Enhancement by sodium salicylate of the blood glucose lowering effect of chlorpropamide—drug interaction or summation of similar effects?

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1 The ability of sodium salicylate (3 g) to enhance the blood glucose lowering action of chlorpropamide (200 mg) has been confirmed in healthy male volunteers who received an oral test dose of 50 g glucose. Salicylate raised the plasma concentration of insulin and lowered that of cortisol but did not alter the concentration of chlorpropamide.

2 The area under the blood glucose concentration-time curve was used as the measure of drug response and the significance of drug effects was assessed by analysis of variance. In one study on five volunteers the effect of combining salicylate and chlorpropamide was additive. In a second study on six volunteers 200 mg chlorpropamide, 3 g sodium salicylate and 100 mg chlorpropamide + 1.5 g salicylate were equi-effective.

3 The enhancement of chlorpropamide action by salicylate in this single dose study is consistent with the summation of similar effects. It is not necessary to postulate an interaction.

Keywords chlorpropamide sodium salicylate hypoglycaemia

Introduction

The enhancement of chlorpropamide induced hypoglycaemia by salicylate is well recognised (American Pharmaceutical Association, 1976) although there are few reports of resultant problems in patients with diabetes mellitus. Salicylate may have contributed to prolonged unconsciousness (Cherner *et al.*, 1963) in one patient receiving tolbutamide. Logie *et al.* (1976) investigated the influence on the quality of diabetic control of drugs with a 'potential to interact'. Salicylates were the most frequently encountered drugs in this category and the frequency of hypoglycaemic episodes was one of the recorded measures of diabetic control. There was however no evidence of impaired control in patients who received both sulphonylureas and salicylates.

Competition for renal tubular secretion (Stowers *et al.*, 1959) and for binding sites on

plasma protein (Wishinsky *et al.*, 1962; Hansen & Christensen, 1977; Davies, 1981) have been suggested as mechanisms for an interaction. It has long been recognised however that salicylates acting alone can increase glucose tolerance (Ebstein, 1876; Williamson, 1901; Ingle, 1950; Field *et al.*, 1967). Suggested mechanisms include (a) increased uptake of glucose by muscle (Randle & Smith, 1958), (b) reduced production of fatty acids by adipose tissue (Fang *et al.*, 1968) and (c) inhibition of gluconeogenesis (Woods *et al.*, 1974). These in turn could be direct effects of salicylate or mediated by increased insulin release (Field *et al.*, 1967; Giugliano *et al.*, 1978; Chen & Robertson, 1979) or antagonism of corticosteroid (Smith, 1952).

Enhancement of hypoglycaemia by the summation of similar effects would therefore be

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expected from the combination of salicylate with chlorpropamide. It may not be necessary to postulate an interaction.

Methods

Subjects

The University of Manchester Ethics Committee gave its approval. Subjects were questioned to reveal any relevant medical history; one was excluded because of a suspicion of peptic ulceration. Blood was taken to screen for renal or hepatic disorder or evidence of chronic blood loss. A consent form was signed after the purpose and hazards of the studies had been explained.

Five healthy male laboratory workers volunteered for the first study. Age 30–38 years; body weight 64–76 kg; fasting blood glucose 3.7–4.8 mmol l⁻¹. One withdrew after experiencing tinnitus with sodium salicylate. The data from his earlier experiments was discarded and a substitute was found. The second, confirmatory study was carried out in six healthy male student volunteers. Age 19–25 years; body weight 60–89 kg; fasting blood glucose 2.9–4.4 mmol l⁻¹. All subjects were non-smokers receiving no medication.

Experimental design

On each experimental day the subject presented at the laboratory after an overnight (10 h) fast during which he had unlimited access to unsweetened, non-alcoholic drinks. A venous cannula was placed in a forearm vein and samples were removed through a three-way tap. Patency was maintained with heparinised saline. Fifty g of glucose in water was administered at 0 min. Samples of venous blood were taken at -30, 0, 5, 10, 20, 25, 30, 60, 90, 120 and 150 min. These were split into aliquots, treated with appropriate anticoagulant and analysed immediately or centrifuged and stored as plasma at -20° C for analysis later.

