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SHORT COMMUNICATIONS

Acute effect of benfluorex on glucose metabolism

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Abstract—The antihyperlipidaemic agent benfluorex [1-(3-trifluoromethylphenyl)-2-(2-benzoyloxyethyl)-aminopropane] and its metabolite S422 [1-(3-trifluoromethylphenyl)-2-(2-hydroxyethyl)-aminopropane] were examined for an acute (after 1–2 hr) effect on glucose metabolism in normal rats. Enteral administration of benfluorex (25 mg/kg) did not affect basal plasma glucose and insulin concentrations. However, enteral and intravenous glucose tolerance were modestly improved without enhancing the insulin response to glucose. Hepatic gluconeogenesis from lactate was not acutely altered by benfluorex (25 mg/kg) *in vivo* or by S422 (1 mM) *in vitro*, but S422 (1 mM) slightly reduced (by 11%) hepatic glycogen mobilization *in vitro* after 2 hr. S422 (1 mM) increased (by 47%) glucose oxidation by diaphragm muscle *in vitro*. The effect was additive to that of insulin. Anaerobic glucose metabolism and glycogenesis of diaphragm muscle were not affected by S422. The results suggest that benfluorex can acutely improve glucose tolerance associated with increased glucose oxidation by muscle.

Benfluorex [1-(3-trifluoromethylphenyl)-2-(2-benzoyloxyethyl)-aminopropane*] is a mild suppressor of food intake with blood lipid-lowering properties in hyperlipidaemic states [1]. Chronic treatment with benfluorex has an antihyperglycaemic effect without enhancing insulin concentrations [1–4]. Indeed, insulin concentrations may be reduced in hyperinsulinaemic states [2–5]. The chronic antihyperglycaemic effect of benfluorex can only be attributed in small part to the food intake-suppressing and body weight-lowering effects of the drug, suggesting an independent effect on glucose metabolism [1–6]. To investigate whether benfluorex exerts an acute effect on glucose metabolism and to assess the mechanism involved, this study examines the acute effects of benfluorex and its metabolite S422 [1-(3-trifluoromethylphenyl)-2-(2-hydroxyethyl)-aminopropane] on glucose tolerance, gluconeogenesis, liver glycogen and muscle glucose utilization by normal rats.

Materials and Methods

Animals and chemicals. Male Wistar rats (about 250 g) were maintained at 22 ± 2° with a 12 hr light–dark cycle (0800–2000 hr light) and fed a standard pellet diet (Rat–Mouse Breeding diet, Heygate and Sons, Northampton, U.K.) and tap water *ad lib*. D-[U-^{14}C]glucose (270 mCi/mmol) and L-[U-^{14}C]lactate (sodium salt, 160 mCi/mmol) were from Amersham International (Amersham, U.K.); GOD-perid glucose oxidase reagent was from Boehringer (Lewes, U.K.); and benfluorex chlorhydrate and metabolite S422 were from Institute de Recherches Internationales Servier (Courbevoie, France). Other chemicals were from the Sigma Chemical Co. (Poole, U.K.) and BDH (Poole, U.K.).

Glucose tolerance tests. Enteral glucose tolerance was determined in rats fed *ad lib*. Anaesthesia was induced with sodium pentobarbitone (45 mg/kg i.p.) and maintained with regular supplements of 5 mg/kg. Time was allowed for glucose homeostasis to stabilize [7], and benfluorex (25 mg/kg) or vehicle only (2% methyl cellulose) was administered as an intrajejunal bolus distal to the ligament of Treitz, and gently massaged distally. After 1 hr glucose (2 g/kg in a 20% w/v solution) was administered intrajejunally.

Intravenous glucose tolerance was determined under sodium pentobarbitone anaesthesia in rats fasted overnight. Benfluorex (25 mg/kg) or vehicle only was administered intrajejunally as above and glucose (0.5 g/kg in a 20% w/v solution) was injected 1 hr later into the inferior vena cava at the junction of the renal veins. During both enteral and i.v. glucose tolerance tests, blood samples for plasma glucose and insulin analysis were taken at intervals using a fine needle inserted into the lower inferior vena cava at the entry of the ilio-lumbar veins.

