Atopaxar
A novel player in antiplatelet therapy?

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Summary
Atopaxar, also known as E 5555 is a novel reversible protease-activated receptor-1 (PAR-1) thrombin receptor antagonist. To date, Atopaxar has been investigated in phase II trials with focus on safety and tolerability in patients with acute coronary syndromes or stable coronary artery disease on top of standard antiplatelet therapy. Atopaxar was generally well tolerated, however a rise in liver enzymes and prolongation of the QTc interval were observed. The data suggest, that atopaxar administration may promote some minor bleeding complications, but does not seem to significantly increase the risk of major bleeding. Although not powered for efficacy, the currently available data suggest potential benefits in patients at high risk for recurrent ischemic events on top of standard antiplatelet therapy. In conclusion, more studies (e.g. phase III) are needed to evaluate efficacy and safety of atopaxar.

Atopaxar – Ein neuer Mitspieler der antithrombozytären Therapie?

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Myocardial infarction and stroke are major causes of death and disability worldwide (1). Platelets play a crucial role in the development of arterial thrombosis resulting in myocardial infarction and ischemic stroke (2). Platelets seem to be also critically involved in the formation of atherosclerotic lesions (3). The disruption of atherosclerotic plaques results in platelet activation and thrombosis. In patients suffering from acute coronary syndrome (ACS), an enhanced excretion of thromboxane metabolites has been described. Consequently, the early administration of aspirin in these patients showed a decrease in mortality (4). Adding the adenosine diphosphate-receptor antagonist clopidogrel to aspirin and fibrinolytic therapy in patients with ST-segment elevated infarction has shown further beneficial effects in reducing recurrent ischemic events (5). Beneficial effects of clopidogrel have also been described in patients with non ST-segment elevation myocardial infarction (6). After percutaneous coronary intervention (PCI), administration of dual antiplatelet therapy comprising aspirin and clopidogrel has shown a significant reduction of recurrent ischemic events (7). However, the response to clopidogrel administration seems to vary among individuals (8, 9). In patients with coronary artery disease after PCI, an inadequate response to clopidogrel was associated with an enhanced incidence of recurrent ischemic events (10).

Further antiplatelet drugs have been successfully evaluated. Prasugrel, a novel thienopyridine and inhibitor of the adenosine diphosphate receptor, seems to be more effective in the prevention of recurrent ischemic events in patients with ACS receiving PCI than clopidogrel, however resulted in a higher rate of major bleeding events (11). Ticagrelor represents the first reversible and direct-acting inhibitor of the adenosine diphosphate receptor P2Y12 on platelets. In patients with ACS, ticagrelor also seems to be more effective in reducing the incidence of ischemic events than clopidogrel without affecting major bleeding and resulted in decreased mortality (12).

Further substances targeting the coagulation cascade have been evaluated in patients with acute coronary syndrome in addition to current antiplatelet therapy. The direct and selective inhibitor of factor Xa, rivaroxaban administered on top of standard antiplatelet therapy reduced the risk of death from cardiovascular causes, as well as myocardial infarction or ischemic stroke in patients with recent ACS (13). However, another direct inhibitor of factor Xa, apixaban, showed no significant reduc-
tion of ischaemic events and its administra-
tion was accompanied by an increased
incidence of major bleeding events (14). In
a phase II trial, the administration of the di-
rect thrombin inhibitor, dabigatran in pa-
tients with recent ACS receiving dual anti-
platelet therapy resulted in reduced coagu-
lation activity but was also accompanied
with increased risk of bleeding (15). Xime-
lagatran, another thrombin inhibitor, ad-
ministered in combination with aspirin,
showed to be more effective than aspirin
alone in preventing recurrent ischaemia in
patients with myocardial infarction (16).

Despite the current availability of vari-
ous potent antiplatelet drugs, in patients at
high risk for recurrent ischaemic events
the combination with further substances for
antithrombotic therapy targeting other
pathways of platelet activation and/or
thrombin generation may provide addi-
tional beneficial effects in preventing re-
current ischaemia.

