USE OF CHOLESTEROL-LOWERING AGENT

The present invention relates to the use of a cholesterol-lowering agent, and more particularly to the administration of a particular dose or dosage range of the HMG CoA reductase inhibitor, (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid and pharmaceutically acceptable salts thereof, hereinafter referred to as "the Agent" and illustrated (as the calcium salt) in formula I hereinafter. The invention further relates to the dosage range, start dose and dosage forms of the Agent.

The Agent is disclosed in European Patent Application, Publication No. 0521471, and in Bioorganic and Medicinal Chemistry, (1997), 5(2), 437-444 as an inhibitor of 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase) which is a major rate-limiting enzyme in cholesterol biosynthesis. The Agent is taught as useful in the treatment of hypercholesterolemia, hyperlipoproteinemia and atherosclerosis. HMG-CoA reductase inhibitors are the most widely used prescription medication for the treatment of hypercholesterolaemia. A number of HMG-CoA reductase inhibitors are marketed, namely lovastatin, pravastatin, simvastatin, fluvastatin, atorvastatin and cerivastatin, and are collectively referred to as 'statins'. Despite the benefits of statin therapy, less than optimal results may be achieved in patients, due to the level of efficacy and safety achieved at the recommended dosages of the currently marketed statins. Accordingly it is important to find dosages of alternative statins which beneficially alter lipid levels to a significantly greater extent than similar dosages of currently used statins and which have a similar or improved safety profile.

Surprisingly it has now been found that when dosed orally to patients with hypercholesterolemia at particular dosages or in a particular dosage range the Agent lowers total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) by an unexpected degree, and without any significant adverse side effects. When dosed at the same dosages or in the same dosage range, the Agent also modifies other lipoprotein levels (such as raising high density lipid cholesterol (HDL-C) levels, lowering triglyceride (TG) levels and lowering apolipoprotein B-100 (Apo-B) levels) to an unexpected and beneficial extent, without any significant adverse side effects. Elevations of alanine aminotransferase (ALT) liver enzyme levels are reported for other HMG-CoA reductase inhibitors. Surprisingly it has now been

A further aspect of the present invention comprises a method of lowering total cholesterol levels by 35% or more in a patient in need thereof by administration of 10 to 80 mg per day of the Agent.

A further aspect of the present invention comprises a method of lowering total cholesterol levels by 40% or more in a patient in need thereof by administration of 40 to 80 mg per day of the Agent.

A further aspect of the present invention comprises a method of treating a hypercholesterolemic patient to bring said patient within recommended NCEP guidelines (or other recognised guidelines) by administration of 5 to 80 mg per day of the Agent. Recommended NCEP guidelines refer to the National Cholesterol Education Program - Adult Treatment Panel (NCEP-ATP) II target LDL-C levels, which are incorporated herein by reference. A summary of these guidelines is given in JAMA, June 16, 1993-Vol 629, No. 23, pages 3015-3023, particularly Figue 1 , page 3018-3019. NCEP guidelines recommend that patients with clinically evident coronary heart disease (CHD), such as documented atherosclerosis, are titrated to LDL-C concentrations less than or equal to 100 mg/dl. Other recognised guidelines includes the European Atherosclerosis Society (EAS) guidelines, which recommend aggressive management of lipids in such patients with a target LDL-C of 115 mg/dl. Where a patient has no clinically evident CHD, but has 2 or more risk factors for such a disease, NCEP guidelines recommend patients are titrated to LDL-C concentrations less than 130 mg/ml. Where a patient has no clinically evident CHD and one or no risk factors, the NCEP guideline target is less than 160 mg/dl.

A further aspect of the present invention comprises a method of treating hypertriglyceridemia by administration of 5 to 80 mg per day of the Agent.

A further aspect of the present invention comprises a method of raising high density lipid (HDL) levels in a patient in need thereof by administration of 5 to 80 mg per day of the Agent.

A further aspect of the present invention comprises a method of raising or maintaining HDL levels whilst lowering LDL-C levels in a patient in need thereof by administration of 5 to 80 mg per day of the Agent. Preferred levels of lowering LDL-C levels, whilst maintaining or raising HDL levels, include the percentage levels of reduction in LDL-C given in the above methods.

