Single dose pharmacokinetics, pharmacodynamics, tolerability and safety of BAY 60–5521, a potent inhibitor of cholesteryl ester transfer protein

Michael-Friedrich Boettcher, Roland Heinig, Carsten Schmeck, Christian Kohlsdorfer, Matthias Ludwig, Anja Schaefer, Sabine Gelfert-Peukert, Georg Wensing & Olaf Weber

Bayer Pharma AG, 42096 Wuppertal, Germany

Correspondence

Mr Michael-Friedrich Boettcher, Clinical Pharmacology, Bayer Pharma AG, 42113 Wuppertal, Germany.
Tel.: +49 202 36 8362
Fax: +49 202 36 4115
E-mail: michael-friedrich.boettcher@bayer.com

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that facilitates the transfer of cholesterol esters from the cardioprotective high density lipoprotein cholesterol (HDL-C) to the proatherogenic low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) leading to lower concentrations of HDL-C but raising the concentrations of proatherogenic LDL-C and VLDL-C.
- Inhibition of CETP is considered a potential approach to treat dyslipidaemia.
- Phase III studies demonstrating no benefit in regard to the defined clinical end points with the CETP inhibitor torcetrapib challenged the future perspectives of CETP inhibitors as potential therapeutic agents although it has been recently discussed whether potential off-target effects of torcetrapib could have contributed to the failure of this CETP inhibitor.
- It has been suggested in recent publications to continue studying other CETP inhibitors for their potential to improve plasma lipid profiles and reduce cardiovascular risk. Currently, several compounds are being investigated in preclinical or clinical studies.

WHAT THIS STUDY ADDS

- This study provides information on the safety, pharmacokinetics (PK) and pharmacodynamics (PD) of a potential new treatment for dyslipidaemia.
- Data from a first in man study with the new CETP inhibitor BAY 60–5521 are presented that demonstrate that BAY 60–5521 is clinically safe and well tolerated. No effects on heart rate, blood pressure and ECG recordings were observed during the study. A clear pharmacodynamic effect on CETP inhibition and HDL-C could be demonstrated.

AIMS

To determine pharmacokinetics (PK), pharmacodynamics (PD), tolerability and safety of BAY 60–5521, a potent inhibitor of cholesteryl ester transfer protein (CETP).

METHODS

The first in man (FIM) study investigated the safety, tolerability, pharmacodynamics and pharmacokinetics in healthy male subjects following administration of single oral doses. The study was performed using a randomized, single-blind, placebo-controlled, single dose-escalation design. Thirty-eight young healthy male subjects (aged 20–45 years) received an oral dose of 5, 12.5, 25 or 50 mg BAY 60–5521 ($n = 28$) or were treated with a placebo ($n = 10$).

RESULTS

In all four dose steps, only one adverse event (25 mg; mild skin rash) was considered drug related. Clinical laboratory parameters showed no clinically relevant changes. A clear dose-dependent CETP inhibition could be demonstrated starting at a dose of 5 mg. At a dose of 25 mg, a CETP inhibition >50% over 18 h was observed. After 50 mg, CETP inhibition >50% lasted more than 50 h. Twenty-four h after administration mean HDL-C-values showed a nearly dose-proportional increase. Following administration of 50 mg, a significant HDL-C increase of about 30% relative to baseline values was found. BAY 60–5521 was slowly absorbed reaching maximum concentrations in plasma after 4 to 6 h. The disposition in plasma was multi-exponential with an estimated mean terminal half-life of 76 to 144 h.

CONCLUSIONS

BAY 60–5521 was clinically safe and well tolerated. No effects on heart rate, blood pressure and ECG recordings were observed during the study. A clear pharmacodynamic effect on CETP inhibition and HDL could be demonstrated.

Introduction

Cardiovascular diseases (CVD) represent the leading cause of death in the developed world [1]. Coronary events are of particular importance and account for more than 50% of all CVD-related deaths [2]. Rupture of atherosclerotic plaques and subsequent thrombosis accounts for approximately 70% of coronary events [3]. Atherosclerosis is a chronic inflammatory disease characterized by endothelial dysfunction, vascular inflammation, and the build up of lipids, cholesterol, calcium and cellular debris within the intima of the vessel wall [3–5].