Targeting PAR-1 mediated platelet activation

Multiple platelet activation pathways have
been characterized yielding haemostasis
and preventing the organism from life-
threatening blood loss. On the other hand,
enhanced stimulation of these pathways
may result in
● inflammation,
● atherothrombosis and
● ischaemia (17, 18).

Von Willebrand factor, adenosine diphos-
phate (ADP), thromboxane A2 (TXA2),
and thrombin are key players in mediating
platelet activation. Activated platelets play
a pivotal role in the development of ACS and
ischaemic stroke. At sites of vascular injury,
an increased generation of the serine pro-
tease thrombin could be observed (4).
Thrombin constitutes a potent activator of
both platelets and of the coagulation cas-
cade and, therefore, plays a key role in the
process of thrombosis, haemostasis, and
blood coagulation (19, 20). Upon activa-
tion, platelets release TXA2 and ADP.
These local mediators, as well as thrombin
promote further platelet activation and ag-
gregation. Thrombin acts via Protease-acti-
vated receptors (PAR) that belong to the G
protein-coupled members of the 7-trans-
membrane domain receptor superfamily
(21). They represent specific substrates for
regulatory proteases like thrombin. PARs
have been identified on the surface of pla-
telets, vascular endothelial cells, smooth
muscle cells, fibroblasts and mononuclear
cells suggesting various effects in vascular
biology. Platelet activation through throm-in is mediated via PAR-1 and PAR-4 re-
ceptors in humans, at which PAR-1 seems
to play a central role (22). PAR-1 and
RAR-4 also seem to form a stable hetero-
dimer arrangement, with PAR-1 represent-
ing a cofactor of PAR-4 in the course of
thrombin mediated platelet activation
(23). On the contrary to PAR-1, PAR-4 does
not have a thrombin binding site. Obvi-
ously, higher thrombin concentrations
are needed for platelet activation via
PAR-4, whereas relatively low thrombin
concentrations can stimulate platelet acti-
vation through PAR-1 (24). The PAR-me-
diated signal transduction across the plas-
ma membrane occurs through proteolytic
cleavage of the N-terminal domain of
PARs, resulting in ligand receptor inter-
action and intramolecular rearrangement
transactions. Further intracellular signal-
ing is exerted by internal G-proteins,
which among others promote the process
of platelet aggregation (25). In peripheral
mononuclear blood cells, cleavage of
PAR-1 seems to be involved in the differ-
etiation into smooth muscle cells (26).
Interestingly, an overexpression of PAR-1 re-
ceptors on cardiomyocytes has been shown
to be associated with myocardial hyper-
trophy which suggests a potential involve-
ment in pathological cardiac remodeling
processes (27). The administration of sta-
tins has proved to reduce the incidence of
myocardial infarction and stroke, poten-
tially in part through inhibition of the pla-
et PAR-1 thrombin receptor, which ap-
pears to be partially responsible for their
pleiotropic effects in the prevention of the
progress of cardiovascular diseases (28).

Therefore, PAR receptors may play a far-
reaching role in vascular biology, with great
impact on haemostasis and blood vessel
physiology. On the other side, they might
be also critically involved in the formation
of thrombosis, the occurrence of restenosis
and development of atherosclerotic lesions
(25). Besides vascular and haemostatic ef-
fects, PAR receptors seem to be also in-
volved in processes like tumor cell motility
and metastasis (29).

