having a unique role in the management of patients with heart failure, particularly as they are not always well tolerated. Flosequinan undoubtedly has the properties theoretically desirable in a vasodilator to be used to treat patients with heart failure, and the improvement in symptoms that we have recorded suggests that it is likely to be a useful alternative to angiotensin converting enzyme inhibitors.

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Combination treatment with cholestyramine and bezafibrate for heterozygous familial hypercholesterolaemia

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Abstract

Cholestyramine and bezafibrate were compared individually and in combination in the treatment of 18 patients with heterozygous familial hypercholesterolaemia. The study used a double blind, placebo controlled block design with a placebo run in period of two months followed by three phases of active treatment, each of two months' duration. Patients were randomly allocated to one of the six possible sequences of medication so that three patients would be treated with each sequence. Two patients withdrew from the study before completion. The median concentration of total cholesterol decreased from 9.65 mmol/l (interquartile range 8.62 to 8.72) to 7.24 mmol/l (6.70 to 7.52) with cholestyramine, to 8.09 mmol/l (7.18 to 8.68) with bezafibrate, and to 6.31 mmol/l (5.84 to 7.27) with the combination. This fall was due almost entirely to a decrease in the low density lipoprotein cholesterol concentration, and the combination was significantly more effective than either drug alone. The 98% confidence intervals for the median differences between the combination and cholestyramine and the combination and bezafibrate were 0.04 to 1.49 mmol/l and 0.51 to 2.18 mmol/l respectively.

These results suggest that this combination is an effective and useful treatment in heterozygous familial hypercholesterolaemia.

Introduction

Familial hypercholesterolaemia is due to a single gene defect affecting the activity of receptors for low density lipoprotein. This leads to a reduction in the number of high affinity receptors for low density lipoprotein on peripheral and hepatic cells and a consequent increase in the plasma low density lipoprotein cholesterol concentration due to reduced plasma clearance. Familial hypercholesterolaemia is one of the commonest single gene disorders, having an incidence of one in 500 and affecting an estimated 100 000 people in the United Kingdom. These patients have a greatly increased risk of coronary heart disease; the heterozygotes are estimated to have a 50% chance of dying

from myocardial infarction before the age of 60,¹ and homozygotes rarely survive to the third decade. It is thus a condition in which there is a clear indication for dietary and pharmacological intervention aimed at reducing the high concentrations of low density lipoprotein. The response to diet is usually poor and treatment with a single agent is often inadequate. Combination treatment, particularly with bile acid sequestrant resins and nicotinic acid, has reduced concentrations of low density lipoprotein.^{2,3} The side effects of nicotinic acid, however, make this approach unsuitable for most patients.

We used an anion exchange resin (cholestyramine), the treatment of choice in familial hypercholesterolaemia, alone and in combination with bezafibrate, one of the newer fibrate drugs, in 18 patients with familial hypercholesterolaemia. The study compared the relative efficacy of cholestyramine and bezafibrate in lowering low density lipoprotein cholesterol concentration in patients with this condition and determined whether combination treatment could reduce low density lipoprotein to acceptable concentrations.

Patients and methods

Eighteen patients with heterozygous familial hypercholesterolaemia were recruited from the outpatient clinic. All had a total cholesterol concentration >7.5 mmol/l or a low density lipoprotein cholesterol concentration >4.9 mmol/l (concentration before treatment or highest concentration while patient was receiving treatment), and they or a first or second degree relative had tendon xanthomas. The 10 women and eight men had a mean age of 41 (range 26 to 52) years.

All patients were maintained on a formal diet low in fat (30% of total calories: 10% each of saturated, monounsaturated, and polyunsaturated fatty acids) and low in cholesterol (less than 300 mg/day); they were provided with regular dietary counselling throughout the study. Patients with diabetes or with liver, kidney, thyroid, or parathyroid diseases were excluded, as were pregnant or lactating women. None of the patients was taking drugs known to affect lipid metabolism.

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All patients were initially given a double placebo for a two month run in period. Throughout the study they were instructed to take one 200 mg tablet (bezafibrate or placebo) three times a day and six 4 g sachets (cholestyramine or placebo) daily. The tablets were taken one hour before the sachets (and at least two hours after the previous dose of cholestyramine) to prevent cholestyramine interfering with the absorption of the bezafibrate.