High plasma high-density lipoprotein cholesterol (HDL-C) is associated with a decreased incidence of coronary events [6]. HDL-C plays a major role in the transfer of excess cholesterol from the peripheral tissues to the liver (reverse cholesterol transport), where it can be cleared from the body.

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that mediates the transfer of cholesterol esters from the cardioprotective HDL-C to the proatherogenic low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) leading to lower concentrations of HDL-C while raising the concentrations of proatherogenic LDL-C and VLDL-C. On the other hand, CETP transfers triglycerides (TG) from VLDL-C or LDL-C to HDL-C leading to TG-enriched HDL-C which is more readily hydrolyzed by hepatic lipase and results in smaller-sized HDL-C particles that more effectively promote reverse cholesterol transport [7]. Thus, CETP inhibitors might be a powerful tool for increasing HDL-C, decreasing LDL-C and reducing the development of atherosclerosis [8].

However, the discussions around the role of CETP-mediated lipid transfer and its inhibition have been controversial [9]. While many animal studies support the hypothesis that inhibition of CETP activity is beneficial [10] phase III studies not meeting clinical end points with the CETP inhibitor torcetrapib challenged the future perspectives of CETP inhibitors as potential therapeutic agents [11–14]. It has been discussed recently whether potential off-target effects of torcetrapib, such as an increase in blood pressure, may have contributed to the failure of this CETP inhibitor [15–17], and it has been suggested to continue studying other CETP inhibitors for their potential to improve plasma lipid profiles and reduce cardiovascular risk. Indeed, currently there are several compounds under investigation in preclinical or clinical studies [17–19].

This study describes the safety, tolerability, pharmacokinetics (PK) and pharmacodynamics (PD) of BAY 60–5521 in healthy male subjects following administration of single oral doses. BAY 60–5521 is a novel tetrahydroquinoline derived CETP inhibitor with a molecular weight of 503.6 g mol⁻¹, a calculated pK_a value of 5.7 and a log P_{OW} of 8.8 (partition coefficient, calculated) [20]. The compound is practically insoluble in water, sparingly soluble in polyethylene glycol (PEG) and freely soluble in ethanol. Preclinical

investigations in transgenic hCETP mice demonstrated that BAY 60–5521 inhibits CETP and elevates serum HDL [21].

Methods

Study participants

All study participants provided written informed consent prior to enrolment. The protocol was approved by the Ethics Committee of the North Rhine Medical Council, Düsseldorf, Germany and by the competent authority (Bundesinstitut fuer Arzneimittel und Medizinprodukte [BfArM]). The study was conducted in accordance with the guidelines on good clinical practice, with the Declaration of Helsinki and the German drug law. Healthy male subjects, 20–45 years old, were included in this study, which was conducted at the study ward, Bayer Healthcare AG, Wuppertal, Germany. Subjects were in good general health according to routine medical history, physical examination, vital signs and laboratory data.

Study design and treatment

The study was performed in a randomized, single-blind, placebo-controlled, single dose-escalation design.

An oral solution of BAY 60–5521 with 4.0 mg drug per 1.0 g solution was used. The solution consists of a two bottle system and was reconstituted before administration from the powder and the diluent, each filled in brown glass bottles separately. After reconstitution, each bottle contained a withdrawal volume of 18.5 g solution. The PEG-based diluent is composed of macrogol, polysorbate 20 (2.5%) and L- menthol. The corresponding placebo formulation contained the same excipients.

In the morning, 38 subjects received a dose of 5 (*n* = 6), 12.5 (*n* = 9), 25 (*n* = 7) or 50 mg (*n* = 6) BAY 60–5521 or were treated with a placebo (*n* = 10) after a fasting period of at least 10 h. Lunch was served 4 h after drug intake.

The dose selection for this study was based on allometric scaling methods that used intravenous and oral PK data from mice, rats and dogs and effective AUC in human CETP-transgenic mice. The starting dose of 5 mg was expected to be a no-effect dose.