Gluconeogenesis. Rats were fasted overnight and benfluorex (25 mg/kg) or vehicle only (2% methyl cellulose) was administered by intragastric gavage. After 1 hr blood was taken for plasma glucose and lactate assay, and [^{14}C]-lactate (2 μCi/kg) was injected into a tail vein. Further blood samples were taken from the tail 15 and 30 min later. Plasma was deproteinized with 0.2 M barium hydroxide and 0.2 M zinc sulphate, and [^{14}C]glucose was separated using a 2-mL ion exchange column comprising Dowex WGR-2 (upper layer) and Dowex 1X2-400 [8].

In vitro studies were performed using isolated hepatocytes from a fasted rat. Hepatocytes were isolated by the collagenase perfusion method as described previously [9]. Cell viability assessed by trypan blue exclusion was 90%. Cells were preincubated for 15 min at 37° in pregassed (95% O_2:5% CO_2) Krebs–Ringer bicarbonate (KRB) buffer, pH 7.4, with continuous agitation. Test incubations were performed for 1 hr at 37° with approximately 10^7 viable cells continuously agitated in 2 mL of pregassed KRB buffer, pH 7.4, containing 20 mg/mL bovine serum albumin, lactate (10 mM) and pyruvate (1 mM) as gluconeogenic substrates, with and without the benfluorex metabolite S422 (1 mM). Glucose released into the medium was measured.

Glycogenolysis. Liver slices from fed rats were incubated at 37° in pregassed (95% O_2:5% CO_2) KRB buffer, pH 7.4, without gluconeogenic substrate and with and without S422 (1 mM). Tissue samples were analysed for glycogen before and after 2-hr incubations.

Glucose metabolism by muscle. Diaphragm segments from fed rats were incubated at 37° in pregassed (95% O_2:5% CO_2) KRB buffer, pH 7.4, containing 2% bovine serum albumin, 20 mM glucose and 0.5 μCi/mL [U-^{14}C]-glucose, with and without S422 (1 mM) and/or insulin

* Abbreviations: benfluorex, 1-(3-trifluoromethylphenyl)-2-(2-benzoyloxyethyl)-aminopropane; S422, 1-(3-trifluoromethylphenyl)-2-(2-hydroxyethyl)-aminopropane; KRB, Krebs–Ringer bicarbonate.

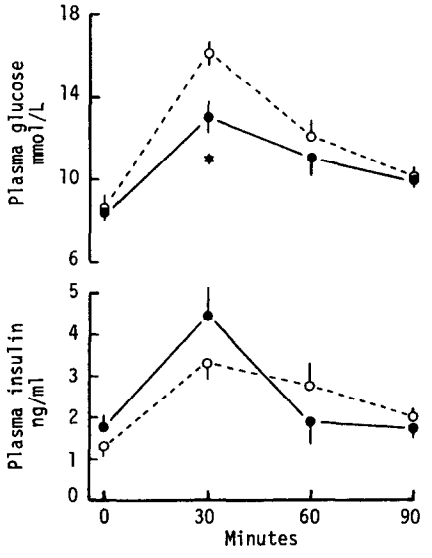

Fig. 1. Effect of benfluorex on enteral glucose tolerance in anaesthetized fed rats. Benfluorex (25 mg/kg) was given as an intrajejunal bolus at −60 min and glucose (2 g/kg) was given as an intrajejunal bolus at 0 min. Benfluorex (●), control (○). Values are means ± SEM, N = 5. * $P < 0.05$ vs control.

