The effects of flosequinan on regional blood flow in normal man

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- 1 The effects of 100 mg and 200 mg flosequinan on limb, hepatic and renal blood flow were investigated in 14 healthy male volunteers in a placebo controlled double-blind randomised three-way crossover study.
- 2 Heart rate, blood pressure, forearm blood flow and venous capacitance measured by volume plethysmography, were recorded sequentially over 4 h, after oral dosing.
- 3 Apparent hepatic and renal blood flows were estimated 2 h post-dose by indocyanine green dye clearance and clearance of ¹²⁵-iodohippuran respectively.
- 4 Flosequinan produced dose-dependent reductions in resting diastolic blood pressure, accompanied by a rise in heart rate.
- 5 Forearm blood flow increased to a maximum 4 h after 200 mg and this was accompanied by a fall in forearm vascular resistance.
- 6 Hepatic blood flow increased, compared with placebo, after 200 mg flosequinan, accompanied by a fall in hepatic vascular resistance. Renal blood flow remained unchanged but renal vascular resistance fell significantly, compared with placebo, following both doses of the drug.
- 7 In normal man blood flow to the limb, hepatic and renal vascular beds is preserved despite dose-dependent reductions in blood pressure following single doses of flosequinan.

Keywords flosequinan regional blood flow plethysmography liver circulation renal circulation

Introduction

Flosequinan (7-fluoro-1-methyl-3-methylsulphonyl-4-quinolone) is a novel vasodilator drug having balanced effects on both venous and arterial vascular beds. It produces dose-dependent relaxation of human subcutaneous resistance vessels (Richards *et al.*, 1989) and its mode of action is thought to involve a reduced release of or decreased sensitivity to intracellular calcium (Yates, 1988; Yates & Holmes, 1988). It has been suggested that this action may be preceded by an elevation of intracellular cyclic GMP, the magnitude of which is less than that observed with sodium nitroprusside and atrial natriuretic factor, for equivalent relaxant concentrations (Allcock *et al.*, 1988). It has also been observed that the vasorelaxant activity of flosequinan *in vitro* is not associated with stimulation of soluble guanylate cyclase (Yates & Holmes, 1988) but with a significant reduction in inositol monophosphate accumulation, an effect common to vasorelaxants which increase cGMP and cAMP resulting in decreased availability of intracellular calcium (Yates *et al.*, 1989).

In normal subjects, flosequinan has been shown to reduce blood pressure and forearm vascular resistance in a dose-dependent fashion (Cowley *et al.*, 1984; Kerth *et al.*, 1986; Wynne *et al.*, 1985). The drug also exerts a hypotensive effect in patients with essential hypertension, while preserving renal blood flow and glomerular filtration rate (Dupont *et al.*, 1988) and it has also been shown to be a potentially useful vasodilator for the treatment of hypertension persisting despite therapy with β -adrenoceptor blockers and diuretics (Cowley *et al.*, 1987).

Flosequinan also exerts balanced cardiocirculatory effects in patients with NYHA grade III–IV chronic congestive heart failure, which have been shown to be cumulative (Kessler & Packer, 1987; Kessler *et al.*, 1988). Flosequinan improves the exercise tolerance of such patients compared with placebo and such improvement is accompanied by increased skeletal muscle blood flow on exercise (Cowley *et al.*, 1988). The ventilatory and metabolic responses during both submaximal and maximal exercise are also improved (Elborn *et al.*,

1989a,b). It has also been suggested that the efficacy of flosequinan in chronic severe congestive heart failure is comparable with captopril (Cowley *et al.*, 1989).

In an effort to preserve flow to the coronary and cerebral circulations in congestive heart failure, blood flow to the limb, renal and hepatic-splanchnic vascular beds is reduced (Zelis *et al.*, 1975). It has been reported that the acute augmentation of cardiac output seen with flosequinan in such patients is not accompanied by preferential alteration of flow to limb, hepatic or renal vascular beds, at rest (Haas *et al.*, 1989).

There is as yet no published data on the effects of flosequinan on regional blood flow in normal man. We have therefore investigated the effects of 100 mg and 200 mg of oral flosequinan on regional blood flow in 14 healthy male volunteers, in a double-blind, placebo controlled randomised three-way crossover study.

Methods

Subjects

After appropriate clinical and biochemical screening, 14 healthy male volunteers were recruited to the study which was approved by the local ethics committee. Body weight was within 10% of ideal weight as defined by Metropolitan Life Assurance Tables. Exclusion criteria included a recent history of blood donation or loss, subjects positive for hepatitis B surface antigen, a history of atopy, asthma or allergy especially to iodine and a history of hypersensitivity to indocyanine green or 125 -iodohippuran. Subjects with a history of psychotic illness, recent neurosis or attempted suicide or parasuicide were also excluded. Written informed consent was obtained.

