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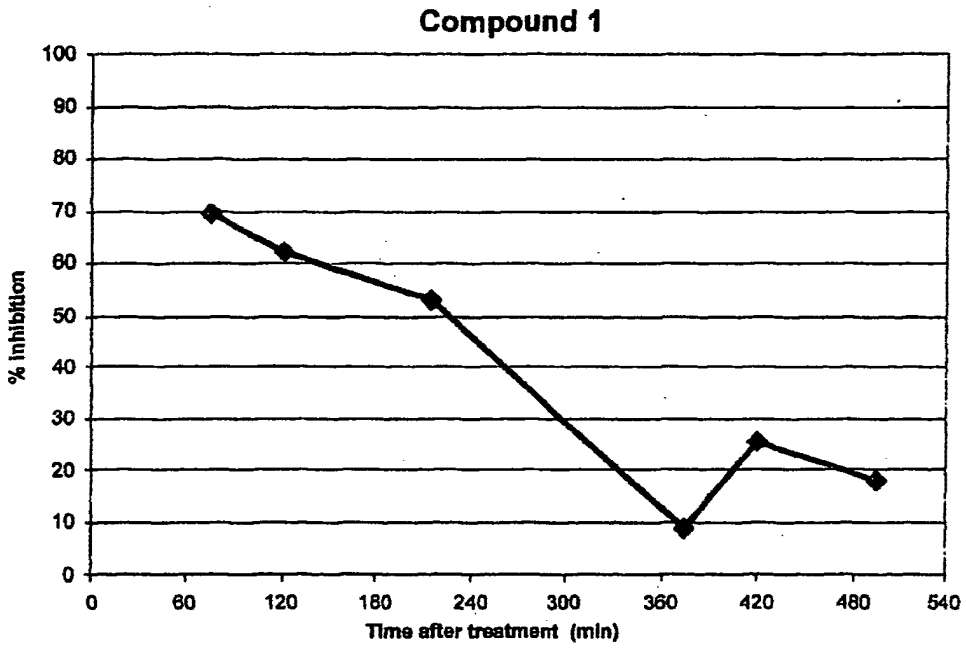
(54) Title: METHOD FOR SCREENING MTP-INHIBITING COMPOUNDS



(57) Abstract: The present invention relates to a screening method for selecting active materials that inhibit microsomal triglyceride transfer protein (MTP) and to a screening kit using the said method.

WO 2006/111238 A1

Method for screening MTP-inhibiting compounds

[0001] The invention relates to a screening method for selecting specific inhibitors of microsomal triglyceride transfer protein (MTP) which are efficient and have a short duration of action.

[0002] MTP ("microsomal triglyceride transfer protein") is a transfer protein located in the reticulum of hepatocytes and enterocytes, which catalyses the assembly of biomolecules that transport triglycerides, the apoB lipoproteins.

[0003] The term apoB more particularly denotes apoprotein B48 of the intestine and apoprotein B100 of the liver, without, however, being limited thereto.

[0004] Mutations in MTP or in the B apoproteins are reflected in man by very low levels or even an absence of apoB lipoproteins. The lipoproteins containing apoB (chylomicrons, "Very Low Density Lipoproteins" = VLDL) and their metabolic residues (chylomicron remnants, "Low Density Lipoproteins" = LDL) are recognised as being a major risk factor in the development of atherosclerosis, a major cause of death in industrialised countries.

[0005] It is observed that, in individuals who are heterozygous for these mutations, levels reduced on average by a half are associated with a low cardiovascular risk (C.J. Glueck, P.S. Gartside, M.J. Mellies, P.M. Steiner, *Trans. Assoc. Am. Physicians*, **90**, 184 (1977)).

[0006] This suggests that modulation of the secretion of triglyceride-rich lipoproteins by means of MTP antagonists and/or of secretion of apoB might be useful in the treatment of atherosclerosis and more broadly of pathologies characterised by an increase in apoB lipoproteins.

[0007] Molecules that inhibit MTP and/or the secretion of apoB might thus be useful for the treatment of diabetes-related hypertriglyceridaemia, hypercholesterolaemia and dyslipidaemia, and also for the prevention of and treating obesity.

[0008] There are already a number of examples in the literature of compounds capable of inhibiting MTP, but it is observed that since the start of the investiga-

tions on these inhibitors, none of them has become commercially available. Indeed, many development projects were stopped after the first clinical trials.

[0009] Specifically, preliminary results of clinical studies support the inhibition of MTP as a means of reducing the level of triglycerides and of cholesterol in man. However, such a therapy requires a long treatment, extending over several years. The *in vivo* administration to man of MTP-inhibiting compounds over such long periods might give rise to toxic effects, for instance an accumulation of lipids in the intestine and the liver, leading, for example, to hepatic steatosis.