There were six possible sequences of medication for the three treatments in the active phase—for example, bezafibrate plus placebo, followed by cholestyramine plus placebo, and finally bezafibrate plus cholestyramine; or bezafibrate plus cholestyramine, followed by bezafibrate plus placebo, and then cholestyramine plus placebo. Patients were randomly allocated to one of these sequences. The study was designed to repeat each sequence three times; thus 18 patients were needed. The active phases of the trial were double blind. Compliance was assessed by counts of tablets and sachets, and adverse reactions were recorded.

Blood was taken into bottles precoated with edetic acid for analysis of lipoproteins after an overnight fast. Plasma lipoproteins were separated by sequential ultracentrifugation using a modification of the method of Havel *et al.*⁴ Cholesterol was measured with the fully enzymatic cholesterol oxidase/peroxidase amidopyrine colorimetric kit, and triglycerides were measured with the glycerol phosphate oxidase/peroxidase amidopyrine enzymatic colorimetric kit (Boehringer Mannheim). Apoproteins A-I and B were measured by radial immunodiffusion using commercial plates (Immuno, Vienna).

Results were analysed with the Wilcoxon signed ranks test. Non-parametric confidence intervals for the median differences after the various treatments were derived from standard tables.⁵

Results

The drugs used in the trial were generally well tolerated, although all the patients found the contents

of the sachets a little unpleasant because of their physical consistency. Five patients complained of nausea and "heartburn" when taking sachets of cholestyramine. No side effects could definitely be attributed to the tablets. Two patients withdrew from the study because they could no longer tolerate the sachets, one during and one after the second cycle of active treatment. Thus four of the six sequences of medication were completed by three patients and two sequences by two. The average compliance for sachets was 83% (range 54 to 98%) and for tablets 85% (range 84 to 90%). Routine monitoring of urea and electrolytes, tests of liver function, and full blood counts showed no clinically important abnormalities throughout the trial.

There were significant reductions in concentrations of total cholesterol ($p < 0.01$), low density lipoprotein cholesterol ($p < 0.02$), and apolipoprotein B ($p < 0.02$) after treatment with each drug individually and in combination, compared with placebo (tables I and II).

TABLE III—Numbers of patients with concentrations of total cholesterol of less than 6.5 mmol/l or low density lipoprotein cholesterol concentrations less than 4.5 mmol/l after treatment with each regimen

	Total cholesterol <6.5 mmol/l	Low density lipoprotein <4.5 mmol/l
Placebo	1	1
Bezafibrate	3	3
Cholestyramine	2	4
Cholestyramine and bezafibrate	9	9

The combination, however, produced significantly greater reductions in all of these variables than either drug alone. With combination treatment many more patients also had their total cholesterol concentrations reduced to less than 6.5 mmol or low density lipoprotein cholesterol to less than 4.5 mmol/l, or both (table III). Seven patients on combination treatment achieved total cholesterol concentrations of less than 6 mmol/l.

TABLE I—Median concentrations (interquartile range) and median differences between concentrations (97.88% non-parametric confidence limits) of plasma lipoproteins after each phase of treatment in 16 patients with heterozygous familial hypercholesterolaemia. Values are mmol/l

