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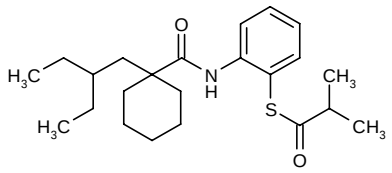
JTT-705

Treatment of Lipoprotein Disorders
Cholesteryl Ester Transfer Protein Inhibitor

2-Methylpropanethioic acid *S*-[2-[1-(2-ethylbutyl)cyclohexylcarboxamido]phenyl] ester



$C_{23}H_{35}N_2S$
Mol wt: 389.6005
CAS: 211513-37-0
EN: 291317

Abstract

The need for novel treatments to treat atherosclerosis and its complications has led to the development of inhibitors of cholesteryl ester transfer protein (CETP). The pro- or antiatherogenic role of CETP has been an important matter of debate over the last 20 years. Despite this, efforts have been made to design new, potent and selective CETP inhibitors such as JTT-705. This compound significantly increases plasma high-density lipoprotein cholesterol (HDL-C) in normolipidemic and hyperlipidemic rabbits. Importantly, administration of JTT-705 causes a marked reduction in the aortic arch atherosclerotic area in moderately, but not in severely hyperlipidemic rabbits. Moreover, in a phase II trial in humans with mild hyperlipidemia, JTT-705 was shown to be safe and effective in decreasing CETP activity and raising HDL-C levels.

Synthesis

Condensation of cyclohexanecarboxylic acid (I) with 1-bromo-2-ethylbutane (II) by means of lithium diisopropylamide (LDA) or LDA/NaH in THF or THF/cyclohexane affords the cyclohexanecarboxylic acid derivative (III), which by treatment with oxalyl chloride in CH_2Cl_2 or CH_2Cl_2/DMF yields the acid chloride (IV). Condensation of acid choride (IV) with bis(2-aminophenyl) disulfide (V) in pyridine provides the symmetric disulfide derivative (VI), which by reduction of its disulfide bond with PPh_3 in dioxane/H_2O gives the benzenethiol (VII). Finally, this compound is coupled with isobutyryl chloride (VIII) in pyridine/$CHCl_3$ (1, 2). Scheme 1.

