howell *et al.* (1972), using ampicillin and cloxacillin, showed that the relative degree of protein binding in plasma and SF was an important determinant of total drug concentration but not of free (unbound) drug concentration.

Our aim was to determine the binding of a number of NSAID and warfarin in simultaneous samples of plasma and SF in patients with arthritis.

Methods

Patients

The study group consisted of nine patients with classical or definite rheumatoid arthritis, according to the American Rheumatism Association criteria and three patients with osteoarthritis. Their mean age was 55.2 ± 17.5 (s.d.) years, with a range of 21–84 years. None of the patients exhibited renal or hepatic dysfunction as shown by usual clinical and laboratory indicators. The mean duration of arthritis was 4.7 ± 2.5 (s.d.) years, with a range of 2 to 10 years. The patients were taking at least one of the following drugs: aspirin, prednisolone, paracetamol, valium, naproxen, D-penicillamine, indomethacin, phenylbutazone, allopurinol, ibuprofen and diflunisal at the time of study. Joint aspiration was performed for diagnostic indications and the study was approved by the Clinical Investigation Committee of Flinders Medical Centre. Samples (20 ml) of blood and SF were collected simultaneously in heparinised tubes, separated and the supernatent stored at −20°C until studied (within 2 weeks). A preliminary experiment on binding of the drugs in fresh plasma and SF in samples stored for 2 weeks (at −20°C) indicated no significant change in the binding of drugs studied.

Methodology

The protein binding of warfarin and seven NSAID, indomethacin, tolmetin, salicylic acid, ibuprofen, flurbiprofen, naproxen and GP53,633, a new basic NSAID, to plasma and SF was determined by equil-

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ibrium dialysis as described previously (Wanwimolruk *et al.*, 1982) using ^{14}C-labelled drugs. GP53,633 (CIBA-Geigy) is a new basic NSAID: 2-tert. butyl-4(5)-phenyl-5(4)-(3-pyridyl)-imidazole, which binds to site I (warfarin site) on albumin. The initial concentration of the drug in the buffer compartment is indicated in Figure 1. The radiochemical purity of free drug (on the buffer side) after equilibrium dialysis was assessed by thin layer chromatography and in each case was > 95% so that interference by poorly bound trace radiochemical impurities could be discounted.

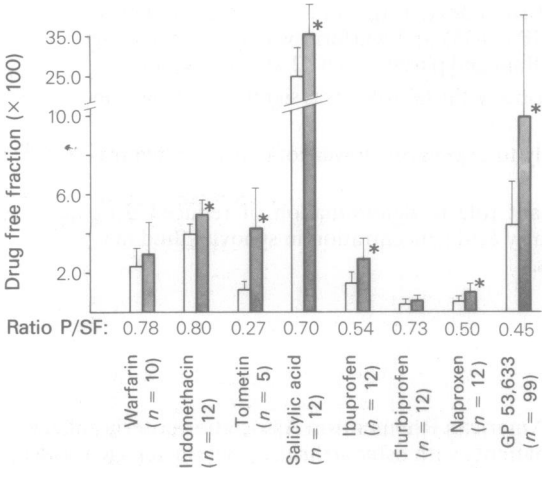

Figure 1 Protein binding of drug in plasma (P, □) and synovial fluid (SF, ■). The initial drug concentrations added were warfarin (2 mg/l), indomethacin (2.5 mg/l), tolmetin (20 mg/l), salicylic acid (200 mg/l), ibuprofen (40 mg/l), flurbiprofen (5 mg/l), naproxen (50 mg/l) and GP53,633 (4 mg/l). * $P < 0.05$.

Total protein and albumin concentrations were measured with the Biuret reagent and bromocresol green reagent, respectively. Free fatty acid concentration was estimated by the method of Duncombe (1964). Sulphydryl group concentrations were measured spectrophotometrically with Ellman's reagent, within 3–4 h of the sample being collected, by a modified method described previously (Pickup *et al.*, 1980).

Statistical analysis of the difference between observations was assessed by unpaired Student's *t*-test.

Results

Concentrations of sulphydryl groups, albumin, total protein and fatty acids in plasma (P) were all considerably higher than in the corresponding synovial fluid (SF) ($P < 0.001$). The mean P/SF ratios varied from 1.54 for sulphydryl concentration to 2.47 for fatty acid concentration.

The binding of indomethacin, tolmetin, salicylic acid, ibuprofen, naproxen and a new NSAID, GP53,633, was significantly lower in SF than in plasma ($P < 0.05$). However, the binding of warfarin and flurbiprofen was not significantly different in SF and plasma (Figure 1). The ratios of drug free fractions in plasma compared to SF are also shown in Figure 1 and ranged from 0.27 for tolmetin to 0.80 for indomethacin. In all cases except for flurbiprofen ($r = 0.39$, $P > 0.05$) the free fraction in plasma was significantly correlated with the free fraction in SF ($r = 0.56$ to 0.76, $P < 0.05$).

In an attempt to define the biochemical parameters that might explain the lower protein binding in SF, the P/SF ratios of drug free fraction and various biochemical parameters (sulphydryl groups, albumin, total protein and fatty acids) were subjected to linear regression procedures. The correlation coefficients are shown in Table 1. None of the drug binding ratios showed a significant correlation with the sulphydryl concentration ratio. For most of the drugs studied, the ratio (P/SF) free fractions show an inverse correlation with the albumin concentration ratio. This correlation was statistically significant for warfarin, indomethacin, salicylic acid, ibuprofen and naproxen. Correlations with total protein were similar to those with albumin. Fatty acid concentration ratios (P/SF) showed a significant negative correlation with the binding ratios in the case of warfarin, indomethacin and GP53,633.

