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In-vitro protein binding interaction between a metabolite of triflusal, 2-hydroxy-4-trifluoromethylbenzoic acid and other drugs

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Abstract—2-Hydroxy-4-trifluoromethylbenzoic acid (HTB) is the main active metabolite of triflusal, an antiplatelet drug. The in-vitro binding of HTB to human serum was studied in the presence of different drugs. The results indicate that no statistically significant changes are observed in the HTB binding in the presence of caffeine, theophylline, glisentide, enalapril, cimetidine or warfarin. The free fraction of HTB increases significantly in the presence of the non-steroidal anti-inflammatory drugs studied: diclofenac, ibuprofen, indomethacin, naproxen, piroxicam and salicylic acid. At high concentrations, HTB displaces these anti-inflammatory drugs and also glisentide and warfarin from their protein binding sites.

Triflusal (2-acetoxy-4-trifluoromethylbenzoic acid) is a salicylate with a significant activity as an inhibitor of platelet aggregation in man (Rutllant et al 1977; Domínguez et al 1985). In clinical trials conducted with daily doses of 600 and 900 mg, triflusal is effective in the treatment and prevention of various thromboembolic diseases (Martí et al 1979; Gala et al 1986; Guiteras et al 1989). Triflusal is rapidly metabolized ($t_{\frac{1}{2}}$, 30 min) to 2-hydroxy-4-trifluoromethylbenzoic acid (HTB) (Ramis et al 1990). Studies conducted in-vitro, ex-vivo and on healthy volunteers have shown that HTB presents a dose-dependent antiplatelet activity (Albors et al 1987; De la Cruz et al 1988). Unlike triflusal, HTB has an elimination half-life of over 24 h, and its plasma concentrations are 200 $\mu g\ mL^{-1}$ for a dosage regime of 300 mg three times a day and 150 $\mu g\ mL^{-1}$ for a regime of 600 mg twice a day (Ramis et al 1990). Recent studies show that HTB binds to plasma albumin in a percentage of over 90% in the rat and man (Mis et al 1992).

The degree of a drug's binding to albumin can vary in the presence of other compounds which also bind to protein. This displacement can give rise to changes in the distribution, elimination kinetics and pharmacological action of the drug. This study examined the in-vitro interactions in the binding to plasma proteins of HTB and various drugs, after incubation in human serum at high therapeutic concentrations. These drugs have been selected because of their possible simultaneous use in the course of a treatment with triflusal.

Materials and methods

Materials. Triflusal, HTB, diazepam, glisentide, enalapril and salicylic acid were synthesized in the Organic Chemistry Department at the J. Uriach & Co. Research Centre. Caffeine, theophylline, cimetidine, warfarin, diclofenac, ibuprofen, indomethacin, naproxen and piroxicam were purchased in their highest degree of purity (Sigma Chemical Co., St Louis, MO, USA). [^{14}C]Warfarin (sp. act. 181 $\mu Ci\ mg^{-1}$, radiopurity 99·4%) was obtained from Amersham (Amersham International, Buckinghamshire, UK).

Binding studies. The interaction study was performed by incubating HTB at concentrations of 100 and 200 $\mu g\ mL^{-1}$ together with the interacting drug in serum at 37°C with the pH adjusted to 7·3–7·5 by a stream of 5% CO_2–95% O_2. The concentration of the interacting drug that was added to the serum corresponded to the highest concentration observed in the therapeutic use of each drug (McEvoy 1989; Benet & Roger 1990). The blood samples were obtained from healthy volunteers who had not taken any medication during the three weeks before the samples were taken. Different pools of serum were obtained by centrifugation and stored at −20°C until they were used.

The fraction of unbound drug (f_u) was determined by ultrafiltration (Mis et al 1992). The degree of retention of the compounds in the system's membrane was first determined by filtration of buffered solutions containing the same concentrations as in the serum samples. No compound was retained in the filter (Amicon Micropartition system MPS-1 with YMT membranes, 10 000 Da cut-off). In the interaction study, all the tests were carried out 5 times, and the results for each concentration are expressed as a mean and its standard deviation.

Determination of the free fraction. The concentration of free (f_u) HTB in serum was determined by HPLC (Ramis et al 1990). The f_u values of glisentide, diclofenac, ibuprofen, indomethacin, naproxen, piroxicam and salicylic acid were determined by HPLC (Applied Biosystems Inc., Foster City, CA, USA) in accordance with the chromatographic conditions described by other authors (Emilsson et al 1986; Lapicque et al 1989; Lee et al 1989; Streete 1989). The radioactivity of the ultrafiltrate with [^{14}C]warfarin was measured in an LS-3800 scintillation counter (Beckman Instruments Inc., Fullerton, CA, USA) after adding 10 mL Optiphase Hisafe II scintillation liquid (LKB Scintillation Products, UK) to each sample.