In each study each subject received four glucose tolerance tests preceded by different drug treatments. The interval between experimental days was not less than 1 week.

Drug treatments

The sequence of drug treatments was different in the two studies. In the first a fixed sequence was followed in order to observe the response to single drugs before exposing subjects to the combination. This precaution proved unnecessary. No subject experienced symptoms attributable to hypoglycaemia.

The treatments were: (a) no drug (control); (b) 200 mg chlorpropamide as tablets of Diabinese (Pfizer Ltd) with water at -90 min; (c) 3 g sodium salicylate in water at -30 min; and (d) the combination of chlorpropamide and salicylate at the above doses and times.

The dose of sodium salicylate was lower than the largest doses used by the early investigators (Williamson, 1901) since an intermediate effect was required. This had to be large enough to be measurable with salicylate alone yet small enough to avoid severe hypoglycaemia in the event of an interaction with chlorpropamide. The provisional choice of 3 g proved adequate and safe in the first subjects exposed and was therefore retained.

In the second, confirmatory study the doses of chlorpropamide and sodium salicylate were cut by half to 100 mg and 1.5 g, respectively for the combined experiment. This was done to test a null hypothesis and not because of adverse effects. In other respects the treatments were as before but they were administered according to a latin square design.

Chemical analysis

Blood glucose was measured by a glucose oxidase method (Richardson, 1977). At a concentration of 5 mmol l⁻¹ the coefficient of variation (CV) was 2.0% within batch and 2.4% between batches. Plasma insulin was measured by competitive, protein binding, radioimmunoassay (Wellcome RD 12); between batch CV 7.5% at 65 m units l⁻¹. Plasma cortisol was measured by the double antibody method of Kane (1979); between batch CV 7.7% at 107 nmol l⁻¹.

Plasma salicylate (after treatments c and d) was measured by application to an Auto Analyzer II (Technicon Instruments Ltd) of the ferric chloride method of Trinder (1954). The mean recovery from serum was 83%; within batch CV 1.5% at 100 mg l⁻¹. Interference from chlorpropamide was not a problem even at higher concentrations than those encountered; 200 mg l⁻¹ salicylate was exaggerated by < 5% in the presence of the same concentration of chlorpropamide.

Plasma chlorpropamide (after treatments b and d) was measured by gas chromatography of a chloroform extract using heptabarbitalone as internal standard. Separation was achieved with 5% OV17 on Chromasorb W-HP (80–100 mesh), Argon 40 ml min⁻¹. Column 230° C, isothermal. Pye 204 two channel chromatograph (Richardson, 1983). Between batch CV 6.5% at 20 mg l⁻¹. Salicylate interference < 0.2 mg chlorpropamide per 100 mg salicylate.

Analysis of data

The area under the blood glucose concentration-time curve (AUC) was calculated by the linear trapezoidal method from 0–150 min. No correction was applied for different zero time blood glucose values on different experimental days.

The AUC glucose data was processed by analysis of variance (Goldstein, 1964). In the first study a 4×5 block design was used to test the significance of differences due to treatments (control and drugs) and to subjects. The administration of treatments in a fixed sequence necessitated the confounding of differences due to days with differences due to treatments. In a second analysis a 2×2 block design was applied to the same data to test the significance of differences due to treatments and to interactions between treatments. In effect this compared the differences due to chlorpropamide (CP) treatment in the absence and in the presence of sodium salicylate (S).

In the second, confirmatory study a paired *t*-test was used to demonstrate a significant difference between CP treatment day and control day. The data for the three drug treatments was then analysed (3×6 block) to test the null hypothesis of no significant difference between drug treatments (namely CP, S and half-dose CP + half-dose S). The balanced latin square design removed the need to confound differences due to days with differences due to treatments.

Results

In the first study on five volunteers the expected enhancement of chlorpropamide action was observed. Chlorpropamide (CP) and salicylate (S) acting alone each reduced the blood glucose concentrations to a comparable extent (Figure 1). The effect was obvious between 30 and 120 min. The combined effect of both drugs (CP + S) was greater and obvious from 0 to 120 min.

