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Diflunisal and blood acid base status in the rabbit

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Both diflunisal and acetylsalicylic acid given orally caused respiratory alkalosis and metabolic acidosis. However the fall in standard bicarbonate was much less in diflunisal-treated rabbits and pH in these animals remained elevated for the duration of the experiment. Whilst the severity of the fall in arterialized venous P_{CO_2} was a good indicator of the degree of acetylsalicylic acid intoxication this was not the case for diflunisal as fatalities occurred with trivial changes in P_{CO_2}. Diflunisal toxicity was associated with markedly elevated temperatures. This was not observed in acetylsalicylic acid-treated rabbits.

Diflunisal 5-(2,4-difluorophenyl)salicylic acid is an anti-inflammatory analgesic related to salicylic acid. It has similar pharmacological actions to aspirin and the main advantages claimed are that it has a more potent analgesic effect (Steelman et al 1978), less tendency to produce gastric haemorrhage (De Schepper et al 1978) and a long duration of action so that it need be given only twice daily (Kaklamanis et al 1978).

Diflunisal is not metabolized to salicylic acid; it is mainly excreted in the urine as unchanged or conjugated drug (Tempero et al 1977).

Acute salicylate toxicity produces a characteristic clinical picture (Flower et al 1980) a prominent feature of which is the disturbance of acid-base balance. Initially a respiratory alkalosis is seen, due to direct stimulation of the medullary respiratory centre by salicylate, even though CO_2 production is increased due to the uncoupling of oxidative phosphorylation.

Later, a combination of respiratory and metabolic acidosis is produced. The respiratory acidosis occurs because higher concentrations of salicylate depress the respiratory centre. The metabolic acidosis is caused by the accumulation of acids from three sources: salicylic acid derivatives, sulphuric, phosphoric and other metabolic acids which are not excreted because of renal impairment due to central vasomotor depression, and lactic, pyruvic and acetoacetic acids which are increased because of deranged carbohydrate metabolism. Also, renal excretion of base during the alkalotic phase impairs the ability to buffer these strong acids in the later stages. In man the acid-base changes may be serious enough to require treatment and produce a useful guide to the severity of the intoxication.

Acute toxicity studies of diflunisal have been carried out on several species and LD50 values have been reported for mice, rats and rabbits (Stone et al 1977). Particular attention has been paid to the gastrointestinal effects after single doses. However, no information is yet available on the effects of diflunisal on blood gases, and the signs of toxicity before death were reported to be non-specific.

Therefore, the aim of this study is to define the acid-base changes, if any, that occur following oral administration of diflunisal in overdose to rabbits, and to compare these with the acid-base changes produced by acetylsalicylic acid.

METHODS

Male and female half lop coloured rabbits (3–4·5 kg) were used to measure pH, P_{CO_2} and standard bicarbonate of arterialized venous blood. The method used was similar to that described by Stainthorp et al (1980) with the exception that the blood gas analyser was an IL 213. The analyser was calibrated for pH using IL precision buffers at pH 6·84 and 7·384. The P_{CO2} electrode was calibrated using 2 IL standardized cylinders containing 5% carbon dioxide and 12% oxygen in nitrogen and 10% carbon dioxide in nitrogen respectively. The analyser was maintained at 37 °C. Rectal temperature of the rabbits was measured using a clinical mercury thermometer so that the analyser read out could be corrected for the rabbit's body temperature.

Initially control blood samples were taken at 5 min intervals until a steady state was reached where the pH of consecutive samples did not differ by more than 0·01 units and the P_{CO2} by more than 1 mm Hg (0·133 kPa).

A size 10 FG feeding tube was then passed into the stomach with the aid of a mouth gag and drugs administered in a maximum volume of 30 ml with a syringe. The feeding tube was then flushed with 5 ml of water.

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In the preliminary studies blood samples were taken at 15 min intervals. It became apparent that this was unnecessary and in later experiments blood samples were taken at 2, 4, 6, 12 and 18 h after oral dosing.

Diflunisal and acetylsalicylic acid were suspended in 5% methylcellulose solution. The dose range was 10 to 400 mg kg^{-1} for diflunisal and 20 to 3200 mg kg^{-1} for acetylsalicylic acid. Only 4 rabbits were given the highest dose of acetylsalicylic acid as it became clear that this was a lethal dose.

All blood gas changes were compared with those obtained in vehicle-treated rabbits.

Unless otherwise stated, results are means ± s.e.m. of not less than six experiments and significance was assessed using the Student's *t*-test.

RESULTS

The control values for acid-base measurements in our rabbits were: pH 7·433 ± 0·006 units; P_{CO2} 4·688 ± 0·076 kPa, standard bicarbonate 24·76 ± 0·48 mmol $litre^{-1}$, n = 62. Rectal temperature was 39·5 ± 0·04 °C.