[0010] In other words, although an *in vitro* primary screening test now makes it possible to identify potential MTP-inhibiting candidates, the *in vivo* confirmation tests show that many of these candidates are responsible for toxic effects on the liver.

[0011] To overcome this problem, it is nowadays proposed, for example, to combine MTP inhibitors with fibrates. However, this type of therapy has the drawbacks associated with the administration of two active materials (problems of dosage, compatibility, etc.).

[0012] There is consequently still a need for MTP-inhibiting active substances that induce few or no toxic side effects, in particular hepatic steatosis.

[0013] Thus, a first object of the invention consists in reducing the levels of triglycerides and cholesterol in the blood, without inducing an accumulation of lipids in the liver and in the intestine.

[0014] Another object of the invention consists in providing a screening test for selecting compounds capable of reducing hypertriglyceridaemia and cholesterolaemia with a lower risk of lipid accumulation in the liver.

[0015] Other objects will become apparent in the description of the invention discussed in further detail hereinbelow.

[0016] The present invention is based on the hypothesis that the liver resecretes the excess accumulated lipids, this excess being inherent to the inhibitory activity of the compound, since it is no longer active.

[0017] The inventors have now discovered, surprisingly, that, given that a certain potency is necessary in order to obtain a sufficient pharmacological effect,

only active principles having a short duration of action and liable to give a sufficient pharmacological effect ("flash" effect) generate fewer or none of the toxic effects observed with inhibitors with a long duration of action.

[0018] In addition, since the use in man involves a chronic treatment, it is necessary for this recovery phase by the liver to take place between two administrations in order to avoid the accumulation of lipids possibly leading to the development of steatosis.

[0019] Consequently, the inventors have, for the first time, used a known qualitative test for the selection of MTP-inhibiting candidates, to determine the kinetics of action of the said candidate.

[0020] Thus, the present invention first proposes a screening method for selecting active materials that inhibit microsomal triglyceride transfer protein (MTP), comprising the steps of:

a) using a candidate compound in a test of kinetic monitoring of inhibition of a parameter associated with the inhibition of MTP (inhibition of secretion of apoB, inhibition of secretion of VLDL and the like);

b) monitoring of the kinetics of inhibition of the said parameter by the said candidate from the start of the test and for a duration of between 3 hours and 24 hours, preferably between 5 hours and 12 hours and more preferably between 6 hours and 10 hours; and

c) selection of the candidate if it has kinetics of inhibition of the said parameter characterised by:

i) a percentage of inhibition for the said parameter of greater than or equal to 50% over a maximum duration of less than 4 hours and preferably less than 3 hours; and

ii) a residual inhibitory activity for the said parameter of less than 20% and preferably less than 10%, beyond 10 hours, preferably beyond 8 hours and more preferably beyond 6 hours, after the start of the test.

[0021] In the screening method of the present invention, the test for kinetic monitoring of inhibition is referred to as "Test A" in the rest of the present description.

[0022] Thus, Test A defined above makes it possible to select compounds having both sufficient MTP-inhibiting activity, and an absence of residual inhibitory activity that might result in adverse effects, such as those mentioned above and in particular hepatic steatosis.

[0023] The term "sufficient MTP-inhibiting activity" means that the percentage of inhibition observed for the parameter associated with the inhibition of MTP (for example apoB or VLDL) is at least equal to 50%.

[0024] The term "residual inhibitory activity" should be understood as meaning inhibitory activity observed for the parameter associated with the inhibition of MTP (for example apoB or VLDL) of less than 20%, preferably less than 10%, and more preferably insignificant inhibitory activity *versus* placebo.

[0025] In the rest of the present description, the term "reversible" qualifies compounds with a short duration of action, i.e. those that have the desired "flash" effect, and "irreversible" qualifies compounds with a long duration of action.

[0026] Test A of the screening method according to the present invention allows the selection of reversible candidates.

[0027] This Test A is a kinetic monitoring of inhibition of a parameter associated with the inhibition of MTP, for example a test of inhibition of the secretion of apoprotein B (apoB), advantageously if it is an *in vitro* test, using hepatic or enteric cells of any type, preferably hepatic cells, such as HepG2 cells, or alternatively a test of kinetic analysis of inhibition of MTP on the secretion of very low density lipoproteins (VLDL), advantageously if it is an *in vivo* test. The examples that follow show, for illustrative purposes, particular modes of implementation of Test A.

[0028] Test A may be performed *in vitro* or *in vivo*. According to one preferred variant of the invention, Test A performed *in vitro* (noted as A_{vitro} in the rest of the description) measures the kinetics of secretion of apoB, and Test A performed *in vivo* (noted as A_{vivo} in the rest of the description) measures the kinetics of secretion of VLDLs.

[0029] In Test A_{vitro}, the candidate compounds are considered as having a short duration of action if they show satisfactory reversibility, i.e. if the secretion of apoB, 24 hours after removal of the test compound, has returned to a level whose

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