Each of the three drug treatments raised the plasma concentrations of insulin (Figure 2). The effect was least obvious with CP alone and most obvious with CP + S combined. The largest changes were in the early peak concentrations. From 60 to 150 min the plasma insulin concentrations after different treatments were indistinguishable.

Each of the three drug treatments was associated with lower plasma concentrations of cortisol (Figure 3) but the combination CP + S had no greater effect than either alone.

Chlorpropamide was detected in plasma at –30 min (1 h after administration). From 30 to

Figure 1 The blood glucose concentration ($\text{mmol l}^{-1} \pm \text{s.e. mean}$) in five healthy volunteers given a 50 g oral glucose load at 0 min. —○— Control values; —□— after 200 mg chlorpropamide (CP) at –90 min; —●— after 3 g sodium salicylate (S) at –30 min; —■— after 200 mg CP at –90 min + 3 g S at –30 min. * $P < 0.05$ on one tail, paired *t*-test applied to difference between treatment and control. The indication of significance has been omitted from some intermediate points on a curve to avoid overcrowding. No further indication is given where $P < 0.01$.

Figure 2 The plasma insulin concentration ($\mu\text{l}^{-1} \pm \text{s.e. mean}$) in five healthy volunteers given a 50 g oral glucose load at 0 min. —○— Control values; —□— after 200 mg chlorpropamide (CP) at –90 min; —●— after 3 g sodium salicylate (S) at –30 min; —■— after 200 mg CP at –90 min + 3 g S at –30 min. * $P < 0.05$ on one tail, paired *t*-test applied to difference between treatment and control. The indication of significance has been omitted from some intermediate points on a curve to avoid overcrowding. No further indication is given where $P < 0.01$.

Figure 3 The plasma cortisol concentration ($\text{nmol l}^{-1} \pm \text{s.e. mean}$) in five healthy volunteers given a 50 g oral glucose load at 0 min. —○— Control values; —□— after 200 mg chlorpropamide (CP) at -90 min; —●— after 3 g sodium salicylate (S) at -30 min; —■— after 200 mg CP at -90 min + 3 g S at -30 min. * $P < 0.05$ on one tail, paired t -test applied to difference between treatment and control.

150 min there was little change in concentration (Figure 4a). The coadministration of sodium salicylate had no apparent effect.

Salicylate was detected in plasma at 0 min (0.5 h after administration). From 20 to 150 min there was little change in concentration (Figure 4b). The co-administration of chlorpropamide had no apparent effect.

More detailed analysis of the blood glucose concentration data was based on the area under the curve (AUC) between 0 and 150 min (Table 1a and b). Table 1a shows the results from the first study. The mean AUC was reduced to a similar extent by CP and S; the combined effect of CP + S was clearly greater. Analysis of variance showed significant differences due to treatments (variance ratio $F = 27.7$, probability $P < 0.01$, degrees of freedom $df = 3$) and due to subjects ($F = 6.5$, $P < 0.01$, $df = 4$).

When the same data were analysed in a 2×2 block design (CP absent and present; S absent and present) a significant effect was observed due to CP ($F = 17$, $P < 0.01$, $df = 1$) and due to S ($F = 17$, $P < 0.01$, $df = 1$) but there was no significant effect due to interaction ($F = 0.7$, $P > 0.05$, $df = 1$).

The AUC glucose data for the second study (Table 1b) was analysed separately. The six volunteers received CP and S as before but the doses in the combination CP + S had been halved. Each drug treatment reduced the mean AUC to a similar extent. The paired t test showed a significant reduction ($t = 2.65$, $P < 0.025$,

Figure 4 The plasma concentration ($\text{mg l}^{-1} \pm \text{s.e. mean}$) of (a) chlorpropamide and (b) salicylate in five healthy volunteers given a 50 g oral glucose load at 0 min. (a) After 200 mg chlorpropamide (CP) as tablets with water at -90 min: —○— CP alone; —●— with the co-administration of 3 g sodium salicylate at -30 min. (b) After 3 g sodium salicylate (S) in water by mouth at -30 min: —○— S alone; —●— with the co-administration of 200 mg chlorpropamide at -90 min. No significant differences were observed.