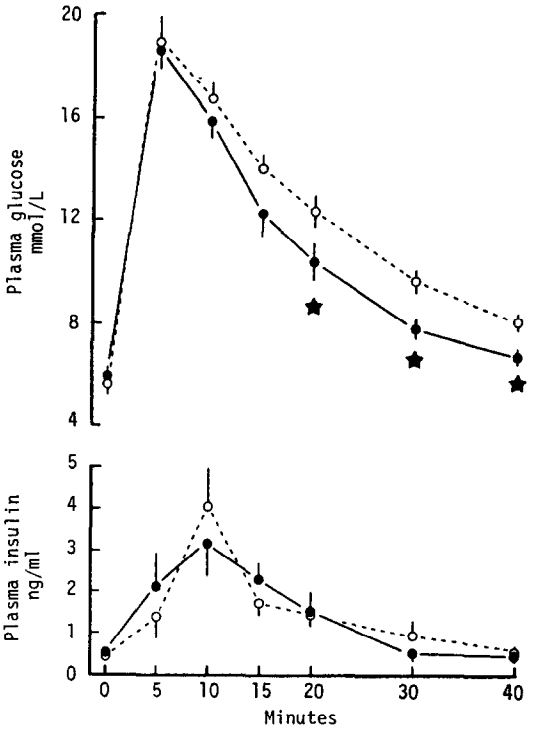

Fig. 2. Effect of benfluorex on intravenous (i.v.) glucose tolerance in anaesthetized fasted rats. Benfluorex (25 mg/kg) was given as an intrajejunal bolus at −60 min and glucose (0.5 g/kg) was given as an i.v. bolus at 0 min. Benfluorex (●), control (○). Values are means ± SEM, N = 5. * $P < 0.05$ vs control.

(1 mM). After 2 hr, $^{14}CO_2$ production, lactate production, and ^{14}C incorporated into glycogen were determined [10].

Analytical methods. Plasma glucose and glucose in the incubation medium were measured by automated [11] and manual [12] glucose oxidase procedures, respectively. Plasma insulin was measured by radioimmunoassay [13] using rat insulin standard and lactate was assayed by a lactate dehydrogenase method [14]. Glycogen was measured by tissue digestion in 1 M KOH, precipitation with ethanol and hydrolysis with H_2SO_4, followed by neutralization with NaOH and manual glucose analysis.

Data are presented as means ± SEM. Groups of data were compared using Student's paired or unpaired *t*-test as appropriate, and differences were considered to be significant if $P < 0.05$.

Results

Glucose tolerance. An intrajejunal bolus of benfluorex (25 mg/kg) did not significantly alter basal plasma glucose or insulin concentrations after 1 hr in fed (Fig. 1) or fasted (Fig. 2) anaesthetized rats. However, the peak plasma glucose response at 30 min after an intrajejunal glucose challenge was reduced (by 19%) in the benfluorex-treated rats, although the plasma insulin response was not altered significantly (Fig. 1). The plasma glucose response to an i.v. glucose challenge was also reduced by benfluorex (mean plasma glucose concentrations at 20, 30 and 40 min were 19% lower), although the plasma insulin response was not altered significantly (Fig. 2).

Gluconeogenesis. Administration of benfluorex (25 mg/kg) by intragastric gavage to fasted rats did not alter plasma lactate after 1 hr compared with rats receiving vehicle only (1.7 ± 0.2 and 2.1 ± 0.2 mmol/L, respectively, mean ± SEM, N = 6). The formation of $[^{14}C]$glucose after i.v. injection of $[^{14}C]$lactate was not significantly affected by the benfluorex treatment (Table 1). Glucose production by isolated hepatocytes was not affected by S422 (1 mM) during 1-hr incubations with lactate and pyruvate. Glucose production (nmol/10^7 viable cells/min, mean ± SEM, N = 6) was 32 ± 1 for control and 29 ± 1 in the presence of S422.

Table 1. Effect of benfluorex on $[^{14}C]$glucose production from $[^{14}C]$lactate in fasted rats

	Plasma $[^{14}C]$glucose (dpm/μmol glucose)	
	15 min	30 min
Control	435 ± 78	511 ± 44
Benfluorex	413 ± 90	476 ± 73

Benfluorex (25 mg/kg p.o.) was given at −60 min, [U-^{14}C]lactate (2 μCi/kg i.v.) was injected at 0 min and plasma $[^{14}C]$glucose was determined at 15 and 30 min.