Introduction

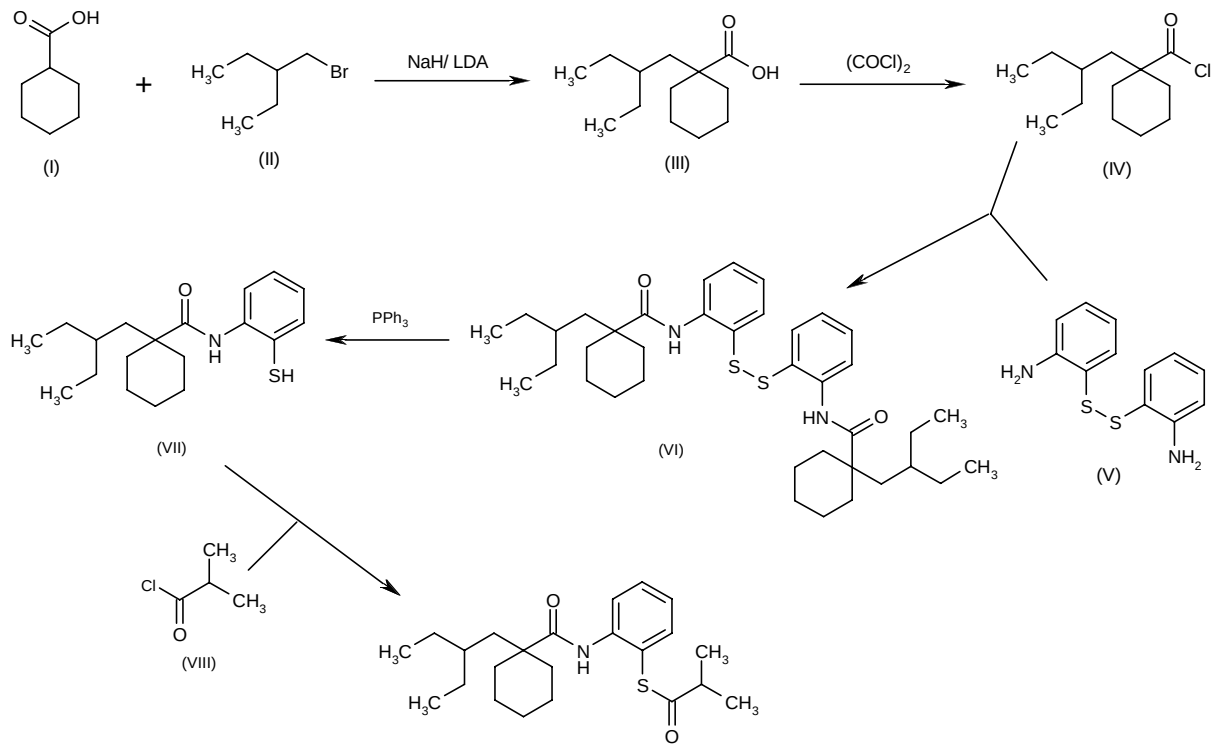
Numerous clinical and epidemiological studies, such as the Framingham Study (3) and the Seven Countries Study (4), have demonstrated a strong, direct relationship between high levels of plasma low-density lipoprotein cholesterol (LDL-C) and the incidence of coronary heart disease (CHD). Therefore, the main strategy for reducing the risk of CHD has been lowering LDL-C levels. The introduction of statins, inhibitors of the enzyme HMG-CoA reductase, confirmed that correction of hypercholesterolemia reduced the risk of developing atherosclerosis-related cardiovascular diseases. Several primary and secondary prevention studies (5-9) have shown that statins can significantly reduce coronary and total mortality. However, when the results of these studies are examined in depth, it appears that, even when LDL-C goals are achieved, there is still an important proportion of patients who are at high risk of dying from CHD. Moreover, it is sometimes difficult to reach the low LDL-C values recommended, particularly in patients at high risk. For these reasons, different approaches aimed at reducing atherosclerosis and its consequences are being tested in addition to cholesterol lowering.

One of these involves increasing high-density lipoprotein cholesterol (HDL-C) levels, as several studies have demonstrated an inverse association between HDL-C levels and the incidence of CHD (10). In fact, every 1% increase in HDL-C is associated with a 3% reduction in cardiovascular events (11).

HDL-C exerts a multitude of antiatherosclerotic effects, but one of the most extensively studied is its role in the pathway termed reverse cholesterol transport (RCT) (12). This process begins with the interaction

Marta Alegret, Unit of Pharmacology, Department of Pharmacology and Therapeutic Chemistry, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain; Jordi S. Silvestre, J. Castañer, Prous Science, P.O. Box 540, 08080 Barcelona, Spain.

Scheme 1: Synthesis of JTT-705



between HDL and peripheral cells, such as macrophages, which deliver cholesterol to these lipoproteins either by aqueous diffusion or through the scavenger receptor class B type I (SR-BI) (13). Lipid-free apolipoprotein A-I (apo A-I) acts also as an acceptor of cholesterol that effluxes from peripheral tissues via the ATP-binding cassette transporter A1 (ABCA1) (14). By this process, pre-β HDL particles are generated, and subsequently converted into spherical HDL particles due to the esterification of their free cholesterol by the action of lecithin-cholesterol acyltransferase (LCAT). The cholesteryl esters (CE) formed are then transferred by means of cholesteryl ester transfer protein (CETP) from HDL to the apo B-containing lipoproteins, very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL) and LDL. LDL and IDL are then catabolized via the LDL receptor, delivering cholesterol to the liver in the last step of RCT.