Statistical analysis. The unpaired *t*-test was used for comparing the interactive drug sample with its own control. For comparison amongst more than two groups, analysis of variance was used. Tukey's multiple comparison method was used to discern significant differences. $P < 0.05$ was taken as the minimum significance criterion.

Results

In the interaction study, it was observed that the free fraction of HTB increases significantly ($P < 0.05$) in the presence of therapeutic concentrations of diclofenac, ibuprofen, indomethacin, piroxicam, naproxen and salicylic acid for a total concentration of 100 and 200 $\mu g\ mL^{-1}$ of HTB (Table 1). The free concentration of HTB remained unchanged in the presence of caffeine, theophylline, glisentide, enalapril, cimetidine and warfarin.

The effect on the binding of different drugs to plasma proteins in the presence of a total concentration in the serum of 100 and 200 $\mu g\ mL^{-1}$ HTB is shown in Table 2. In the presence of HTB (100 $\mu g\ mL^{-1}$) there is a statistically significant displacement of diclofenac, ibuprofen, naproxen and piroxicam in their binding to the proteins. The displacement of warfarin, glisentide and indomethacin is not statistically significant at this total concentration of HTB. At a total concentration of HTB in the serum of 200 $\mu g\ mL^{-1}$, all the compounds studied showed a statistically significant increase of the f_u.

In view of the structural similarity between HTB and salicylic

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Table 1. Free fraction of HTB (100/200 μg mL^{-1}) in human serum in the absence and in the presence of caffeine, theophylline, glisentide, enalapril, cimetidine, warfarin, diclofenac, ibuprofen, indomethacin, naproxen, piroxicam or salicylic acid.

Interacting drug (μg mL^{-1})	Free fraction of HTB (100 μg mL^{-1})		Free fraction of HTB (200 μg mL^{-1})	
	Absence of interacting drug (%)	Presence of interacting drug (%)	Absence of interacting drug (%)	Presence of interacting drug (%)
Caffeine (2)	0·62±0·09	0·63±0·04	1·25±0·12	1·25±0·12
Theophylline (15)	0·53±0·07	0·76±0·05	1·36±0·11	1·41±0·14
Glisentide (0·30)	0·62±0·09	0·55±0·06	1·25±0·12	1·29±0·09
Enalapril (0·25)	0·62±0·09	0·64±0·01	1·24±0·12	1·37±0·13
Cimetidine (2)	0·70±0·10	0·71±0·05	1·14±0·15	1·39±0·33
Warfarin (1)	0·62±0·09	0·54±0·15	1·24±0·12	1·28±0·09
Diclofenac (10)	0·67±0·14	1·39±0·03	1·33±0·02	2·15±0·05
Ibuprofen (80)	0·67±0·14	1·34±0·03	1·33±0·02	4·27±0·15
Indomethacin (10)	0·67±0·14	1·16±0·04	1·33±0·02	1·84±0·30
Naproxen (80)	0·67±0·14	0·98±0·07	1·33±0·02	3·15±0·04
Piroxicam (10)	0·43±0·03	1·34±0·03	1·14±0·02	1·84±0·06
Salicylic acid (100)	0·53±0·27	1·2±0·07	1·14±0·10	3·04±0·33

Mean values ± s.d.

Table 2. Free fraction in plasma of different drugs tested in the presence of HTB.

Drug (μg mL^{-1})	Free fraction (%) HTB (μg mL^{-1}) 0	100	200
Warfarin (1)	2·22±0·11	2·70±0·41	5·97±0·57
Glisentide (0·4)	2·27±0·36	2·85±0·66	3·37±0·30
Diclofenac (10)	0·67±0·06	1·09±0·14	1·46±0·28
Ibuprofen (80)	0·70±0·12	2·32±0·14	3·05±0·14
Indomethacin (10)	2·56±0·37	2·90±0·72	4·10±0·40
Naproxen (80)	0·20±0·02	1·43±0·02	2·21±0·06
Piroxicam (10)	0·77±0·06	2·86±0·87	3·70±0·34

Mean values ± s.d.

Table 3. Free fraction (%) of salicylic acid in the presence of HTB.