Values are means ± SEM, N = 5. For comparison, control values in fed rats were 142 ± 24 and 86 ± 12 dpm/μmol glucose (N = 5) at 15 and 30 min, respectively. The control values in fed rats were significantly lower ($P < 0.05$) than in fasted rats.

Glycogenolysis. The glycogen content (μM glucose equivalent/g, N = 6) of liver slices from fed rats was 409 ± 29 (mean ± SEM) before incubation, and decreased to 131 ± 15 after incubation for 2 hr in the absence of gluconeogenic substrate. When S422 (1 mM) was added to the incubation medium the glycogen content decreased

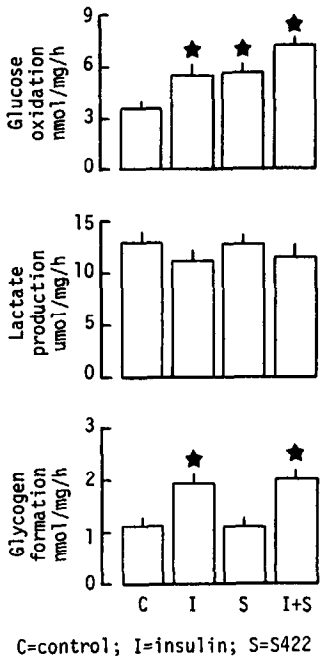

Fig. 3. Effect of S422 (1 mM) on glucose oxidation, lactate production and glycogen formation by diaphragm segments of fed rats incubated for 2 hr with glucose (20 mM) in the absence and presence of insulin (1 μM). Values are means ± SEM, N = 6. * $P < 0.05$ vs control.

significantly less ($P < 0.05$) to 176 ± 13, representing an 11% greater retention of tissue glycogen.

Glucose metabolism by muscle. Diaphragm segments of fed rats showed a 47% increase in glucose oxidation to CO_2 after incubation for 2 hr with 1 mM S422 (Fig. 3). This effect was similar in magnitude to 1 μM insulin, and the effect was additive to that of insulin when both agents were present. Lactate production was not significantly altered, and glycogen formation was increased by insulin but not by S422.

Discussion

Benfluorex is absorbed rapidly. An oral dose achieves maximum circulating concentrations at 1–4 hr [1]. Benfluorex is also metabolized rapidly in the liver. The first metabolite, S422, is metabolically active [1], and this compound was used for *in vitro* experiments because it is readily soluble in physiological media. The concentration of S422 (1 mM) used in these experiments is about 100-fold greater than the maximum circulating concentration of this metabolite after a therapeutic dose of benfluorex.

The present study demonstrates that benfluorex can act acutely (after 1 hr) to improve modestly enteral and intravenous glucose tolerance in normal rats. This is consistent with evidence that chronic benfluorex treatment can improve glucose homeostasis in non-insulin-dependent states of hyperglycaemia in animal models [1, 15] and man [2, 3]. The improvement cannot be attributed to increased insulin secretion and we have shown in other acute studies (unpublished) that benfluorex does not impede intestinal glucose absorption.

The lack of an acute effect of benfluorex on basal glucose and insulin concentrations in normal rats supports the view that this agent exerts an antihyperglycaemic effect without causing excessive lowering of blood glucose concentrations below the normal range [1]. Indeed, there have not been any reports of overt hypoglycaemia during chronic benfluorex treatment. This correlates with present evidence that benfluorex did not affect gluconeogenesis from lactate. A contrary claim that benfluorex acutely and markedly inhibits hepatic gluconeogenesis [16] cannot readily be explained. However, a small reduction in hepatic glucose output may be anticipated due to reduced hepatic glycogenolysis. Similar observations have been made during the culture of hepatocytes with S422 [17]. Hepatic glucose output is raised in diabetic states [18], and there is evidence that improved glucose homeostasis is associated with reduced hepatic glucose output during chronic benfluorex treatment of rats made diabetic by neonatal administration of streptozotocin [15].