Current standard oral antiplatelet ther-
apy, comprising aspirin and a P2Y12 ADP
receptor antagonist result in impairment of
platelet activation through TXA2 and ADP
mediated pathways. However, few patients
still suffer from the recurrence of ischaemic
events despite receiving dual antiplatelet
therapy. Interestingly, these patients are
also at increased risk of the occurrence of
serious bleeding events, as important path-
ways in the process of primary haemostasis
are inhibited. Thus, other pathways of pla-
etlet activation and aggregation may be re-
sponsible for the recrudescence occurrence
of ischaemic events in patients receiving
dual antiplatelet therapy (30). In the after-
math of an acute coronary syndrome in
some patients receiving dual antiplatelet
therapy comprising aspirin and clopi-
grel, the persistence of biomarkers of co-
agulation, including thrombin generation
and activity may be an indicator for recur-
rent ischaemic events (31). Therefore,
thrombin receptor antagonists may repre-
sent a novel promising approach in anti-
thrombotic therapy and may yield further
beneficial effects on blood vessel biology
beyond platelet inhibition. The currently
available two PAR-1 receptor antagonists,
vorapaxar (SCH 530348; Merck, Sharp and
Dohme, USA) and atopaxar (E 5555; Eisai
Inc., Japan) have been the objects of exten-
sive clinical studies.
● Vorapaxar has already passed phase III
trials, whereas
● atopaxar has been evaluated in phase II
trials thus far.

Vorapaxar will be discussed in detail in this
issue elsewhere.

Atopaxar

Atopaxar is also known as E 5555
[(1-(3-tert-buty1-4-methoxy-5-morpholi-
nophenyl)-2-(5,6-dietoyo-fluoro-1-
imino-1,3-di-hydro-2H-isindol-2 yl)
ethanone hydrobromide], an orally active
and potent PAR-1 antagonist that has been
developed by Eisai. Atopaxar is a small molecule with a molecular weight of 608.54. Thrombin binding to PAR-1 seems to be inhibited by atopaxar near the site of the tethered ligand-binding epitope (22). In humans, platelet aggregation mediated by thrombin is attenuated with a half maximal inhibitory concentration (IC50) value of 0.064 μmol/L (32). Atopaxar causes a range of changes in expression of platelet major surface receptors, including GP IIb/IIIa, platelet-endothelial cell adhesion molecule (PECAM)-1, vitronectin and thrombospondin, suggesting an effect on platelet status beyond direct PAR-1 blockade (33). After oral administration, terminal half-life of atopaxar is 89 hours, with predominant gastrointestinal metabolism and to a lesser degree renal metabolism (<10%). On the other hand, the other PAR-1 antagonist vorapaxar has a terminal half-life of 126–269 hours and is metabolized predominantly via hepatic (cytochrome P450 3A4) and little via renal (5%) pathways. Apart from their antiplatelet efficacy, clinical and pharmacogenetic characteristics, as well as potential drug interactions have to be taken into account in the decision-making process for atopaxar versus vorapaxar (22). Atopaxar has initially been investigated in various animal models: In a guinea pig model of thrombosis, administration of atopaxar successfully inhibited thrombus formation, but did not result in prolonged bleeding or coagulation time (32). In a rat model of restenosis upon balloon injury, oral administration of atopaxar showed a significant reduction in the occurrence of neointima formation. Therefore, atopaxar could also be beneficial in atherothrombotic disease and lead to a lower rate of restenosis in patients after PCI (34). Administration of atopaxar has been evaluated in three phase II trials (LANCELOT-ACS, LANCELOT-CAD, J-LANCELOT). In LANCELOT-ACS, one to three hours after administration of the atopaxar loading dose (400 mg), an inhibition of platelet aggregation could be measured, with a mean inhibition of platelet aggregation by 74%. At three to six hours after administration of the loading dose, 90% of the participants showed a >80%-inhibition of platelet aggregation. The extent of platelet inhibition seemed to be dose-related, with an apparent trend towards higher doses of atopaxar. In patients receiving 50 mg, 100 mg or 200 mg atopaxar per day, mean inhibition of platelet aggregation accounted 66.5%, 71.5% and 88.9%, respectively, during the 12th week of administration (p for trend = 0.07) (35).

