Correlation of plasma phenformin concentration with metabolic effects in normal subjects

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Summary

1. Circulating concentrations of intermediary metabolites have been measured after administration of 50 mg of phenformin to normal subjects.
2. Phenformin caused a significant increase in blood lactate, alanine and the lactate/pyruvate ratio but did not affect blood glucose or serum insulin concentrations.
3. There was a significant correlation between the increase in blood lactate concentration after phenformin and the plasma concentration of the drug.

Key words: alanine, gluconeogenesis, lactate, phenformin.

Introduction


However, phenformin does not lower the blood glucose concentration of normal subjects (Fajans et al., 1960) unless administration is combined with prolonged fasting (Lyngsøe & Trap-Jensen, 1969). On the other hand, after phenformin administration blood lactate concentration is increased after an overnight fast (Lyngsøe, Bitsch & Trap-Jensen, 1972) and during oral glucose and pyruvate tolerance tests (Fajans et al., 1960).

We have studied the metabolic effects of phenformin in normal subjects and their relationship to plasma phenformin concentration.

Methods

Subjects

Six healthy volunteer subjects (age range 21–61 years; percentage ideal body weight 90–137) were studied. None had evidence of hepatic or renal impairment, as judged by routine liver and renal function tests, and none had a first-degree relative with diabetes mellitus. All gave informed consent and the study was approved by the local Ethical Committee.

Procedure

Each subject was studied on two occasions at least a month apart. On the day of the study, after an overnight fast, an indwelling intravenous cannula was inserted into an antecubital vein at 08.25 hours and at 08.30 hours breakfast was eaten. At the beginning of breakfast, phenformin (50 mg sustained-release capsule) or placebo of identical appearance was taken. The order of the two studies was randomized. Blood samples were withdrawn at 08.30 and 09.00 hours and hourly until 18.00 hours. Lunch was eaten at 12.05 hours and snacks at 10.05 and 15.05 hours, and each subject ate a diet of identical carbohydrate and calorific content.
on the 2 days of the study. Subjects were encouraged to take gentle exercise between samples, but rested for 10 min before each blood sample.

**Blood sampling procedure and assays.** At each sample time approximately 8 ml of blood was withdrawn; 1–2 ml was mixed with ice-cold 5% (v/v) perchloric acid and refrigerated; 3–4 ml was placed in a lithium/heparin tube (Searle Diagnostics, High Wycombe, Bucks, U.K.), centrifuged, and plasma separated immediately and stored at −20°C for plasma phenformin assay. The remainder of the blood was allowed to clot, and serum was separated and stored at −20°C for subsequent insulin assay.

Glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate were assayed in the perchloric acid extract by automated enzymic fluorimetric assays (Lloyd, Burrin, Smythe & Alberti, 1978). Acetoacetate was assayed in the perchloric acid extract by the kinetic, spectrophotometric method (Price, Lloyd & Alberti, 1977). Plasma phenformin was assayed by gas/liquid chromatography (Mottale & Stewart, 1975) and serum insulin by double-antibody radioimmunoassay (Soeldner & Slone, 1965).

**Statistical analysis.** The average concentration during the study for each patient has been used to derive the mean ± SEM for the six subjects. Significant differences between studies have been sought by using Wilcoxon's rank sum test. Significant correlations were sought for plasma phenformin concentration and the differences in concentrations of metabolite on placebo and on phenformin by Spearman's rank correlation.

**Results**

There were no significant differences in fasting metabolite or hormone concentrations on the 2 study days.

The minimum detectable concentration of phenformin in plasma was 0.05 μmol/l and it was not reached in our subjects until 0.5–1.5 h after ingestion of a 50 mg sustained-release capsule. Thereafter concentrations ranged up to 0.29 μmol/l with the mean peak concentration occurring 3.5 h after ingestion (0.19 ± 0.03 μmol/l; mean ± SEM). Plasma phenformin concentration 9.5 h after ingestion was 0.12 ± 0.02 μmol/l.

Overall no significant differences were found between the placebo and the phenformin days for the concentration of blood glucose, serum insulin, blood glycerol, total ketone bodies (sum of 3-hydroxybutyrate and acetoacetate concentrations) or the 3-hydroxybutyrate/acetoacetate ratio.

The mean concentrations of blood lactate, alanine and pyruvate were higher on the phenformin day. These increases were significant for lactate (0.95 ± 0.05 vs 0.77 ± 0.04 mmol/l; P < 0.001), and alanine (0.42 ± 0.02 vs 0.38 ± 0.01 mmol/l; P < 0.01). The increase in blood pyruvate concentration was also significant (0.08 ± 0.01 vs 0.07 ± 0.01; P < 0.01), although proportionately less than the increase in lactate.

![Fig. 1. Scatter diagram of plasma phenformin concentration and change in blood lactate concentration after 50 mg of phenformin orally compared with placebo. Symbols represent individual patients (n = 6).](image-url)
Metabolic effects of phenformin

hence resulting in a significant increase in the lactate/pyruvate ratio (11.3 ± 0.3 vs 10.7 ± 0.7 ± 0.02; P < 0.001). There was no correlation between plasma phenformin concentration and the changes in blood glucose, alanine, pyruvate and the lactate/pyruvate ratio produced by phenformin. In contrast there was a significant correlation between plasma phenformin and the change in blood lactate concentration (rs = 0.41; P < 0.01) (Fig. 1).

Discussion

Beckmann (1966) reported phenformin concentrations of 1.7 μmol/l after 100 mg given orally with a half-life of 3.2 h. Plasma concentrations in the present study are compatible with these results although the time-course is altered by the use of a sustained-release capsule. The present study demonstrates that some of the metabolic changes after phenformin in maturity-onset diabetic subjects (Nattrass et al., 1977) also occur in normal subjects after 50 mg of phenformin. The blood glucose concentration was not changed but the blood lactate, alanine and lactate/pyruvate ratio were significantly increased.

There was a correlation between plasma phenformin concentration and the effect upon blood lactate which has not previously been reported. The results suggest that the effect of phenformin upon blood lactate concentration may precede the effect upon blood glucose.

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References