(ii) 8% or more by administration of 5 to 20 mg per day of the Agent; or

(iii) 10% or more by administration of 5 to 10 mg per day (especially 10 mg per day) of the Agent;

(4) a method of lowering apolipoprotein B-100 levels in a patient in need thereof by

(i) 30% or more by administration of 5 to 80 mg per day (particularly 5 to 40 mg per day, more particularly 10 to 20 mg per day, especially 10 mg per day) of the Agent;

(ii) 35% or more by administration of 5 to 80 mg per day (particularly 5 to 40 mg per day, more particularly 10 to 20 mg per day, especially 10 mg per day) of the Agent;

(iii) 40% or more by administration of 10 to 80 mg per day (particularly 5 to 40 mg per day, more particularly 10 to 20 mg per day, especially 10 mg per day) of the Agent;

(iv) 45% or more by administration of 20 to 80 mg per day of the Agent; or

(v) 50% or more by administration of 40 to 80 mg per day of the Agent;

(5) a method of lowering triglyceride levels levels in a patient in need thereof by

(i) 10% or more by administration of 5 to 80 mg per day (particularly 5 to 40 mg per day, more particularly 10 to 20 mg per day, especially 10 mg per day) of the Agent; or

(ii) 20% or more by administration of 20 to 40 mg per day of the Agent;

Further independent aspects of the present invention comprise the following:-

(1) a method of lowering LDL-C levels in a patient in need thereof by 30% or more by administration of 1 to 5 mg per day, more particularly 2.5 to 5 mg per day, of the Agent;

(2) a method of lowering total cholesterol levels in a patient in need thereof by 30% or more by administration of 2.5 to 5 mg per day of the Agent;

(3) a method of raising HDL-C levels in a patient in need thereof by 5 % or more by administration of 2.5 to 5 mg per day of the Agent;

(4) a method of lowering apolipoprotein B-100 levels in a patient in need thereof by 30% or more by administration of 2.5 to 5 mg per day of the Agent; or

(5) a method of lowering triglyceride levels in a patient in need thereof by 10% or more by administration of 2.5 to 5 mg per day of the Agent.

(4) a method of lowering LDL-C levels by 55% or more and lowering apolipoprotein B-100 levels by 45% or more by administration of 20 to 40 mg per day of the Agent; or

(5) a method of lowering LDL-C levels by 60% or more and lowering apolipoprotein B-100 levels by 50% or more by administration of 40 to 80 mg per day of the Agent.

Further aspects of the present invention comprise the following:-

(1) a method of lowering LDL-C levels by 30% or more and lowering triglyceride levels by 10% or more by administration of 1 to 5 mg per day of the Agent;

(2) a method of lowering LDL-C levels by 45% or more and lowering triglyceride levels by 10% or more by administration of 5 to 20 mg per day (particularly 5 to 10 mg per day) of the Agent;

(3) a method of lowering LDL-C levels by 50% or more and lowering triglyceride levels by 10% or more by administration of 10 to 20 mg per day of the Agent;

(4) a method of lowering LDL-C levels by 55% or more and lowering triglyceride levels by 10% (more especially 20%) or more by administration of 20 to 40 mg per day of the Agent; or

(5) a method of lowering LDL-C levels by 60% or more and lowering triglyceride levels by 10% (more especially 20%) or more by administration of 40 to 80 mg per day of the Agent.

Further aspects of the present invention comprise the following:-

(1) A method of raising HDL-C levels by 5 % (particularly 8% and more particularly 10%) or more and lowering apolipoprotein B-100 levels by 25 % or more in a patient in need thereof by administration of 1 to 5 mg per day of the Agent; or

(2) A method of raising HDL-C levels by 5 % (particularly 8% and more particularly 10%) or more and lowering triglyceride levels by 10 % or more in a patient in need thereof by administration of 5 to 80 mg per day (particularly 5 to 40 mg per day, more particularly 10 to 20 mg per day, especially 10 mg per day) of the Agent.

to 80 mg per day (particularly 5 to 40 mg per day, more particularly 10 to 20 mg per day, especially 10 mg per day) of the Agent.