HTB (μg mL^{-1})	Total salicylic acid (μg mL^{-1}) 100	200
0	12·54±1·39	16·25±1·26
100	25·81±0·45	33·62±0·10
200	31·28±3·84	36·48±1·37
400	39·23±2·39	45·44±3·14

Mean values ± s.d.

acid, a more detailed study was conducted on the interaction of the former with salicylic acid using various concentrations of both compounds (Table 3). In all cases, salicylic acid is displaced from its binding to the serum proteins, with the percentage of displaced drug increasing significantly ($P<0·05$) as the HTB concentration increases.

Discussion

The variability of the control values observed in Tables 1 and 2 is explained by the utilization of different pools of plasma. The results show that of the drugs tested, only non-steroidal anti-inflammatory drugs (NSAIDs) salicylic acid, ibuprofen, indomethacin, naproxen and piroxicam, displace HTB from its binding to albumin. In the presence of therapeutic concentrations of these NSAIDs, the free concentration of HTB increases between 1·2 and 3·2 times. This increase is similar for the two concentrations of total HTB in plasma studied. HTB also displaces the NSAIDs from their binding to albumin. The f_u values observed for the interacting drugs in plasma in the absence of HTB are similar to those described in the literature (McEvoy 1989; Benet & Roger 1990). Indomethacin, piroxicam and ibuprofen show a displacement from their free concentration of between 1·2 and 3 times in the presence of a total concentration of 100 and 200 μg mL^{-1} HTB. The largest displacement is shown by naproxen with an increase of 7–11 times in its f_u. The free fraction of salicylic acid at two different drug concentrations and in the presence of HTB in the concentration interval from 100 to 400 μg mL^{-1} increases between 2 and 3·3 times. In this study, it is observed that the displacement of salicylic acid by HTB is dependent on the concentration over the range studied.

Acid compounds such as NSAIDs and HTB have a high affinity for albumin (Sellers & Koch-Weser 1971; Mis et al 1992), binding to this protein generally being over 90%. In addition, the affinity constants of these compounds for the primary binding site are similar, of the order of 104–105 M^{-1} (Lin et al 1987). These data and the fact that there is a mutual displacement between HTB and the NSAIDs suggest that they compete for the same albumin binding sites and that they displace among themselves in different percentages, depending on their affinity constant for the protein and the total concentration of the displacing agent.

The free concentration of warfarin increases at concentrations of HTB in the serum of 200 μg mL^{-1}. The risk of this interaction must be taken into account, since as the free fraction of warfarin increases in the presence of high concentrations of HTB, it could give rise to an increase in potency of the anticoagulant effect of the drug. However, it was observed in the presence of acid drugs such as ibuprofen, indomethacin, naproxen and diflunisal that, although warfarin was displaced from its binding to albumin, no clinically important increase in the degree of hypoprothrombinaemia was detected (Penner & Abbrecht 1975; Jain et al 1979; McElnay & D'Arcy 1980). To explain the minor clinical incidence of the warfarin displacement due to the NSAIDs, Sellers (1978) suggested that the increase of the free fraction of warfarin is transient because it is compensated by the process of elimination and redistribution of the drug. Sellers concluded that for a unique interaction mechanism, the displacement of the

binding to plasma proteins is important if there exists an additional decrease in plasma clearance. In fact these processes can take place quite readily, and drugs which compete with others for binding sites to proteins can also compete for the elimination and distribution processes (Gillette 1973).

The drug interaction with sulphonylureas can cause hypoglycaemia because of the displacment in the binding to the plasma proteins (Griffin & D'Arcy 1984; George et al 1990). HTB slightly increases the concentration of glisentide, a sulphonylurea which is clinically similar to glibenclamide (Marín & Morell 1975), by 1·14 and 1·49 times depending on the total HTB concentration in plasma. Glibenclamide presents a lower non-competitive displacement with respect to acid drugs, which corresponds to its binding parameters and a low incidence of possible adverse effects (Brown & Crooks 1975). The small increase in the free fraction of glisentide, even at high HTB levels, suggests that glisentide could present a similar behaviour to that of glibenclamide.

In conclusion, HTB is an active metabolite which binds to proteins. In the in-vitro interaction studies, HTB displaces warfarin, glisentide and non-steroidal anti-inflammatory drugs from their binding to albumin. Considering the plasma levels of HTB that can be reached during the clinical use of triflusal (Ramis et al 1990), interactions in the binding to plasma proteins could occur if these drugs are administered together with triflusal at therapeutic doses.

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