Table 1 Correlations between ratios (P/SF) of drug free fractions and ratios (P/SF) of biochemical parameters. Values given are the simple correlation coefficient (*r*).

Ratio (P/SF) of parameter	*Ratio (P/SF) of drug free fraction*							
	Warfarin	*Indomethacin*	*Tolmetin*	*Salicylic acid*	*Ibuprofen*	*Flurbiprofen*	*Naproxen*	*GP53,633*
Sulphydryl	−0.19	−0.18	−0.23	−0.36	−0.28	−0.28	−0.53	−0.28
Albumin	−0.69*	−0.65*	−0.60	−0.58*	−0.71**	−0.20	−0.72*	−0.64
Total protein	−0.74*	−0.64*	−0.42	−0.59	−0.62*	−0.29	−0.61	−0.64
Fatty acids	−0.64*	−0.78**	−0.20	−0.23	−0.44	−0.30	−0.24	−0.81*

Significant correlation: * $P < 0.05$; ** $P < 0.01$

Discussion

Drug binding in SF as well as in plasma is an important determinant of drug distribution between plasma and SF and of the total concentration of drug in SF. Only free (unbound) drug diffuses across the synovial membrane and binds to target receptors to produce a pharmacological effect.

The protein binding of the drugs evaluated in this study was significantly lower in SF than in plasma ($P < 0.02$), except for warfarin and flurbiprofen ($P > 0.05$). These results are in agreement with previous studies reporting lower binding of salicylate (Rosenthal *et al.*, 1964; Soren, 1979; Trnavska & Trnavsky, 1980) and ibuprofen (Whitlam *et al.*, 1981) in SF than in the corresponding plasma. It has been suggested that the lower total drug concentrations in SF than in plasma is due to lower protein binding in SF. In agreement with this, *free* concentrations of ibuprofen (Whitlam *et al.*, 1981) and cloxacillin (Howell *et al.*, 1972) were found to be the same in plasma and SF. The present results show that the protein binding of a number of drugs is substantially lower in SF than in plasma and suggest that free drug concentrations should be measured in studies of the pharmacokinetics of drugs in SF.

The variability in the ratio drug free fraction in plasma compared to free fraction in SF was surprisingly large, ranging from 0.27 for tolmetin to 0.80 for indomethacin (Figure 1). Rheumatoid arthritis (RA) is a systemic disease and is associated with hypoalbuminaemia (Ballantyne *et al.*, 1971) and possibly with an altered amino acid composition of albumin (Denko *et al.*, 1970). Some studies have reported reduced drug protein binding in serum from RA patients (Selley *et al.*, 1978) but we have recently shown that the binding of warfarin and four acidic NSAID (indomethacin, salicylic acid, ibuprofen and flurbiprofen) was similar in patients with RA and osteoarthritis (Wanwimolruk *et al.*, 1982). Albumin and total protein concentrations have been reported to be lower in SF than in plasma (Ropes *et al.*, 1954; Lorber *et al.*, 1971; Ott & Meier, 1978) and it has been suggested that this accounts for the lower drug binding in SF (Rosenthal *et al.*, 1964; Howell *et al.*, 1972).

For the patients reported in this study, SF albumin and total protein concentrations were 62% of those in plasma and the ratio of albumin concentration in SF versus plasma was a significant determinant of the relative binding of these fluids of five of the eight drugs studied (Table 1). The importance of albumin concentration was confirmed by comparing drug binding in two albumin solutions of different concentrations. One solution was made up to the mean plasma albumin concentration for the 12 patients studied and the other to the mean SF albumin concentration. The difference in binding between these albumin solutions was very close to that observed for the plasma and SF samples for all drugs except tolmetin and GP53,633. Thus the differences in drug binding between plasma and SF could largely be explained by differences in albumin concentration.

Part of the variability in binding and binding ratios may have been contributed to by other protein bound drugs being taken by some patients. Such effects, however, should be similar in plasma and synovial fluid.

Another important determinant of drug protein binding in plasma is fatty acid concentration. Addition of fatty acids to albumin can increase binding of some drugs such as warfarin (Birkett *et al.*, 1977) and indomethacin (Wanwimolruk *et al.*, 1982). Free fatty acid concentrations in joint fluid were markedly lower than in plasma and the difference was greater than could be accounted for by the lower albumin and total protein concentrations. As expected, the P/SF fatty acid concentration ratio was a significant determinant of the binding ratios for warfarin, indomethacin and GP53,633 (Table 1).

The concentration of serum sulphydryl (SH) groups is decreased in patients with RA and the extent of the decrease reflects disease activity (Lorber *et al.*, 1971). SH group concentrations observed in the 12 arthritis patients in this study (397 ± 56 μM) were considerably lower than those observed in normal subjects by us (590 ± 41 μM, $n = 4$) and by others (Lorber *et al.*, 1971). SH group concentrations in SF have not been reported before but in this study were 65% of the plasma concentration, a reduction very similar in extent to that observed for albumin and total protein which probably accounts for most SH groups in plasma. SH concentration did not however seem to be a determinant of relative drug binding in plasma and SF (Table 1).

In conclusion, this study has shown that NSAID and warfarin are more highly bound in plasma than in SF. This difference may explain the lower total concentrations found for a number of drugs in SF than in plasma. A lower albumin concentration is the major determinant of reduced drug binding in SF compared to plasma.

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