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Letter to the Editor

NaF and two other esterase inhibitors unaffect acetyl salicylic acid enzyme hydrolysis

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Prevention of chemical or enzyme hydrolysis of drugs such as esters, amides and carbamates during analytical manipulation is a mandatory need in pharmacokinetics (Stella 1990). This hydrolysis occurs through the action of non-specific esterases which are present in blood, plasma and tissues, and it is common to add NaF or other esterase inhibitors to plasma during extraction procedures (Mays et al 1984). We have investigated the enzyme hydrolysis of acetyl salicylic acid as a model for evaluating the inhibitory effect of sodium fluoride (NaF), diisopropyl-fluorophosphate (DFP) and 1,5 bis(4-allyl dimethyl ammonium phenyl)-pentan-3-one dibromide (ADAPP).

Materials and methods

A pool of plasma was obtained from heparinized blood drawn from 15 male rats (Sprague-Dawley, 200 g) killed by decapitation.

One millilitre of fresh plasma diluted with Na_2HPO_4 0·2 M buffer at pH 8·6 in a 1:1 ratio was added to 1·1 μmol mL^{-1} of acetyl salicylic acid and to each of the different inhibitors tested at the concentrations shown in Table 1. The mixture was incubated at 37°C for 0, 30, 60, 90, 120 and 180 min. The reaction was stopped by adding 0·5 mL 1 M HCl and the sample was extracted with ethylacetate. After stirring and centrifuging, the organic phase was separated, evaporated under N_2, reconstituted with 100 μL acetonitrile and injected into the analytical column. Each test was carried out in duplicate.

The hydrolysis rate was monitored, evaluating both acetyl salicylic acid and salicylic acid, by HPLC under the following conditions. A LiChrosorb RP 18 column (5 μm 250 × 4 mm i.d.), a 10 μL loop, a mixture of H_2O + 1% acetic acid-acetonitrile (1:1) at a flow rate of 1 mL min^{-1} and 3-methyl benzoic acid as an internal standard were used. Detection was at 238 nm. Retention times were as follows: acetyl salicylic acid 4·56 min; salicylic acid 5·11 min; internal standard 6·24 min. The assay proved to be linear in the range 1–200 μg mL^{-1} for both analytes, with intra-assay %CV of 1·29 for acetyl salicylic acid and 1·35 for salicylic acid. The lowest detectable concentration was 0·5 μg mL^{-1} for both analytes.

The decrease of acetyl salicylic acid concentration over time was linearized according to an exponential function using linear regression. The slopes measured in the various tests were compared according to the parallelism method to ascertain any statistically significant differences.

Results and discussion

NaF depletes metallic ion Mg^{2+}, the organophosphorous derivative DFP irreversibly phosphorylates the enzyme and the quaternary derivative ADAPP reversibly binds its active site, thus covering three different enzyme inhibition mechanisms (Taylor 1990).

In the analytical conditions selected in this investigation, plasma esterases hydrolyse acetyl salicylic acid at a rate that seems to be unaffected by any of the three substances added (Table 1).

Table 1. Hydrolysis of acetyl salicylic acid in plasma (200 μg mL^{-1}) in the presence of esterase inhibitors.

Inhibitor	$t_{\frac{1}{2}}$ (min)	r^2
NaF (μg mL^{-1})		
5	59	0·920
10	85	0·830
15	68	0·970
DFP (μg mL^{-1})		
200	86	0·960
ADAPP (μg mL^{-1})		
600	64	0·930
No inhibitor	82	0·915

We conclude that NaF and other esterase inhibitors are not always effective for all substrates; in fact they completely failed in inhibiting enzyme hydrolysis of acetyl salicylic acid. Acetyl salicylic acid, when administered orally, gives rise to both parent drug and the hydrolysate in plasma, so a carefully validated procedure is required in order to avoid any conversion during analytical manipulations (Bochner et al 1988). In such cases a rapid deproteinization method avoids enzyme hydrolysis and manipulation at 0°C avoids chemical hydrolysis (Marzo et al 1990); these precautions would therefore be sufficient without the addition of esterase inhibitors.

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