Further aspects of the present invention comprise the following:-

(1) a method of reducing the LDL-C/HDL-C lipid ratio to below 2.2 in a patient in need thereof by administration of 1 to 5 mg per day of the Agent;

(2) a method of reducing the LDL-C/HDL-C lipid ratio to below 2.0 in a patient in need thereof by administration of 5 to 10 mg per day of the Agent;

(3) a method of reducing the LDL-C/HDL-C lipid ratio to below 1.5 in a patient in need thereof by administration of 20 to 40 mg per day of the Agent;

(4) a method of reducing the LDL-C/HDL-C lipid ratio to below 1.2 in a patient in need thereof by administration of 80 mg per day of the Agent;

(5) a method of reducing the non-HDL-C/HDL-C lipid ratio to below 2.6 in a patient in need thereof by administration of 1 to 5 mg per day of the Agent;

(6) a method of reducing the non-HDL-C/HDL-C lipid ratio to below 2.2 in a patient in need thereof by administration of 5 to 10 mg per day of the Agent;

(7) a method of reducing the non-HDL-C/HDL-C lipid ratio to below 2.0 in a patient in need thereof by administration of 20 to 40 mg per day of the Agent;

(8) a method of reducing the non-HDL-C/HDL-C lipid ratio to below 1.5 in a patient in need thereof by administration of 1 to 5 mg per day of the Agent;

(9) a method of reducing the TC/HDL-C lipid ratio to below 3.6 in a patient in need thereof by administration of 1 to 5 mg per day of the Agent;

(10) a method of reducing the TC/HDL-C lipid ratio to below 3.3 in a patient in need thereof by administration of 5 to 10 mg per day of the Agent;

(11) a method of reducing the TC/HDL-C lipid ratio to below 3.0 in a patient in need thereof by administration of 20 to 40 mg per day of the Agent; or

(12) a method of reducing the TC/HDL-C lipid ratio to below 2.5 in a patient in need thereof by administration of 80 mg per day of the Agent.

A particularly suitable starting dose of the Agent in the methods referred herein is 5 to 10 mg per day, especially 10 mg per day. After initiation and/or upon titration of the Agent,

Preferably the Agent is bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt. Where herein a dose of (or a pharmaceutical composition comprising) 5 mg, 10 mg, 20 mg, 40 mg or 80 mg of the Agent is referred to, this includes a dose (or pharmaceutical composition comprising) of 5.2 mg, 10.4 mg, 20.8 mg, 41.6 mg and 83.2 mg respectively of the calcium salt of formula 1. Such doses (or compositions) are calculated to provide the equivalent of 5 mg, 10 mg, 20 mg, 40 mg and 80 mg respectively of the free acid form when dosed.

Pharmaceutical compositions

The following Example illustrates, but is not intended to limit, pharmaceutical dosage forms which are suitable for use in the invention as defined herein:

Capsule	mg
The Agent	5.0
Lactose	42.5
Corn Starch	20.0
Microcrystalline cellulose	32.0
Pregelatinised starch	3.3
Synthetic Hydrotalcite	1.1
Magnesium stearate	1.1

Capsules containing 1, 2.5 or 10 mg of the Agent may be obtained similarly using more or less lactose as appropriate to maintain a total fill weight of 105 mg.

Preferred such formulations are those in which the Agent is bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt.

To illustrate the invention, a randomised, dose response parallel-group study with bis[(E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]-pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid] calcium salt (hereinafter referred to as ZD4522) and atorvastatin (ATORV) in subjects with primary hypercholesterolaemia was carried out.

level. This has been increased to 12 subjects per group to adjust for multiple comparisons of groups against placebo while preserving a power of at least 90% (based on simulations). This sample size also leads to an estimate of the dose-response curve for percentage decline in LDL-C with a width of the confidence band less than 10% for most of the dose range.

Inclusion Criteria

For inclusion in the dietary run-in period, subjects had to fulfil all of the following criteria:

(1) fasting LDL cholesterol (>4.14 but <6.21 mmol/L);

(2) fasting levels of triglycerides (<3.39 mmol/L);

(3) male subjects, age 18 to 70 years. Female subjects age 50 to 70 years;

(4) absence of menstruation for more than two years and follicle stimulating hormone (FSH), luteinising hormone (LH) and oestradiol within the post-menopausal range (using local laboratory ranges);

(5) body mass index ≤ 30 kg/m^2.