The present study provides evidence to suggest that benfluorex can enhance glucose utilization by increasing glucose oxidation in muscle. Although S422 increased glucose oxidation by diaphragm without added insulin, it must be remembered that some insulin would be retained in the interstitial fluid. However, the apparently additive effect of S422 and insulin, and the lack of effect of S422 on glycogenesis, suggest a mode of action that is not directly dependent upon insulin. Increased peripheral glucose disposal and the amelioration of insulin resistance by skeletal muscle have recently been observed after chronic benfluorex administration to rats fed high-fat and high-fructose diets [19]. The lipid-lowering effect of chronic benfluorex treatment in hyperlipidaemic states is likely to contribute to improved glucose homeostasis by improving the glucose–fatty acid (Randle) cycle, as noted with other antihyperlipidaemic agents [20].

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The influence of ebselen on the toxicity of cisplatin in LLC-PK$_1$ cells

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Abstract—LLC-PK$_1$ cells have been used as an *in vitro* model to study the nephrotoxicity of the antitumor drug cisplatin. A concentration-dependent cytotoxicity of cisplatin, measured as lactate dehydrogenase leakage and amount of protein remaining attached to the culture plate, was observed. At a cisplatin concentration of 0.4 mM cell viability was reduced to 21% after 72 hr. Ebselen, a seleno-organic compound capable of forming selenol intermediates through reaction with thiols, was found to protect LLC-PK$_1$ cells against cisplatin-induced toxicity at low concentrations (5–15 μM). The ebselen-induced protection against cisplatin toxicity in this *in vitro* test system apparently correlates well with a similar protection previously observed *in vivo* in mice and rats.

Cisplatin (*cis*-diamminedichloroplatinum II) is an important cytotoxic drug which is used in the treatment of a variety of human neoplasms [1]. However, severe side-effects, notably toxicity to the kidneys, the gastrointestinal tract, the peripheral nerves and the bone marrow lower the therapeutic index of cisplatin [2]. Nephrotoxicity is one of the most important toxicities of cisplatin in humans. The events responsible for the toxicity of cisplatin occur shortly after administration of the drug [3]. Signs of kidney toxicity, however, occur after several days, mainly in the S_3 segment of the proximal tubule [4, 5]. The precise molecular mechanism of cisplatin-induced nephrotoxicity is unknown. In cells, due to the low chloride concentration, cisplatin is hydrolysed to positively charged monoaquo- and diaquo species [6]. These species are probably the active toxicants. Based on *in vitro* experiments it has been suggested that lipid peroxidation is not a major cause in cisplatin-induced nephrotoxicity [7]. The nephrotoxicity of cisplatin has been attributed to binding of platinum to critical protein sulfhydryl groups [8]. Renal brush border enzymes, however, have been excluded as primary targets of toxicity *in vivo* [9]. Alteration of glutathione (GSH*) levels and depression of macromolecule synthesis in the kidney have also been suggested to play a role in cisplatin-induced nephrotoxicity [6].

Several attempts have been made to reduce cisplatin-induced nephrotoxicity [10]. Induction of chloruresis by hypertonic saline infusions, for example, protects against cisplatin nephrotoxicity because this treatment leads to a reduction in the concentrations of cisplatin and reactive metabolites in the kidney, during the first hours after administration of cisplatin [11]. An important alternative approach is the use of chemoprotectors. Most chemoprotectors contain a nucleophilic moiety [12]. Diethyldithiocarbamate, for example, reduces the nephrotoxicity but not the antitumour activity of cisplatin, probably because this compound can selectively remove platinum from platinum–sulfhydryl complexes but not from platinum–DNA adducts [13]. An important problem in the clinical

* Abbreviations: GSH, glutathione; LDH, lactate dehydrogenase.