Diflunisal had no significant effects on any of these measurements in doses below 200 mg kg^{-1} and then a dose dependent fall in P_{CO_2} was seen (Table 1) which had not returned to normal by the 18 h reading. A similar persistent dose dependent fall in P_{CO2} was observed in acetylsalicylic acid-treated rabbits (Table 2). The fall in P_{CO_2} was associated with a rise in pH in both groups of rabbits. However, pH returned to control values 6 to 12 h after acetylsalicylic acid but persisted for more than 18 h in diflunisal-treated rabbits. Although a fall in standard bicarbonate occurred with both drugs, the severity was much greater in acetylsalicylic acid treated rabbits (Tables 3, 4).

No rabbits died with 1600 mg kg^{-1} acetylsalicylic acid, but all died within 6 h with twice this dose. This was characterized by rapid falls of P_{CO_2} of 1·3 to 2·0 kPa and variable changes in pH and standard bicarbonate.

Rabbits died with much lower doses of oral diflunisal. Three out of 12 died with 300 mg kg^{-1} and 5 out of 7 died with 400 mg kg^{-1}. Death occurred 6 to 24 h after administration of the drug. Although some of these rabbits exhibited quite marked changes in acid-base balance, some rabbits died with fall of P_{CO_2} as little as 0·266 kPa and no significant change in standard bicarbonate or pH. The routine measurement of rectal temperature for correction of the acid-base measurements showed some surprising changes. Rabbits given 400 mg kg^{-1} diflunisal showed a sudden increase in rectal temperature 6 to 18 h after administration. The mean last recorded temperature in all rabbits before death or at 24 h in the survivors was 2·6 ± 0·4 °C (7). One rabbit which was destroyed after the reading had an increase in rectal temperature of 4·7 °C. These changes were not observed in rabbits dying of acetylsalicylic acid

Table 1. Effect of oral diflunisal on the P_{CO_2} (kPa) of arterialized venous blood from the rabbit. All values are the mean change from pre drug values ± s.e.m. Figures in parentheses indicate the number of observations. Differences in this figure from the 2 h reading are indicative of deaths.

		diflunisal dose		
Time (h)	Vehicle	200 mg kg^{-1}	300 mg kg^{-1}	400 mg kg^{-1}
2	0·067 ± 0·040(6)	−0·289 ± 0·124(9)*	−0·539 ± 0·145(12)*	−1·131 ± 0·242(7)*
4	0·013 ± 0·067(6)	−0·182 ± 0·154(9)	−0·692 ± 0·128(12)*	−1·290 ± 0·267(7)*
6	−0·027 ± 0·080(6)	−0·314 ± 0·249(9)	−0·652 ± 0·149(12)*	−1·224 ± 0·197(7)*
12	0·133 ± 0·146(6)	−0·306 ± 0·146(9)	−0·785 ± 0·278(11)*	−1·463 ± 0·231(6)*
18	−0·027 ± 0·069(6)	−0·321 ± 0·127(9)*	−0·412 ± 0·250(9)	−1·842 ± 0·311(4)*

* $P>0·05$ when compared with vehicle control values.

Table 2. Effect of oral acetylsalicylic acid on the P_{CO_2} (kPa) of arterialized venous blood from the rabbit. All values are the mean change from pre drug values ± s.e.m. Figures in parentheses indicate the number of observations.

Time (h)	Vehicle	400 mg kg^{-1}	800 mg kg^{-1}	1,600 mg kg^{-1}
2	0·067 ± 0·040(6)	−0·234 ± 0·215(7)	−0·154 ± 0·126(8)	−1·290 ± 0·235(6)*
4	0·013 ± 0·067(6)	−0·217 ± 0·263(7)	−0·446 ± 0·132(8)*	−1·676 ± 0·255(6)*
6	−0·027 ± 0·080(6)	−0·057 ± 0·254(7)	−0·730 ± 0·188(8)*	−1·210 ± 0·282(6)*
12	0·133 ± 0·146(6)	−0·065 ± 0·323(7)	−0·557 ± 0·120(8)*	−1·835 ± 0·209(6)*
18	−0·027 ± 0·069(6)	−0·028 ± 0·215(7)	−0·410 ± 0·162(8)	−1·542 ± 0·215(6)*

* $P>0·05$ when compared with vehicle control values.

Table 3. Effect of oral diflunisal on the standard bicarbonate (mmol litre^{-1}) of the arterialized venous blood from the rabbit. All values are the mean change from pre drug values ± s.e.m. Figures in parentheses indicate the number of observations. Differences in this table from the 2 h reading are indicative of deaths.

		diflunisal dose		
Time (h)	Vehicle	200 mg kg^{-1}	300 mg kg^{-1}	400 mg kg^{-1}
2	−0·10 ± 0·41(6)	−2·28 ± 1·62(9)	0·25 ± 0·67(12)	−4·80 ± 1·16(7)*
4	−0·05 ± 0·16(6)	−2·54 ± 1·40(9)	0·69 ± 0·74(12)	−3·10 ± 1·50(7)
6	0·40 ± 0·30(6)	−1·49 ± 1·52(9)	1·41 ± 0·74(12)	−1·80 ± 1·41(7)
12	2·10 ± 1·20(6)	−3·56 ± 1·42(9)*	−0·95 ± 0·82(11)	−1·90 ± 1·51(6)
18	−0·50 ± 0·50(6)	−3·00 ± 1·36(9)	−2·02 ± 0·89(9)	−2·70 ± 1·68(4)

* $P>0·05$ when compared with vehicle control values.