For inclusion in the randomised treatment phase of the trial, subjects had to fulfil all of the following criteria:

(6) fasting LDL cholesterol >4.14 mmol/L but < 5.69 mmol/L from measurements taken at weeks -2 and -1 before randomisation, with the lower value within 15% of the higher value;

(7) fasting TG < 3.39mmol/L at Weeks -6,-2 and -1, before randomisation;

(8) a Food Record Rating Score (FRR) ≤ 15 at Weeks -2 and -1, before randomisation, to demonstrate compliance with the American Heart Association Step -1 diet.

Exclusion Criteria

Any of the following was regarded as a criterion for exclusion from the trial:

(1) Subjects using cholesterol lowering drugs (this therapy must have been discontinued at least 4 weeks before the start of the dietary run-in period. Subjects taking probucol should have discontinued 12 months before inclusion in this study).

(2) History of serious or hypersensitivity reactions to other HMG-CoA reductase inhibitors.

DISALLOWED MEDICATIONS

CLASS OF DRUG	GENERIC NAME
Antibiotics/ antifungals	Erythromycin Base Erythromycin Ethyl Succinate, Acetyl Sulfisoxazole Rifampicin Fluconazole Ketaconazole Itraconzole
Anti-epileptics/ antidepressants	Phenytoin Phenobarbitone Fluoxetine Carbemazepine
Acne treatment	Isotretinoin
Antiulcer drugs	Cimetidine Cisapride
Systemic Steroids	Triamcinolone Acetonide Triamcinolone Diacetate Betamethasone Sodium Phosphate Betamethasone Acetate Hydrocortisone Hydrocortisone Acetate Hydrocortisone Sodium Phosphate Hydrocortisone Sodium Succinate Cortisone Acetate Dexamethasone Dexamethasone Acetate Dexamethasone Sodium
Anti arrhythmic	Digoxin
Anticoagulant	Warfarin
Antihistamine	Astemizole Terfenadine
Systemic Steroids Systemic Steroids continued	Phosphate Prednisolone Methylprednisolone Methylprenisolone Acetate Methylprednisolone Sodium Succinate Prednisolone Tebutate Prednisolone Sodium Phosphate Methyltestosterone Fluoxymesterone any hormone replacement therapies (HRT)

Trial Methods

Summary

The subjects were requested to take the study drug once daily, not less than 3 hours after the evening meal. At Visits 1, 2, 3, 4, 6, 7, 8, 9 and 10 the trial subjects reported to the clinic after fasting for twelve hours (water permitted) and blood was drawn to determine fasting lipids. Subjects provided a urine sample, and blood samples for haematology and clinical biochemistry evaluation. Where lipid and clinical biochemistry measurements fell at the same visit, only one sample was taken. At Visits 4, 6, 7 and 8 pharmacokinetic samples were collected. Weight, blood pressure and heart rate were measured and recorded at all visits. At Visits 1 and 10 an ECG was performed.

The analytical laboratory used is certified for standardisation of lipid analysis as specified by the Standardisation programme of the Centres for Disease Control and Prevention and the National Heart, Lung and Blood Institute.

Analysis

(a) Lipid

Concentrations of the following lipids were determined LDL-C, HDL-C, TG, TC, apo A-I, apo A-II, Lp(a), apo B, LDL-C (by Friedewald equation and β-quantification method).

(b) Biochemistry

Concentrations of the following were determined, AST, ALT, CK[1], γGT, ALP and total bilirubin, sodium, potassium, calcium, chloride, phosphate, bicarbonate creatinine, fasting blood glucose and serum thyroxine (T_4) and thyroid stimulating hormone (TSH).

Unless surgically sterilised female subjects had post menopausal status confirmed by measurement of FSH, LH and oestradiol at Visit 1.

Haematology

Full blood count was performed and included the following; erythrocyte count, haemoglobin, haematocrit, leucocyte cell count, platelets, red cell distribution width, percentage differential leukocyte count (including percentage large unstained cells), mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration. A clotting screen was performed at Visits 1 and 10 only.

3 **Study Arm** **Lipid Ratios (mean value at week 6)**

	non HDL-C/HDL-C	TC/HDL-C	LDL-C/HDL-C
Placebo	4.3	5.3	3.8
ZD4522 1 mg	2.5	3.5	2.2
ZD4522 2.5 mg	2.5	3.5	2.1
ZD4522 5 mg	2.2	3.2	1.9
ZD4522 10 mg	2.2	3.2	1.8
ZD4522 20 mg	1.8	2.8	1.4
ZD4522 40 mg	1.5	2.5	1.3
Atorvastatin 10 mg	2.5	3.5	2.1
Atorvastatin 80 mg	2.1	3.1	1.7

4 Effects in each study arm on percentage change of total cholesterol from baseline are given in Fig.1. Dosing began at BASELINE after an initial dietary run in period of 6 weeks, as per the clinical protocol above. Dosing stopped at 6 weeks after BASELINE.