Design

Investigations were carried out on each of three study days when flosequinan 100 mg, 200 mg or matching placebo was administered orally in a double-blind randomised fashion. Study days were separated by a minimum washout period of 2 weeks.

Subjects took no alcohol for the 24 h period prior to and following the study period. After a light breakfast at least 1 h earlier subjects attended a supervised investigational area and were placed on bed rest in a semi-supine position. A 20 g intravenous cannula was then inserted into a vein in each forearm for blood sampling and dye injection. After 30 min rest blood was taken for routine haematology and biochemistry. Baseline heart rate, blood pressure, forearm blood flow and venous capacitance were recorded and the study medication given. These measurements were repeated at 30 min intervals up to and including 2 h and again at 4 h. Blood was also taken for flosequinan levels with additional sampling at 3 h. At 2 h apparent hepatic blood flow was estimated by the technique of indocyanine green dye clearance. This was immediately followed by renal blood flow estimation, employing 125 -iodohippuran clearance to measure effective renal plasma flow. The cannulae were removed after 4 h, a light lunch given and the subjects remained under observation for a further 2 h.

Heart rate and blood pressure were measured in the dominant arm by a semi-automatic sphygmomanometer (Hitachi HME-20). The mean of two consecutive readings was recorded, and mean blood pressure was calculated as diastolic blood pressure + $\frac{1}{3}$ pulse-pressure.

Forearm blood flow

Forearm blood flow and venous capacitance were measured in the non-dominant forearm by venous occlusion plethysmography employing mercury-in-silastic strain gauges (Greenfield *et al.*, 1963), and a standard plethysmograph (Medimatic SP2, Hellerup, Denmark). The mean of four consecutive readings was taken for forearm blood flow and forearm venous capacitance was measured at a venous occlusion pressure of 30 mm Hg with the subject in the recumbent position and the forearm supported at the level of the right atrium. Forearm blood flow was expressed in $\text{ml } 100 \text{ ml}^{-1} \text{ tissue min}^{-1}$ and forearm vascular resistance calculated by dividing mean blood pressure by forearm blood flow at each time point and then expressed in resistance units.

Apparent hepatic blood flow

Indocyanine green clearance was used to estimate apparent hepatic blood flow, following a bolus injection of 0.5 mg ICG kg^{-1} (Bax *et al.*, 1980). Samples for estimation of ICG concentration were taken 6 min post-injection and then every 2 min up to and including 14 min. ICG concentration was then estimated spectrophotometrically (Pye-Unicam SP6-500 spectrophotometer, Cambridge, England) and clearance calculated from the product of the apparent volume of distribution and elimination rate constant.

Renal blood flow

ERPF was measured by estimating plasma clearance of 125 -iodohippuran, following a bolus injection of 2 MBq (Lawson, 1986; Shields, 1986). Plasma samples were obtained at 10 min intervals for 60 min post-injection and sample radioactivity measured by gamma counter (Canberra Packard, Pangbourne, England). ERPF was calculated using an in-house BASIC programme (Medical Physics, Leicester Royal Infirmary) on a Sirius micro-computer.

Both ERPF and ICG clearance were converted to renal and hepatic blood flows respectively by dividing by 1-haematocrit, and expressed in $\text{ml min}^{-1} \text{ m}^{-2}$. Organ vascular resistance was calculated by dividing mean blood pressure at 2 h by regional blood flow in l min^{-1} , and expressed in resistance units.

Plasma flosequinan assay

Blood was taken into heparinised tubes, immediately centrifuged at 1500 g for 10 min and the plasma separated and stored at -20°C until analysis. Levels of flosequinan and its principal active metabolite (BTS 53554) were determined by high performance liquid chromatography (h.p.l.c.) employing u.v. detection (Slegowski *et al.*, 1988) and the analysis was performed by The Boots Company PLC, Nottingham, England.

Statistics

All values were expressed as mean (s.e. mean). All statistical tests performed were two-tailed and the null hypothesis was always that the treatments were equivalent.

Repeated measures analysis of covariance was carried out on heart rate, blood pressure, forearm blood flow, venous capacitance and forearm vascular resistance, with the hour 0 value for each investigational day fitted as covariate. Factors were included for treatment, carry-over period, volunteer, time post-dose and interaction of time with treatment, period and volunteer. Due to non-normality of data, the analyses for systolic blood pressure and forearm vascular resistance were conducted following a rank transformation (Conover & Iman, 1981).

Analysis of covariance was carried out on hepatic and renal blood flows and vascular resistances with factors included for treatment, carryover period and volunteer.