Table 4. Effect of oral acetylsalicylic acid on the standard bicarbonate (mmol litre^{-1}) of arterialized venous blood from the rabbit. All values are the mean change from pre drug values ± s.e.m. Figures in parentheses indicate the number of observations.

Time (h)	Vehicle	400 mg kg^{-1}	800 mg kg^{-1}	1,600 mg kg^{-1}
2	−0·10 ± 0·41(6)	−0·47 ± 1·03(7)	0 ± 2·29(8)	−1·37 ± 1·56(6)
4	−0·05 ± 0·16(6)	−1·80 ± 1·08(7)	−0·14 ± 1·63(8)	−1·35 ± 0·66(6)
6	0·40 ± 0·30(6)	−0·89 ± 1·57(7)	−1·54 ± 1·73)8)	−3·57 ± 0·78(6)*
12	2·10 ± 1·20(6)	−2·43 ± 1·39(7)*	−3·86 ± 1·44(8)*	−6·83 ± 0·67(6)*
18	−0·50 ± 0·50(6)	−2·24 ± 1·29(7)	−4·64 ± 1·66(8)*	−7·12 ± 1·44(6)*

* $P>0·05$ when compared with vehicle control values.

poisoning indeed a slight fall in temperature occurred with most animals. For example, 18 h after 1600 mg kg^{-1} acetylsalicylic acid the mean change in rectal temperature was −0·48 ± 0·23 °C.

DISCUSSION

Rabbits given high doses of acetylsalicylic acid exhibited a decrease in arterialized venous P_{CO2} and an increase in pH characteristic or respiratory alkalosis. The P_{CO_2} remained low but the pH gradually returned to control values after a few hours, giving a state of compensated respiratory alkalosis. This was associated with a metabolic acidosis as reflected by the falls in standard bicarbonate which persisted for the duration of the experiment. Very similar changes in acid-base balance have been observed in adult patients suffering from salicylate poisoning (Proudfoot & Brown 1969).

Fatalities amongst the rabbits given acetylsalicylic acid were associated with a precipitous fall in P_{CO2}. Respiratory acidosis was not observed nor was their a consistent acidaemia (fall in pH). Adult patients show a similar picture and it may be that respiratory acidosis occurs more readily in children (Proudfoot & Brown 1969). Flower et al (1980) stated that toxic doses of salicylate may result in pyrexia due to the uncoupling of oxidative phosphorylation and the dissipation of excess energy as heat. No pyrexia was seen in the rabbits dying of salicylate poisoning although the fatalities occurred during the period of continuous surveillance. Proudfoot & Brown (1969) observed neither hyperpyrexia nor hypopyrexia in adult patients poisoned with salicylate and again it may be that this symptom is age-dependent.

Nevertheless pyrexia was observed in rabbits given high doses of diflunisal. There was a highly significant rise in rectal temperature at the time of the last reading before death. This was recorded despite the fact that death occurred at a time of infrequent observations. Since the pyrexia was an unexpected finding the experiments had not been designed to obtain more information on this aspect of diflunisal's toxicity. However, if pyrexia can be explained by uncoupling of oxidative phosphorylation (Flower et al 1980) it would seem that diflunisal is more potent in this respect than acetylsalicylic acid.

Whilst both diflunisal and acetylsalicylic acid produced respiratory alkalosis this is no way indicated the likelihood of fatalities in the case of diflunisal. Some rabbits died with quite minor changes in P_{CO_2}. Diflunisal also differed from acetylsalicylic acid in that the respiratory alkalosis was apparently uncompensated as the pH of the blood remained elevated. Furthermore, diflunisal produced much less metabolic acidosis and this may be due to the lack of salicylic acid formation during diflunisal's metabolism and elimination (Tempero et al 1977).

Diflunisal was approximately ten times more acutely toxic than acetylsalicylic acid in coloured half lop rabbits. The oral dose of diflunisal causing deaths was somewhat lower than the LD50 of 603(503-788) mg kg^{-1} orally reported by Stone et al (1977) for New Zealand rabbits. This may be a strain variation.

In summary, this study has shown that at least in the rabbit, diflunisal in high doses does cause changes in blood acid base status, but that in contrast to acetylsalicylic acid-treated animals this is not a good predictor of the severity of intoxication. In the rabbit, monitoring of rectal temperature would seem more useful.

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