The CETP enzyme catalyzes the bidirectional transfer of CE and triglycerides (TG) between plasma lipoproteins (15, 16). The overall effect of CETP under normal conditions is to promote a net transfer of CE from HDL to triglyceride-rich lipoproteins (VLDL and chylomicrons), and of TG from these lipoproteins to HDL and LDL. As a result, HDL particles become enriched in TG, and are hydrolyzed by hepatic lipase, rendering smaller HDL particles named HDL3. Therefore, CETP plays an important role not only in the regulation of plasma HDL-C levels, but also in the remodeling of HDL particles.

The interest in CETP as a drug target was raised when it was discovered that a deficiency of this molecule in humans caused hyperalphalipoproteinemia, with markedly high serum HDL-C levels (17). However, the pro- or antiatherogenicity of CETP has been a matter of debate for a long time. On the one hand, it was suggested that CETP could inhibit atherogenesis by enhancing the rate of RCT; moreover, CETP remodels HDL particles to form smaller ones, which are better acceptors of cholesterol effluxed from peripheral cells (18). Supporting the antiatherogenicity of CETP, several epidemiological studies showed that individuals with a CETP genetic deficiency have a high risk for atherosclerosis (19-22). However, it is interesting to note that when CETP deficiency is associated with high HDL-C levels (> 80 mg/dl), there is no evidence of an increase in CHD (16, 17, 23). Therefore, the atherogenicity of CETP deficiency is highly dependent on the metabolic background.

On the other hand, CETP may be considered a proatherogenic enzyme, as it can transfer CE from antiatherogenic HDL to apo B-containing lipoproteins, thereby increasing LDL-C and lowering HDL-C levels

(24). Supporting this hypothesis, studies in cholesterol-fed rabbits showed that inhibition of CETP with antisense oligonucleotides (25), generation of autoantibodies against CETP (26) or chemical compounds (27) caused significant reductions in atherosclerotic lesions. The discrepancy between human CETP deficiency and CETP inhibition in rabbits may be attributed to the fact that, although in both cases HDL-C was higher in comparison with the respective controls, total cholesterol was markedly reduced in rabbits but not in humans with CETP deficiency (18).

Despite the controversial role of CETP in atherogenesis, this enzyme has become an interesting target for raising HDL-C and several compounds that inhibit CETP activity have been synthesized (1, 24, 28). One of the most promising compounds is JTT-705, which is currently in phase II clinical trials in Europe. This compound was obtained as a result of a strategy to design CETP inhibitors developed by investigators from Japan Tobacco (1). A series of disulfides, thiols, thioesters and sulfides were studied, searching for orally active molecules able to selectively inhibit CETP activity. Bis[2-(acetylamino)-phenyl]disulfide was selected for structure-activity studies because it inhibited CETP in human plasma by 50% at a concentration of 500 μM. It was shown that the alkyl moiety markedly increased the potency of the tested compounds, and that the amide and disulfide groups were essential for inhibition of CETP. It was also found that thiols functioned like disulfides, and that a dimeric structure was not necessary to inhibit CETP. However, disulfides are not orally available and thiols are unstable, so the thioester JTT-705, which can be administered orally and is readily converted to the active thiol after its administration, was considered the optimum compound for further development.

Pharmacological Actions

Mechanism of CETP inhibition

JTT-705 inhibits CETP activity by forming a disulfide bond with a cysteine residue of the enzyme. Although no CETP/JTT-705 complexes have been identified in the plasma of treated rabbits, studies using monoclonal antibodies against CETP suggested that JTT-705 induces structural changes in the enzyme that prevent binding to the antibody (27). These results are consistent with the formation of a bond between the inhibitor and CETP. To investigate which cysteine was involved, Shinkai *et al.* (1) constructed several CETP mutants in which the cysteine at residue 1, 13 or 131 was replaced by a serine. From these studies, it was concluded that JTT-705 binds to the cysteine located at position 13 of the CETP molecule. According to the model proposed by Sikorski and Connolly (24), disulfide-based CETP inhibitors act in two phases: first, they bind to a hydrophobic site that is near the neutral lipid-binding site of CETP, disturbing the binding of CE and TG to the enzyme. This reaction is fast and reversible, and it is followed by a second reaction involving a disulfide exchange between a cysteine group of the enzyme and the disulfide moiety of the drug, leading to irreversible inhibition of CETP.