$df = 5$) in AUC by CP relative to control. Analysis of variance applied to the three drug treatments (control excluded) showed significant differences between subjects ($F = 5.2$, $P < 0.025$, $df = 5$) but not between days ($F = 1.7$, $P > 0.05$, $df = 2$) or drug treatments ($F = 0.2$, $P > 0.05$, $df = 2$).

Thus the response to the combination of 100 mg CP and 1.5 g S was indistinguishable from the response to 200 mg CP or 3 g S acting alone.

Discussion

The ability of sodium salicylate to lower blood glucose (Ebstein, 1876; Williamson, 1901; Ingle, 1950; Field *et al.*, 1967) has been confirmed (Figure 1, Table 1a,b). Similarly, elevation of plasma insulin concentration (Field *et al.*, 1967; Guigliano *et al.*, 1978; Chen & Robertson, 1979) has been confirmed (Figure 2). In addition sali-

Table 1 Influence of chlorpropamide 200 mg (CP), sodium salicylate 3 g (S) and a combination of both (a) 200 mg + 3 g, (b) 100 mg + 1.5 g (CP + S) on the area ($\text{mmol l}^{-1} \text{min}$) under the blood glucose concentration–time curve (0–150 min) after 50 g glucose by mouth to healthy male subjects

Subject	Treatments			
	Control	CP	S	CP + S
(a) 1	630	569	581	389
2	675	684	656	531
3	699	573	555	494
4	819	671	728	513
5	756	593	564	408
Mean	716	618	617	467
(b) 6	570	564	529	574
7	550	541	474	503
8	656	543	528	470
9	755	647	732	635
10	599	590	590	593
11	553	424	404	597
Mean	614	552	543	562

cylate appeared to lower plasma cortisol concentration (Figure 3). Both these hormonal changes are consistent with the hypoglycaemic effect. In terms of plasma concentrations of drug necessary for a similar lowering of blood glucose, salicylate appeared to have about one tenth of the potency of chlorpropamide (Figure 4a, b).

During the course of the glucose tolerance test the presence of salicylate appeared to have no effect on the total plasma concentration of chlorpropamide. No attempt was made however to prolong the experiment in order to detect an effect on chlorpropamide concentration half-time or plasma clearance. The question of whether it is necessary to postulate a drug interaction to explain the enhancement of chlorpropamide hypoglycaemia has been approached rather by a more detailed analysis of the blood glucose response.

The 2×2 analysis of variance for the first study showed no significant effect due to inter-

action; the reduction in blood glucose AUC attributable to CP was not significantly greater in the presence of S ($\text{AUC}_S - \text{AUC}_{CP+S}$) than in its absence ($\text{AUC}_{\text{control}} - \text{AUC}_{CP}$). Allowing for the different 'baseline' the blood glucose response to CP appeared to be uninfluenced by the presence of S. This is not a rigorous test however for nothing is known about the influence of baseline AUC on the size of the CP response.

The chance observation that 200 mg CP and 3 g S were equi-effective in lowering blood glucose AUC (Table 1a) suggested a more rigorous test based on the isobologram (Gaddum, 1959). This is the curve obtained by drawing a line through the combinations of doses of drugs A (say CP) and B (say S) which are equi-effective. Potentiation is revealed by bowing of the curve towards the origin and antagonism by bowing away from the origin. The absence of any interaction is shown by a straight line.

The demonstration in the second study that 100 mg CP + 1.5 g S is equi-effective with 200 mg CP or 3 g S alone (Table 1b) is consistent with a straight line isobologram and the null hypothesis that there is neither potentiation nor antagonism. The same apparent result would be obtained if each of these doses was supra-maximal but the demonstration of a greater blood glucose lowering effect with the combination treatment in the first study excludes this possibility.

It is concluded that in the context of this healthy volunteer single dose study the summation of similar effects is an adequate explanation for the enhancement by sodium salicylate of the blood glucose lowering effect of chlorpropamide. There is no need to postulate an interaction. A confirmatory clinical study would be necessary to justify extrapolation to patients with diabetes mellitus.

We gratefully acknowledge the gifts of reagents and standards for the plasma cortisol assay from Dr J. W. Kane and the gift of chlorpropamide chromatographic standard from Pfizer Ltd.

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(Received 29 August 1985,
accepted 26 February 1986)