The study period consisted of an examination, admission to the ward 25 h before dosing, a single dose administration of BAY 60–5521 or placebo on the profile day and an in-house observational period of 96 h, followed by an ambulatory phase up to 10 days. The final examination was performed approximately 7 days after the last study day.

Pharmacokinetic blood samples were collected at pre dose and 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 24, 36, 48, 72, 96, 120, 144, 168, 192*, 216*, 240*, 312*, 336* h after administration. In dose step 1 samples were taken up to 168 h after drug intake whereas in dose steps 2–4 additional samples (*) were taken up to 336 h after drug administration.

Safety and tolerability

The safety and tolerability of BAY 60–5521 were assessed throughout the study by scheduled physical examination, vital signs, 12-lead ECG and laboratory safety tests, and adverse events were identified by subject questioning or self-reporting. Laboratory safety tests were performed 24 h before, immediately before (pre dose) and 24, 48, 72, 96 and 120 h after administration.

Bioanalysis and pharmacokinetic data analysis

Blood plasma samples were collected for up to 336 h (168 h at starting dose) after administration of BAY 60–5521 and stored at or below -15°C until the time of analysis. BAY 60–5521 concentrations were determined after protein precipitation by a fully validated assay using liquid chromatography coupled with a tandem mass spectrometer (HPLC-MS/MS). A structural analogue of BAY 60–5521 was used as internal standard. The validated working range was comprised of 0.2 ng ml^{-1} (the lower limit of quantification, LLOQ) to 2000 ng ml^{-1} . Inter-day accuracy and precision of the assay were equal to 91.1–107% and 1.80–12.4%, respectively. BAY 60–5521 concentrations in plasma were unchanged after three freeze-thaw cycles and stability after storage at -15°C could be demonstrated for at least 3.5 months. Quality control (QC) samples (0.600 to 1800 ng ml^{-1}) were analyzed together with study samples with an accuracy of 91.0–106% and a precision of 2.30–11.5%. Plasma concentration vs. time data were evaluated using non-compartmental techniques (WinNonlin, version 4.1, Pharsight Corporation). C_{max} and the times needed to reach these concentrations (t_{max}) were assessed by inspection of the concentration vs. time plots. The AUC was calculated using the log-linear trapezoidal rule up to the last time point with a concentration above LLOQ (t_n) plus the extrapolated portion $[\text{AUC}(t_n, \infty)]$ calculated by the terminal phase rate constant (λ_z) which was the slope of the log-linear regression of the last n data points (where $n \geq 3$) and the estimated concentration at t_n ($C'(t_n)$) using the formula $C'(t_n)/\lambda_z$. The $\text{AUC}(0, t_n)$ was the AUC from time 0 to the last data point. Mean residence time (MRT) was calculated as ratio $\text{AUMC} : \text{AUC}$. AUC/D and C_{max}/D were dose-normalized exposure parameters (AUC and C_{max} divided by dose) and AUC_{norm} and $C_{\text{max, norm}}$ were dose- and body weight-normalized parameters (AUC and C_{max} divided by dose (mg) per kg body weight).

Pharmacodynamic data analysis

CETP inhibition was determined involving only modest dilution (1.27-fold) during the assay procedure of the serum samples collected, at pre dose and 0.25, 0.50, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 24, 36, 48, 72, 96, 120 h after administration of the test compounds.

The preparation of fluorogenic donor liposomes for the microemulsion-based CETP activity assay was performed

as previously described [22], using a modification of the method of Bisgaier [23]. In brief, $72\text{ }\mu\text{l}$ undiluted serum samples were mixed with $20\text{ }\mu\text{l}$ of a donor liposome preparation in a microtitre plate and the fluorescence of the assay mixture was determined at an excitation wavelength of 485 nm and an emission wavelength 535 nm before and after a 24 h incubation period at 37°C protected from light, using a Tecan GENios microtitreplate fluorimeter. All samples were analyzed in quadruplicate. As a direct measure of CETP activity in the sample, the mean difference between pre and post incubation fluorescence values of the quadruplicate assays was calculated, and the inhibition of CETP activity was determined relative to the individual's pre dose sample activity value. The precision and accuracy of the method were 12.1% and 110.8%, respectively.