CETP-inhibitory activity

The inhibitory effect of JTT-705 on CETP activity has been measured *in vitro* as the ability to mediate the transfer of CE from HDL to apo B-containing lipoproteins in plasma from several species. The IC_{50} values obtained were 5.5-6 μM for humans (1, 29), 1 μM for rabbits, 2.4 μM for cynomolgus monkeys, 6.3 μM for marmosets and around 12 μM for hamsters (29). The *in vitro* inhibitory activity of the thiol derivative (JTT-25203) was superior to that of JTT-705, and the IC_{50} values for this compound are quite similar among different animal species (29). It is believed that JTT-705 similarly inhibits CETP activity in different animal species, but its conversion to the thiol form may differ.

The inhibitory effect of JTT-705 is specific for CETP, as demonstrated in *in vitro* and clinical studies where the compound did not inhibit other enzyme activities related to lipid metabolism, such as ACAT, HMG-CoA reductase (27), phospholipid transfer protein (PLTP) and LCAT (30).

The ability of JTT-705 to inhibit CETP was confirmed by measuring *ex vivo* CETP activity in the plasma of Japanese white rabbits 3 h after oral administration of the compound. At a dose of 10 mg/kg, JTT-705 inhibited CETP activity by 35%, while at a dose of 30 mg/kg CETP activity was inhibited by 95% (1). In a similar study, Okamoto *et al.* (29) determined CETP activity in the plasma of rabbits treated orally with JTT-705 at doses of 10, 30, 100 and 300 mg/kg for 1 and 3 days. The compound inhibited CETP activity dose-dependently, with significant effects seen from 30 mg/kg.

Effects on plasma lipids, lipoproteins and atherosclerotic lesions

The acute effect of JTT-705 on plasma HDL-C levels was determined in normolipidemic Japanese white rabbits. Administration of the compound for 3 days at doses of 10, 30, 100 and 300 mg/kg raised plasma HDL-C levels by 16%, 27%, 54% and 59%, respectively (1, 29). Although JTT-705 did not increase non-HDL-C levels, plasma total cholesterol was increased at the highest dose tested. Conversely, HDL-TG concentrations were significantly reduced after treatment with JTT-705 (100 and 300 mg/kg) for 3 days (29). Similar results (*e.g.*, reduction in CETP activity, increase in HDL-C and decrease in HDL-TG) were obtained after oral administration of JTT-705 to hamsters and marmosets (29).

In another experiment in Japanese white rabbits fed regular chow, the administration of JTT-705 (0.06%, 0.2% and 0.6% in the diet) for 7 days caused a dose-dependent decrease in plasma CETP activity and an increase in

plasma HDL-C, which achieved statistical significance at the highest dose tested. The increase in HDL-C was considered to be a consequence of CETP inhibition, as both phenomena were strongly correlated ($r = -0.695$, $p < 0.01$) and there was no increase in HDL levels when JTT-705 was administered to rats, a species devoid of CETP activity (27).

A long-term study was performed in cholesterol-fed rabbits treated with JTT-705 (0.75%) or simvastatin (0.075%) for 6 months (27). Plasma CETP activity was progressively reduced in rabbits treated with JTT-705, reaching 97% inhibition at the end of treatment. Although simvastatin also reduced CETP activity after 6 months of treatment, the differences were not statistically significant. On the other hand, the effects of JTT-705 and simvastatin in reducing non-HDL-C levels were comparable, but the CETP inhibitor raised HDL-C to a markedly greater extent than the statin (90% and 28%, respectively). Although in view of the physiological role of CETP it would be logical to expect that CETP inhibition would cause a shift towards larger HDL2 particles, it was shown that JTT-705 increased both HDL2 and HDL3; however, the effect was more intense on HDL2 (27, 31), which represents the antiatherogenic fraction. By contrast, simvastatin only increased HDL3. Finally, the effect of JTT-505 and simvastatin on atherosclerosis development in this rabbit model of hypercholesterolemia was examined. Both compounds caused a marked, significant reduction (70% and 80%, respectively) in the aortic arch atherosclerotic area compared with progression in controls.