Pairwise comparisons were then made between treatments using the least significant difference *t*-test. Significance was determined with reference to the 5% level.

Figure 1 Change in heart rate vs time following placebo (■, *n* = 11), flosequinan 100 mg (▲, *n* = 13) and 200 mg (●, *n* = 12). Overall treatment effect, *P* = 0.049. Placebo vs 100 mg, *P* = 0.028. Placebo vs 200 mg, *P* = 0.03. 100 mg vs 200 mg, *P* = 0.80.

Figure 2 Change in diastolic blood pressure vs time following placebo (■, *n* = 11), flosequinan 100 mg (▲, *n* = 13) and 200 mg (●, *n* = 12). Overall treatment effect, *P* = 0.0001. Placebo vs 100 mg, *P* = 0.0005. Placebo vs 200 mg, *P* = 0.0001. 100 mg vs 200 mg, *P* = 0.026.

Results

The mean age of the volunteers was 23.1 (0.69), with a mean weight of 76.1 (2.5) kg.

Haemodynamic changes

Treatment with flosequinan increased heart rate (Figure 1), both 100 mg and 200 mg producing significant effects when compared with placebo. No significant effect on systolic blood pressure was demonstrated at either dose but flosequinan did reduce diastolic blood pressure (Figure 2). This reduction was highly significant for 100 mg and 200 mg doses compared with placebo and dose dependent.

Limb blood flow

There was a trend for flosequinan to increase forearm blood flow (Figure 3). This trend reached significance after 200 mg of the drug and was maximal 4 h after dosing. No significant reduction of forearm vascular resistance was documented after 100 mg of flosequinan compared with placebo but 200 mg produced a significant fall compared with placebo which was also maximal after 4 h (Figure 4). The effect of 200 mg was also significant compared with 100 mg. No significant change in forearm venous capacitance occurred following either dose of the drug.

Organ blood flow

Apparent hepatic blood flow tended to increase after flosequinan (Table 1), the rise after 200 mg reaching statistical significance when compared with placebo. This was accompanied by a trend towards a fall in hepatic vascular resistance which was again significant after 200 mg. Flosequinan tended to increase renal blood flow but this trend did not reach statistical significance. Renal vascular resistance, however, was significantly reduced following flosequinan, the effects of 100 mg and 200 mg being found to be similar, when compared with placebo.

Figure 3 Change in forearm blood flow vs time following placebo (■, *n* = 11), flosequinan 100 mg (▲, *n* = 13) and 200 mg (●, *n* = 12). Overall treatment effect, *P* = 0.09. Placebo vs 200 mg, *P* = 0.03. Placebo vs 100 mg, *P* = 0.25. 100 mg vs 200 mg, *P* = 0.19.

Plasma drug concentrations

Plasma levels of flosequinan and its principal metabolite BTS 53554 are represented in Figure 5.

Adverse effects

No significant haematological or biochemical changes were produced by flosequinan during the course of this study. Six subjects reported 10 adverse events, three reporting headache following 100 mg of flosequinan and three after 200 mg of the drug. One subject reported dizziness following 200 mg and another reported dizziness associated with hypotension following 200 mg.

There were three withdrawals. One volunteer withdrew after study day 1 because of headache unrelated to study medication. The second was withdrawn following the discovery that he was being investigated because of an unproductive cough. The third was withdrawn during study day 2 following the development of a sinus tachycardia up to 150 beats min^{-1} approximately 30 min post the 200 mg dose. The subject was aware of 'palpitations'

Figure 4 Change in forearm vascular resistance vs time following placebo (■, $n = 11$), flosequinan 100 mg (▲, $n = 13$) and 200 mg (●, $n = 12$). Overall treatment effect, $P = 0.01$. Placebo vs 200 mg, $P = 0.003$. 100 mg vs 200 mg, $P = 0.04$. Placebo vs 100 mg, $P = 0.11$.

and requested immediate withdrawal from the study. The episode resolved spontaneously with no intervention and there were no late sequelae.

Discussion

This study is the first to report the effects of flosequinan on limb, hepatic and renal blood flow in normal subjects. We have shown that when compared with placebo, flosequinan 200 mg increases forearm blood flow, reaching a maximum 4 h after dosing, this being associated with a significant fall in forearm vascular resistance. The fall in forearm vascular resistance observed following 200 mg was also significant when compared with 100 mg of the drug. No change in forearm venous capacitance was observed during the course of this study. These changes in forearm blood flow were accompanied by an increase in hepatic blood flow and preservation of renal blood flow, associated with significant reductions in organ vascular resistance. In addition 100 mg of flosequinan produced a significant reduction in renal vascular resistance, when compared with placebo.