5 Effects in each study arm on percentage LDL cholesterol (Friedewald equation calculation) percentage change from baseline are given in Fig 2. Dosing began at BASELINE after an initial dietary run in period of 6 weeks, as per the clinical protocol above. Dosing stopped at 6 weeks after BASELINE.

6 Effects on each study arm on HDL cholesterol percentage change from baseline are given in Fig. 3. Dosing began at BASELINE after an initial dietary run in period of 6 weeks, as per the clinical protocol above. Dosing stopped at 6 weeks after BASELINE.

7 Effects on each study arm on Apolipoprotein B-100 percentage change from baseline are given in Fig. 4. Dosing began at BASELINE after an initial dietary run in period of 6 weeks, as per the clinical protocol above. Dosing stopped at 6 weeks after BASELINE.

CLAIMS

1. The use of the compound (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid, or a pharmaceutically acceptable salt thereof, for the manufacture of an oral dosage form comprising 5 to 80 mg of the compound for use in lowering LDL-C levels by 40% or more, and/or lowering total cholesterol levels by 30% or more, and/or lowering triglyceride levels by 10% or more, and/or lowering apolipoprotein B-100 levels by 30% or more, and/or raising HDL-C levels by 5% or more in a human patient in need thereof, wherein the compound is administered at 5 to 80 mg per day.

2. The use of the compound or a pharmaceutically acceptable salt thereof as claimed in claim 1 for the manufacture of an oral dosage form comprising 5 to 10 mg of the compound for use in lowering LDL-C levels by 40% or more, and/or lowering total cholesterol levels by 30% or more, and/or lowering triglyceride levels by 10% or more, and/or lowering apolipoprotein B-100 levels by 30% or more, and/or raising HDL-C levels by 5% or more in a human patient in need thereof, wherein the compound is administered at 5 to 10 mg per day.

3. The use of the compound or a pharmaceutically acceptable salt thereof as claimed in claim 2 for the manufacture of an oral dosage form comprising 5 to 10 mg of the compound for use in lowering LDL-C levels by 45% or more in a human patient in need thereof.

4. The use of the compound or a pharmaceutically acceptable salt thereof as claimed in claim 2 for the manufacture of an oral dosage form comprising 5 to 10 mg of the compound for use in lowering total cholesterol levels by 35% or more in a human patient in need thereof.

5. The use of the compound or a pharmaceutically acceptable salt thereof as claimed in claim 2 for the manufacture of an oral dosage form comprising 5 to 10 mg of the compound for use in lowering triglyceride levels by 10% or more in a human patient in need thereof.

6. The use of the compound or a pharmaceutically acceptable salt thereof as claimed in claim 2 for the manufacture of an oral dosage form comprising 5 to 10 mg of the compound

more, in a human patient in need thereof, by oral administration of 5 to 80 mg per day of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid or a pharmaceutically acceptable salt thereof.

14. A method as claimed in claim 13 wherein 5 to 10 mg per day of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid or a pharmaceutically acceptable salt thereof is administered.

15. A method as claimed in claim 14 which is a method of lowering LDL-C levels by 45% or more.

16. A method as claimed in claim 14 which is a method of lowering total cholesterol levels by 35% or more.

17. A method as claimed in claim 14 which is a method of lowering triglyceride levels by 10% or more.

18. A method as claimed in claim 14 which is a method of lowering apolipoprotein B-100 levels by 35% or more.

19. A method as claimed in claim 14 which is a method of raising HDL-C levels by 10% or more.

20. A method of reducing the LDL-C/HDL-C lipid ratio to below 2.0 in a patient in need thereof by administration of 5 to 10 mg per day of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid or a pharmaceutically acceptable salt thereof.

21. A dosing regime comprising administration of a dose of 5 to 10 mg per day of (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)amino]pyrimidin-5-yl]-(3R,5S)-3,5-dihydroxyhept-6-enoic acid or a pharmaceutically acceptable salt thereof to a patient having

* C L M S *