	Total cholesterol	Triglyceride	Low density lipoprotein	High density lipoprotein	Very low density lipoprotein
<i>Median concentrations</i>					
Placebo	9.65 (8.62-10.72)	1.06 (0.91-1.23)	7.19 (5.85-7.61)	1.10 (0.84-1.34)	0.53 (0.31-0.60)
Bezafibrate	8.09 (7.18-8.68)	0.87 (0.74-1.07)	5.80 (5.35-6.15)	0.99 (0.89-1.07)	0.40 (0.29-0.66)
Cholestyramine	7.24 (6.70-7.52)	1.07 (0.98-1.29)	5.20 (4.58-5.48)	1.21 (0.98-1.27)	0.46 (0.32-0.66)
Bezafibrate and cholestyramine	6.31 (5.84-7.27)	0.86 (0.81-0.96)	4.41 (4.05-5.30)	1.12 (0.82-1.38)	0.37 (0.30-0.43)†
<i>Median differences</i>					
Placebo v bezafibrate	1.93 (0.25 to 2.99)***	0.13 (0.02 to 0.39)***	1.46 (0.23 to 2.08)***	-0.01 (-0.12 to 0.24)	0.18 (-0.01 to 0.27)*†
Placebo v cholestyramine	2.16 (0.58 to 3.20)***	0.02 (-0.46 to 0.38)	1.49 (0.42 to 2.58)***	0.01 (-0.38 to 0.23)	0.01 (-0.21 to 0.21)
Placebo v bezafibrate and cholestyramine	3.58 (1.82 to 3.81)***	0.20 (0.04 to 0.38)***	2.15 (0.99 to 3.52)***	-0.1 (-0.42 to 0.32)	0.08 (-0.11 to 0.31)
Bezafibrate v cholestyramine	0.32 (-1.10 to 1.37)	-0.13 (-0.47 to 0.07)**	0.36 (-0.64 to 1.17)	-0.09 (-0.42 to 0.10)	-0.16 (-0.33 to 0.02)***†
Bezafibrate v bezafibrate and cholestyramine	1.45 (0.51 to 2.18)***	0.01 (-0.07 to 0.19)	1.18 (0.47 to 1.52)***	-0.08 (-0.38 to 0.37)	0.00 (0.27 to 0.18)†
Cholestyramine v bezafibrate and cholestyramine	1.05 (0.04 to 1.49)***	0.18 (0.02 to 0.52)***	0.76 (0.04 to 1.61)**	-0.08 (-0.17 to 0.45)	0.11 (-0.02 to 0.23)*

* $p < 0.05$, ** $p < 0.02$, *** $p < 0.01$, Wilcoxon signed ranks test.

†n = 15, 96.5% confidence interval.

TABLE II—Median concentrations (interquartile range) and median difference between concentrations (96.5% non-parametric confidence intervals) of apolipoproteins A-I and B after each phase of treatment in 15 patients with heterozygous familial hypercholesterolaemia. Values are mg/l

	Apolipoprotein A-I	Apolipoprotein B
<i>Median concentrations</i>		
Placebo	11.4 (8.9-13.0)	15.1 (13.2-18.0)***
Bezafibrate	12.2 (10.8-15.6)	14.0 (11.9-14.6)***
Cholestyramine	12.7 (10.4-15.3)	11.9 (10.1-14.6)
Bezafibrate and cholestyramine	12.8 (11.7-14.0)	10.5 (19.4-12.0)
<i>Median differences</i>		
Placebo v bezafibrate	-2.0 (-4.1 to -0.5)**	2.0 (0 to 3.6)***
Placebo v cholestyramine	-1.9 (-3.0 to -0.6)	2.4 (0.4 to 6.7)***
Placebo v bezafibrate and cholestyramine	-2.6 (-3.7 to -0.6)**	3.2 (2.6 to 7.4)***
Bezafibrate v cholestyramine	-0.2 (-0.7 to 2.3)	0.9 (-1.5 to 3.8)
Bezafibrate v bezafibrate and cholestyramine	-0.4 (-1.6 to 2.0)	3.2 (0.7 to 4.6)***
Cholestyramine v bezafibrate and cholestyramine	-0.7 (-3.1 to 2.3)	0.9 (0.5 to 3.7)**

** $p < 0.02$, *** $p < 0.01$, Wilcoxon signed ranks test.

There were small changes in high density lipoprotein cholesterol concentrations, but these were not significant. There were, however, significant increases in concentrations of its major apoprotein, apolipoprotein A-I, during treatment with bezafibrate and with the combination compared with placebo ($p < 0.02$; table II).

Total triglyceride concentrations were significantly lower after treatment with bezafibrate and with the combination than after placebo ($p < 0.01$). Concentrations of total triglycerides and of very low density lipoprotein triglyceride were also significantly lower after treatment with bezafibrate and the combination than after cholestyramine ($p < 0.01$ and $p < 0.05$, respectively).