Statistical methods

The statistical evaluation was performed by using the SAS (Version 9.1) software package. Thirty-eight subjects completed the study and were included in the safety population. Thirty-five subjects were valid for PD analysis and 28 subjects for PK analysis.

Data were analyzed using descriptive summary statistics (arithmetic mean, SD, median, minimum, maximum and frequency counts). All statistical tests were performed in an explorative manner and therefore without adjustment for multiplicity.

The CETP inhibition was calculated with reference to the pre-administration value of each subject and summarized using descriptive statistics for each sampling point separated by treatment. Additionally, the time during which CETP inhibition exceeds 50% was calculated and 95% confidence intervals were provided. HDL-C at 24 h was compared vs. baseline using summary statistics and an explorative t -test with comparison to an unadjusted significance level of $\alpha = 0.05$.

For plasma concentrations, descriptive statistics were calculated (geometric mean and CV) by timepoint and dose step. Means at any time were only calculated if at least 2/3 of the individual data were measured and were above the lower limit of quantification (LLOQ). For the calculation of the mean value, a data point below LLOQ was substituted by one half of this limit. Pharmacokinetic characteristics (t_{max} excluded) were summarized by descriptive statistics (geometric mean and CV). For t_{max} , median and range were calculated. To investigate dose-proportionality without any assumptions about the form of the relationship between dose and pharmacokinetic parameters an ANOVA (including the factor 'treatment') was performed on the log-transformed values of AUC/D , AUC_{norm} , C_{max}/D and $C_{\text{max, norm}}$. Based on these analyses point estimates (LS-means) and 95% confidence intervals for the treatment ratios were calculated by retransformation of the logarithmic data using the intra-individual standard deviation of the ANOVA. For the analysis of the correlation between BAY

Figure 1

CETP inhibition (mean, SD) after single oral administration of BAY 60–5521 or placebo. Placebo, $n = 10$ (—○—); 5.0 mg, $n = 6$ (—■—); 12.5 mg, $n = 6$ (—▲—); 25.0 mg, $n = 7$ (—▼—); 50.0 mg, $n = 6$ (—●—)

60–5521 plasma concentration and CETP inhibition hysteresis plots were evaluated. Signs for hysteresis were not evident suggesting a direct link between BAY 60–5521 plasma concentration and CETP inhibition. Individual plasma-concentrations (C) of BAY 60–5521 and CETP inhibition (I) data collected within 12 h after administration were pooled and the parameters $I_{\max}$ (maximum inhibition) and concentration resulting in 50% of maximum inhibition (IC_{50}) were estimated by fitting the equation $I = \frac{I_{\max} \times C^n}{IC_{50}^n + C^n}$ to the data using SigmaPlot 2001 for Windows Version 7.0.

Results

Pharmacodynamic effects

In healthy subjects, BAY 60–5521 at doses of 5, 12.5, 25 and 50 mg led to dose-dependent CETP inhibition and increases in HDL-C.

The mean CETP inhibition observed in this single dose escalation study is depicted in Figure 1. A dose-dependent CETP inhibition could be demonstrated. Starting at a single dose of 25 mg, a CETP inhibition exceeding 50% was observed over a time interval of more than 18 h and following administration of 50 mg, CETP inhibition exceeding 50% lasted for more than 50 h (Table 1). The 50 mg dose group revealed a mean duration of 50.17 h with CETP inhibition >50% (95% confidence interval 23.17, 77.16 h). The

Table 1

Time of CETP inhibition above 50% (h)

Treatment	CETP inhibition Mean	SD	95% confidence interval
Placebo ($n = 10$)	0.00	–	–
5.0 mg ($n = 6$)	3.95	3.11	0.68, 7.21
12.5 mg ($n = 6$)	10.84	3.36	7.31, 14.37
25.0 mg ($n = 7$)	18.57	4.23	14.66, 22.49
50.0 mg ($n = 6$)	50.17	25.72	23.17, 77.16

profiles of CETP activity in the placebo treated subjects pointed to an increase of endogenous CETP activity starting with the 6 h time point. Due to insufficient serum volume in the sample tubes, CETP inhibition could not be determined for three subjects (#17, #20, #22) in the 12.5 mg dose step.