In a study by Okamoto *et al.* (27), feeding rabbits with diets containing 0.2% cholesterol resulted in moderate hypercholesterolemia, with plasma cholesterol levels of 129 mg/dl. A slight increase in the cholesterol content of the diet (from 0.2% to 0.25%) caused a huge increase in plasma cholesterol levels of these rabbits, which reached 757 mg/dl (32). In this model of severe hypercholesterolemia, treatment with JTT-705 at a high dose (300 mg/kg) for 3 months significantly increased HDL-C, but did not reduce the progression of atherosclerotic lesions. It should be noted that, in this study, the levels of non-HDL-C were very high, and treatment with JTT-705 caused only a minor decrease (about 25%) in this parameter. Therefore, in a situation of extremely high non-HDL-C levels, an increase in HDL-C due to CETP inhibition is not sufficient to protect from atherosclerosis. On the other hand, the unexpected increase in TG levels found in the study performed by Huang *et al.* (32) could account for the lack of effect of JTT-705 on atherosclerosis development. Thus, a significant correlation between the increase of TG and non-HDL-C levels and the development of atherosclerosis was found, while CETP activity and HDL-C levels were not correlated with the atheromatous area (32). These results suggest that it will be necessary to carefully monitor plasma TG levels in individuals treated with JTT-705.

CETP inhibition by JTT-705 also decreased the phospholipid (PL) content of HDL and the serum levels of PL (31). It has been reported that JTT-705 does not affect the activity of PLTP (30). On the other hand, the introduction of the CETP gene in PLTP knockout mice results in lower levels of HDL-PL (33). Thus, it has been proposed that CETP may mediate PL exchange between lipoproteins, and therefore, CETP inhibition may cause a reduction in HDL-PL.

Antiatherogenic mechanisms

Several studies have been conducted in order to establish the mechanism responsible for the antiatherogenic effects of JTT-705. One possibility is that HDL from treated animals has a greater ability to stimulate cholesterol efflux from lipid-loaded macrophages. To test this hypothesis, normolipidemic Japanese white rabbits were treated with JTT-705 at a dose of 300 mg/kg/day for 1 week and plasma HDL was obtained (34). HDL isolated from treated rabbits concentration-dependently prevented cholesterol accumulation in an *in vitro* model of foam cell formation (acetyl-LDL-loaded J774 macrophages). However, there were no differences between HDL from control and treated rabbits in terms of reductions in macrophage intracellular CE levels, suggesting that the antiatherosclerotic effects of JTT-705 are not related to an increase in the efficiency of HDL as a cholesterol acceptor, but rather to an increase in HDL concentration (34).

The mechanism by which JTT-705 raises HDL levels may be related to an increase in its main apolipoprotein, apo A-I. It has been shown that administration of JTT-705 (0.75% in the diet) for 6 months increases serum apo A-I levels in rabbits by 78% (27). The increase in apo A-I could be due to increased synthesis or to reduced catabolism of the apolipoprotein. To clarify the mechanism, the *in vivo* kinetics of apo A-I were examined in rabbits treated with 0.75% JTT-705 for 7 months (31). The results of this study revealed that there were no differences in the fractional catabolic rate of apo A-I between control and treated rabbits. On the other hand, treatment with JTT-705 significantly increased apo A-I synthesis, and there was a positive correlation between plasma apo A-I levels and the synthetic rate of this apolipoprotein. In the same study, apo A-I gene expression was determined in the liver and intestine of control and JTT-705-treated rabbits by quantitative RT-PCR. The results showed an increase in hepatic but not intestinal apo A-I mRNA levels. Given that in rabbits apo A-I is synthesized primarily in the intestine, the increase in hepatic apo A-I mRNA can not account for the increase in apo A-I synthetic rate in this animal model.