The effects of flosequinan on heart rate in this study are similar to those demonstrated by previous workers and represent a physiologic response to systemic vasodilatation which partly attenuates the reduction in blood pressure produced by the drug (Cowley *et al.*, 1984; Kerth *et al.*, 1986; Wynne *et al.*, 1985). We did not document a reduction in systolic blood pressure, but were able to demonstrate a significant fall in diastolic blood pressure after both 100 mg and 200 mg, which was dose-dependent and reached a maximum 2 h after 100 mg and 1 h after 200 mg. Cowley *et al.* (1984) demonstrated similar falls in diastolic blood pressure after 200 mg and 400 mg, but not after 100 mg.

In the same study, Cowley *et al.* (1984) demonstrated dose-dependent increases in forearm blood flow following 200 mg and 400 mg, which were accompanied by corresponding reductions in forearm vascular resistance, and were maximal after 6–8 h. A trend towards a decrease in forearm venous tone was also suggested. We found no change in forearm venous capacitance, but our

Table 1 Hepatic and renal blood flows and vascular resistances 2 h post placebo ($n = 11$), flosequinan 100 mg ($n = 13$) and 200 mg ($n = 12$). Overall treatment effect on apparent hepatic blood flow, $P = 0.08$. Overall treatment effect on hepatic vascular resistance, $P = 0.06$. Overall treatment effect on renal vascular resistance, $P = 0.008$.

	Placebo	Flosequinan	
		100 mg	200 mg
Hepatic blood flow ($\text{ml min}^{-1} \text{m}^{-2}$)	426 (38)	448 (31)	456 (31)*
Hepatic vascular resistance (units)	117.6 (12.6)	97.6 (9.8)	94.8 (10.1)**
Renal blood flow ($\text{ml min}^{-1} \text{m}^{-2}$)	577 (36)	585 (27)	597 (33)
Renal vascular resistance (units)	82.6 (6.7)	70.7 (5.1)***	69.1 (4.5)†

* $P = 0.03$ vs placebo, ** $P = 0.02$ vs placebo, *** $P = 0.006$ vs placebo,

† $P = 0.007$ vs placebo.

Figure 5 Plasma concentrations of flosequinan and principal metabolite BTS 53554 vs time following flosequinan 100 mg (a, $n = 13$) and 200 mg (b, $n = 12$).

forearm blood flow findings are consistent with those previously described.

The baseline mean value for apparent hepatic blood flow is lower than has been described previously (Leithe *et al.*, 1984). This may be accounted for by differences in technique with respect to timing of clearance study and sampling, age of subjects or variations in the time of clearance studies post-absorption. All our subjects were studied at least 3 h post-prandially. The baseline mean value for renal blood flow is well within the range of those previously described.

Renal blood flow was preserved by significant falls in renal vascular resistance in the presence of dose-dependent reductions in blood pressure following both 100 mg and 200 mg of flosequinan, when compared with placebo. Dupont *et al.* (1988) described 10 patients with essential hypertension who received 30–100 mg of flosequinan daily as monotherapy for 12 weeks and experienced significant reductions in resting and ambulatory systolic and diastolic blood pressure. Renal vascular resistance was significantly reduced while renal blood flow and glomerular filtration remained unchanged. Our findings in normal man are similar, but no data are available following chronic administration of flosequinan in normal subjects. In the only other published study concerning the combined regional vascular effects of flosequinan, Haas *et al.* (1989) reported 10 patients with chronic congestive heart failure who received 100 mg of flosequinan in single-blind non-randomised fashion. Regional haemodynamic measurements made between 30 min and 2 h after dosing found no significant alteration

in limb, renal or hepatic-splanchnic blood flow or vascular resistance. Peripheral venous capacitance was found to increase, however.

The levels of flosequinan and metabolites recorded in this study are consistent with those previously reported. It is thought that the sustained effects of flosequinan on blood pressure, forearm blood flow and forearm vascular resistance are associated with persisting levels of the active metabolite in plasma (Paran *et al.*, 1987; Wynne *et al.*, 1985).

In summary, flosequinan produces dose-dependent reductions in diastolic blood pressure in normal subjects after 100 mg and 200 mg when compared with placebo. This is accompanied by an increase in forearm blood flow, at the higher dose and a corresponding fall in forearm vascular resistance. Hepatic and renal blood flows are preserved, associated with reductions in hepatic and renal vascular resistances. These findings have important implications with respect to the effects of flosequinan on regional blood flow in certain disease states, i.e. hypertension, acute and chronic congestive heart failure and further studies are required to evaluate the effects of flosequinan on altered regional blood flow states in these conditions.

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