HDL-C values were determined prior to drug administration and 24 h after drug intake. Mean HDL-C values showed a nearly dose-proportional increase (Figure 2). A significant ($P \leq 0.003$) HDL-C increase by about 30% relative to baseline values was found after a single oral dose of 50 mg BAY 60–5521.

Similarly to HDL-C, LDL-C, triglycerides and total cholesterol were determined. Compared with baseline, LDL and total cholesterol showed no changes. Triglycerides were

Figure 2

Mean (SD) HDL-C differences (24 h value – baseline [pre-administration value]) after placebo, 5 mg, 12.5 mg, 25 mg, and 50 mg BAY 60–5521. Placebo, $n = 10$ (—○—); 5.0 mg, $n = 6$ (—■—); 12.5 mg, $n = 9$ (—▲—); 25.0 mg, $n = 7$ (—▼—); 50.0 mg, $n = 6$ (—●—)

slightly reduced by about 26% 24 h after administration of 50 mg. However, this effect was statistically not significant.

Pharmacokinetics

BAY 60–5521 plasma concentration vs. time profiles (geometric mean values) are shown in Figure 3. Table 2 summarizes the pharmacokinetic parameters of BAY 60–5521. The compound was slowly absorbed reaching maximum concentrations in plasma after 4 to 6 h (median $t_{\max}$). The disposition in plasma was multi-exponential with estimated mean terminal half-lives ($t_{1/2}$) of 76 to 144 h and plasma concentrations were above the LLOQ in all subjects throughout the entire observation period. The value of 76 h may underestimate the actual $t_{1/2}$ as it was obtained after the lowest dose, when the observation period was 7 days instead of 14 days utilized at higher doses. Systemic exposure increased in a dose-dependent manner. The dose-normalized exposure parameters $C_{\max}/D$ and AUC/D displayed pronounced variability between the cohorts but did not change with dose in a consistent fashion. AUC/D (180.6 and $219.0 \text{ h} \times 10^{-3} \text{ l}^{-1}$) and $C_{\max}/D$ values (11.23 and $11.35 \times 10^{-3} \text{ l}^{-1}$, geometric mean) at the lowest and highest investigated dose were similar. The 95% confidence intervals of the treatment ratios (50 mg/5 mg) for AUC/D (0.8191, 1.7952; point estimate 1.2126 (geo. LS mean)) and $C_{\max}/D$ (0.6515, 1.5670; point estimate 1.0104) included unity supporting the assumption of dose-proportionality. The apparent oral clearance CL/F of BAY 60–5521 was low ($4.6\text{--}7.2 \text{ l h}^{-1}$). Figure 4 depicts the individual data of the

concentration–effect relationship between BAY 60–5521 plasma concentrations and CETP inhibition up to 12 h following the administration of 5 mg, 12.5 mg, 25 mg and 50 mg doses. A time-delay between plasma concentrations of the drug and inhibition of CETP (hysteresis) was not evident and the PK/PD relationship could be described with an $I_{\max}$ model with estimates for IC_{50} and $I_{\max}$ of $32.2 \mu\text{g l}^{-1}$ and 98.6%, respectively, and a small sigmoidicity factor (exponent) of 1.1.

Safety and tolerability

Thirty-eight young healthy male subjects completed all four dose steps. Twenty-eight of them received BAY 60–5521 and 10 received placebo.

In all four dose steps, treatment emergent adverse events were observed in eight subjects. Only one adverse event (mild skin rash) was considered drug related (Table 3).

Shortly after manipulation at the indwelling cannula, a vasovagal syncope followed by atrial fibrillation of 57 min duration occurred after 50 mg BAY 60–5521 treatment in one subject. Atrial fibrillation returned spontaneously to sinus rhythm. These adverse events were classified as medically important, but not related to study medication. This diagnosis was confirmed by an external cardiologist. Thereafter, the subject stated he had already had similar reactions on two occasions under directly comparable circumstances before the start of the study.