Discussion

This study was designed to test rigorously the hypothesis that treatment with a combination of cholestyramine and bezafibrate could produce significantly better results than treatment with a single agent in familial hypercholesterolaemia. The randomised block design of the study enabled a limited number of patients to be used in a relatively short period and offered some compensation for inadvertent bias due to an effect of the sequence of medications.

Treatment with cholestyramine is known to decrease serum concentrations of low density lipoprotein cholesterol by enhancing its catabolism.⁶ Its effectiveness is subsequently limited by a compensatory increase in hepatic synthesis of cholesterol.⁷ Bezafibrate is a clofibrate analogue and is thought to act by reducing the activity of β -hydroxy- β -methylglutaryl coenzyme A reductase, the enzyme catalysing the rate limiting step in cholesterol synthesis,⁸ and increasing the activity of lipoprotein lipase.^{9,10} Theoretically, therefore, the two drugs should have a complementary action, and this study confirms the efficacy of combined treatment. The doses of the drugs used were at the upper limit of the range for normal maintenance treatment recommended by the manufacturers.

The combination of the new inhibitors of β -hydroxy- β -methylglutaryl coenzyme A reductase with a bile acid sequestrant has been found to be highly effective,^{11,12} and this offers hope for the future, when these agents have been fully evaluated and are readily available. Treatment with bezafibrate and cholestyramine has been reported in uncontrolled studies of patients with different types of hypercholesterolaemia and small groups with familial hypercholesterolaemia,^{13,14} but this study is, to our knowledge, the first placebo controlled double blind trial of this combination to be reported. The comparisons with the double placebo are not as rigorous as those between the active groups since the double placebo phase was not randomised. The information is, however, comparable with the results of other published trials of combination treatments, none of which had randomisation of treatment and some of which did not have placebo control.

The decreases in apolipoprotein B and the increases in apolipoprotein A-I concentrations may be important as several studies have found that changes in concentrations of apoproteins are more closely related to atherosclerotic disease than are the corresponding concentrations of lipoprotein cholesterol fractions.¹⁵⁻¹⁷

Primary prevention trials using drug treatment to lower cholesterol concentrations have not achieved any reduction in overall mortality despite statistically significant reductions in cardiovascular endpoints.¹⁸ The populations that were treated in these studies were heterogeneous and would have included many patients with polygenic hypercholesterolaemia and with a more benign prognosis than that of patients with familial hypercholesterolaemia. Only modest reductions in cholesterol concentrations were achieved in these trials, and it was estimated that a more dramatic reduction in the incidence of coronary heart disease could have been achieved if the treatment had been more effective.¹⁹ We looked at one way of achieving large reductions in plasma concentrations of cholesterol in a selected population of people at high risk as treatment based on such an approach may be more effective.

We conclude that the combination of cholestyramine and bezafibrate is more effective than either drug alone in treating familial hypercholesterolaemia. In compliant individuals serum cholesterol concentration may be reduced substantially, and this may improve the prognosis of this group of high risk patients.

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ANY QUESTIONS

Citrus fruit is used to prepare fruit juices; as these fruits are commonly treated with pesticides is use of the peel a health hazard?

In the production of citrus juices the juice is expressed so as to minimise the expression of materials from the peel, such as peel oil and those constituents of citrus peel that would impart a strong and bitter orange flavour to the juice.¹ Thus the possibility of contamination from pesticides is reduced. Comminuted pulp, which is used for the production of some orange soft drinks, does include the whole fruit and thus may include some surface contamination although the fruit is washed thoroughly.² In practice citrus fruit intended for use in this way would not be treated with preservatives that are not permitted for human consumption, and most responsible producers will have observed the proper interval between treating the crop and its harvesting. Wax coating and surface fungicides are sometimes used on citrus fruit intended for consumption as fruit. The preservatives (diphenyl derivatives) used in this way are absorbed into the wax coatings and would be released into a product such as marmalade or slices of citrus fruit added to an alcoholic beverage. The amounts are quite small and there is little or no evidence of risks to health. Even so, when fruit that has been treated in this way is being used to prepare juice it would be as well to wash it thoroughly before use, a caveat that applies to all fruit.—D A T SOUTHGATE, head, AFRC Institute of Food Research, Norwich.

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