Other effects

Administration of JTT-705 (0.75% in the food) to normolipidemic rabbits for 7 months decreased body weight gain. The effect was not attributable to a reduction in food intake, but was rather related to a decrease in CE accumulation in adipocytes due to inhibition of

CETP-mediated transfer of CE from HDL to this tissue (31).

Clinical Studies

JTT-705 has been tested in three phase I clinical studies (30). In a single-dose study, the compound (100-1800 mg/day) was well tolerated in healthy human volunteers and did not cause significant toxicity. Another crossover bioavailability study demonstrated that JTT-705 was more effective in the postprandial period than in the fasted state. The third study was a 14-day multiple-dose study in which JTT-705 was administered at doses of 600 and 900 mg/day. The results of this study confirmed that the compound significantly increased HDL-C and reduced LDL-C levels.

In a randomized, double blind, placebo-controlled phase II study, the efficacy and safety of JTT-705 (300, 600 and 900 mg/day for 12 weeks) were evaluated in hyperlipidemic patients (30). The study cohort consisted of individuals with mild hyperlipidemia (mean plasma total cholesterol of 5.6 mmol/l and plasma TG levels of 1.5 mmol/l). JTT-705 was well tolerated even at the highest dose, causing no changes in vital signs or hepatic or renal toxicity. The main side effects reported, especially at the dose of 900 mg/day, were gastrointestinal (diarrhea, flatulence, nausea or constipation). However, these effects were mild, and there were no withdrawals due to gastrointestinal complaints. JTT-705 caused a clear dose-dependent inhibition of CETP activity which was accompanied by an increase in CETP mass (maximal inhibition of CETP activity of about 37% and maximal increases in CETP concentration of 67%, both after 4 weeks of treatment at the highest dose). The increase in CETP mass could be a compensatory mechanism by which the organism tries to overcome the reduction in the enzyme activity caused by the drug. However, inhibition of CETP by antisense oligonucleotides does not increase, but rather causes a decrease in plasma levels of CETP protein (25), arguing against the hypothesis of a compensatory mechanism. Therefore, de Grooth *et al.* (30) suggest that the formation of a complex between JTT-705 and CETP delays the hepatic clearance of the protein, an effect that may result in an increase in CETP plasma concentration. Regarding the effect of JTT-705 on plasma lipids and lipoproteins, the compound dose-dependently increased plasma HDL-C and apo A-I. The effect was already seen at 1 week, reached a plateau from then on, and was maximal in patients treated for 4 weeks with the highest dose (increase of approximately 34%). In accordance with the results obtained in animal models, JTT-705 increased both HDL2 and HDL3 subfractions. Together with the increase in HDL-C, JTT-705 caused a modest but significant decrease (7%) in LDL-C in the group treated with 900 mg/day. This effect was positively correlated with baseline LDL-C levels, so that a greater effect on LDL-C was observed in individuals with high baseline LDL-C levels.

Conclusions

JTT-705 is a potent and selective inhibitor of CETP that has shown efficacy in increasing plasma HDL-C in both rabbits and humans. Moreover, administration of this compound to mildly hyperlipidemic rabbits results in an impressive reduction in the development of atherosclerotic lesions. However, it should be mentioned that in rabbits with more severe hypercholesterolemia, administration of JTT-705 did not reduce aortic cholesterol content despite causing a significant increase in HDL-C. These results indicate that the consequences of CETP inhibition will greatly depend on the metabolic, environmental and racial background. Therefore, CETP inhibitors will probably be beneficial in patients with moderate hypercholesterolemia and possibly in combined hyperlipidemia, but their role in hypertriglyceridemia or in a situation of defective RCT has to be carefully evaluated. In this sense, the only clinical study published to date was carried out in patients with moderate hypercholesterolemia, in which administration of JTT-705 significantly increased HDL-C. However, more studies are needed to confirm the potential of JTT-705 and other CETP inhibitors for reducing the development of atherosclerosis in humans.

Source

Japan Tobacco, Inc. (JP).

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