Figure 3

BAY 60–5521 plasma concentration vs. time profiles by dose step (geometric mean values). 5.0 mg, $n = 6$ (—●—); 12.5 mg, $n = 9$ (—▲—); 25.0 mg, $n = 7$ (—▼—); 50.0 mg, $n = 6$ (—■—)

Table 2

Non-compartmental pharmacokinetic parameters for BAY 60–5521 following administration of an oral liquid formulation at doses of 5, 12.5, 25, and 50 mg (geometric means/geometric CV% and median (range) for $t_{\max}$)

Parameter	5 mg ($n = 6$)	12.5 mg ($n = 9$)	25 mg ($n = 7$)	50 mg ($n = 6$)
AUC $_{\infty}$ ($\mu\text{g l}^{-1} \text{h}$)	905.5/44.5	1737/34.8*	4012/17.9*	10 960/32.0
AUC $_{\text{norm}}$ (kg h l^{-1})	15.46/35.8	10.87/32.6*	12.62/21.5*	18.06/29.9
AUC/D ($\text{h } 10^{-3} \text{ l}^{-1}$)	180.6/44.5	138.4/34.9*	160.1/17.8*	219.0/32.0
AUC(0, t_n) $_{\dagger}$ ($\mu\text{g l}^{-1} \text{h}$)	831.8/47.8	1475/27.8	3688/18.4	10 310/32.3
$C_{\max}$ ($\mu\text{g l}^{-1}$)	56.32/64.5	88.03/28.4	217.5/25.4	568.1/33.2
$C_{\max, \text{norm}}$ (kg l^{-1})	0.9616/49.7	0.5733/29.6	0.6925/32.5	0.9358/30.4
$C_{\max}/D$ (10^{-3} l^{-1})	11.23/64.4	7.015/28.4	8.680/25.4	11.35/33.1
$t_{\max}$ (h)	6.0 (2.0–6.0)	4.0 (2.0–6.0)	6.0 (3.0–6.0)	6.0 (2.0–6.0)
$t_{1/2}$ (h)	76.42/21.1	143.4/79.6*	144.1/29.8*	117.4/21.3
MRT (h)	48.67/23.0	93.74/88.8*	93.81/23.1*	78.98/13.4
V_z/F (l)	610.6/64.1	1495/80.8*	1298/45.1*	773.4/47.8
CL/F (l h^{-1})	5.538/44.5	7.226/34.9*	6.244/17.8*	4.567/32.0

* $n = 6$ as $t_{1/2}$ could only be estimated in six subjects. †The extrapolated portion AUC(t_n, ∞) accounted for 7.6, 6.3, 7.6 and 5.7% of AUC at 5, 12.5, 25 and 50 mg (geometric mean).

$_{\dagger}t_n = 168 \text{ h}$ (5 mg) and 336 h (12.5, 25 and 50 mg).

No clinically relevant changes in clinical laboratory parameters, urinalysis, vital signs (blood pressure, heart rate) or ECG were observed.

Discussion

In this first in man (FIM) study we investigated the safety, tolerability, pharmacodynamics and pharmacokinetics of

the new CETP inhibitor BAY 60–5521 in healthy male subjects following administration of single oral doses. The target indication for CETP inhibition is the treatment of dyslipidaemia in humans.

BAY 60–5521 was clinically safe and well tolerated.

In general, after the administration of BAY 60–5521, the incidence of treatment emergent adverse events ranged between 22%–33% (Table 3) and was comparable with other FIM studies [24, 25]. However, the incidence of treat-

Figure 4

Concentration–effect relationship between CETP inhibition and BAY 60–5521 plasma concentration (≤ 12 h) after single oral administration. $I_{\max} = 98.6\%$; $IC_{50} = 32.2 \mu\text{g l}^{-1}$ estimated using the Hill equation with a sigmoidicity factor of 1.1

Table 3

Incidence of subjects with treatment emergent adverse events by treatment group (all subjects treated, $n = 38$)

MedDRA preferred term	Placebo ($n = 10$)	BAY 60–5521 5 mg ($n = 6$)	BAY 60–5521 12.5 mg ($n = 9$)	BAY 60–5521 25 mg ($n = 7$)	BAY 60–5521 50 mg ($n = 6$)
Number of subjects with any AE	0 (0%)	2 (33%)	2 (22%)	2 (29%)	2 (33%)
Atrial fibrillation					1 (17%)
Puncture site pain			1 (11%)		
Seasonal allergy		1 (17%)			
Wound					1 (17%)
Blood creatine phosphokinase increased				1 (14%)	
Headache		1 (17%)	1 (11%)		
Syncope vasovagal					1 (17%)
Skin rash				1 (14%)*	

*Adverse event considered drug-related.

ment emergent drug-related adverse events per subject was 3.5% (1 out of 28 volunteers treated with BAY 60–5521) and therefore very low. This event was a mild skin rash on the trunk observed approximately 10 days after 25 mg BAY 60–5521 intake following a sunbath. Symptoms disappeared later without any medical intervention. A few days later, the same subject took a sunbath again without showing any skin reaction. The symptoms of this subject were discussed with two consulting dermatologists and a phototoxic reaction was excluded. BAY 60–5521 showed no evidence of skin related toxicity in albino rats, which are known to be very sensitive to UV radiation. Furthermore no

relevant accumulation of BAY 60–5521 in the skin was found in the autoradiographic investigations. Most likely, the skin rash was caused by a virus infection; however, at this stage of development, a relation to study drug could not be excluded. Therefore, this event was judged as drug related.

Heart rate, blood pressure and ECG readings were not affected by BAY 60–5521.

A consistent pharmacodynamic effect on CETP-inhibition and on HDL-C concentrations was observed in healthy volunteers, using a fluorimetric microemulsion-based activity assay, which involved only marginal dilution

of the serum sample to be analyzed and thus closely reflected the *in vivo* profile of enzyme activity under treatment. In the placebo-treated subjects, a trend to enhanced endogenous CETP activity was observed starting at the 6 h time point, which may be related to food intake (lunch) after the 4 h sampling time point as suggested by Syeda [26]. BAY 60–5521 and placebo were administered as an oral solution with non-sparkling water (total volume 240 ml). Due to the clear effect of BAY 60–5521 on CETP and the exploratory character of the study, drop-outs were not replaced. It was originally planned to include 12 subjects in each dose-step with nine receiving active treatment and three receiving placebo.

CETP inhibition in BAY 60–5521-treated subjects was dose dependent. This finding is consistent with the observed pharmacokinetic profiles and the close relationship between CETP inhibition and concentration of BAY 60–5521 in plasma. After administration of 50 mg, CETP inhibition >50% lasted more than 50 h which translated into a statistically significant increase of target HDL-C by about 30% relative to baseline levels. A CETP-Inhibition of >50% over 24 h is necessary to increase HDL-C concentrations significantly. This was also demonstrated for torcetrapib doses ≥ 60 mg [27] in healthy subjects as well as in the patient population. Therefore, it seems recommendable, that for the development of further CETP inhibitors, at least a CETP inhibition of >50% over 24 h is desirable and could be used as a proof of concept criteria.

The perspective of CETP inhibitors as potential therapeutic agents [11–14] has been negatively influenced by the results of phase III studies with the CETP inhibitor torcetrapib. Although potential off-target effects of torcetrapib could have contributed to the failure of this CETP inhibitor [15–17], it remains to be demonstrated in the currently ongoing studies with other CETP inhibitors [17–19] whether longer-term CETP inhibition has the potential to reduce cardiovascular risk in patients with dyslipidaemia.

The results from this FIM study suggest that the CETP inhibitor BAY 60–5521 may offer potential as a treatment option for dyslipidaemia. BAY 60–5521 demonstrated consistent effects on CETP inhibition and HDL-C concentrations in healthy volunteers. Moreover, BAY 60–5521 was clinically safe and well tolerated. No effects on heart rate, blood pressure and ECG recordings were observed during the study. Further studies are needed to define the profile and the utility of BAY 60–5521 as a potential novel therapeutic agent.

Competing Interests

All are employees of Bayer and have worked on the project described in this paper.

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