In the LANCELOT-CAD trial, patients with coronary artery disease were included. At 4 to 6 hours after drug administration in the 100 mg and 200 mg dosing groups an almost complete inhibition of platelet aggregation was observed, whereas in the 50 mg dosing group only 38% of platelet inhibition was evident. In the 50 mg group, the degree of platelet inhibition decreased rather rapidly and reached levels comparable to the placebo group within 24 hours, whereas in the 100 mg and 200 mg groups the degree of platelet aggregation inhibition still persisted on a high inhibitory level. In the course of the study, all atopaxar dosing groups exhibited a highly effective inhibitory level of platelet aggregation. While there were considerable fluctuations in the inhibitory effect in the 50 mg-group intake of 100 or 200mg achieved a stable inhibitory level of platelet aggregation (36). These results were confirmed by data attained from the J-LANCELOT trial (37). In LANCELOT-ACS trial, the anti-inflammatory effects of atopaxar have also been investigated among patients through determination of high-sensitivity C-reactive protein and interleukin-6, –18, and –1. There was no significant anti-inflammatory effect observed. However, a reduced expression of the platelet inflammatory marker CD40 ligand from platelets in the atopaxar treated groups was evident (35).

Studies

The clinical development of atopaxar in phase II has been investigated in the Lessons from Antagonizing the Cellular Effects of Thrombin (LANCELOT) -CAD and -ACS trials and a smaller trial conducted in Japan (J-LANCELOT). In these randomized, double-blind, placebo-controlled phase II trials, attention was mainly focused on safety and tolerability of atopaxar administration. The primary end points were bleeding events according the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) criteria (38), as well as the Thrombolysis in Myocardial Infarction (TIMI) criteria (39). Secondary end points comprised the incidence of recurrent ischaemic events and adverse effects, especially a rise in liver enzymes and QTcF (Fridricia’s formula) prolongation. In all LANCELOT studies, patients were randomized into a matching placebo group and atopaxar treatment groups at doses of 50 mg, 100 mg and 200 mg in a 1:1:1:1 manner.

In the LANCELOT-ACS trial 603 patients with non-ST-elevation ACS within 72 hours after onset of symptoms were included. For inclusion either ECG changes related to myocardial ischaemia or elevated markers of myocardial necrosis were required. Patients were treated according to current guidelines. Patients with anaemia, thrombocytopenia, history of transient ischaemic attack or stroke or increased bleeding risk were excluded. Further exclusion criteria were pathological intracranial findings and known hepatic or kidney disease (creatinine clearance below 30 ml/min). Patients receiving other oral antiplatelet drugs than aspirin, clopidogrel or ticlopidin or oral anticoagulants (e.g. warfarin) were excluded. The treatment was conducted over 12 weeks and there were 4 weeks follow up survey after the drug was discontinued. Study baseline characteristics were well balanced between placebo and treatment arms. Initially, all patients in the atopaxar treatment arms received a 400 mg loading dose (35).

In the LANCELOT-CAD trial 720 patients with coronary artery disease (CAD) including patients with (1) previous ACS (including myocardial infarction or unstable angina) at least 4 weeks previously, (2) percutaneous coronary revascularization at least 12 weeks previously, and (3) angina with documented ischaemia by provocative testing or angiographically evident CAD were enrolled. All patients received antiplatelet therapy with aspirin (75 to 325 mg daily) and/or a thienopyridine (clopidogrel or ticlopidine) for at least one month before screening. Patients with increased risk of bleeding, congestive heart failure (NYHA III/IV), elevated liver enzymes or kidney disease have also been excluded (36).
In the J-LANCELOT trial, 241 patients with ACS consisted of non-ST-elevation myocardial infarction (NSTEMI) (67.2%) and unstable angina (UA) (32.8%) were included, as well as 263 patients with high-risk CAD. In the CAD group, all patients were receiving aspirin (75–325 mg) for at least 4 weeks before enrollment, fewer than half of the patients received dual antiplatelet therapy (39.4% in placebo and 42.1% in atopaxar arm). In the ACS group, the majority of patients were treated with aspirin and thienopyridine (95.1% in placebo and 91.7% in atopaxar group) (37).

Safety

In the LANCELOT-ACS trial, there were 8 major bleeds and 9 minor bleeds according to the CURE bleeding criteria reported with no statistically significant differences between placebo and the combined atopaxar groups (p = 0.63). However, there was a numeric preponderance in CURE major bleedings in the atopaxar than placebo group (p = 0.12). Also no dose related trend could be observed (p = 0.8). The occurrence of minor bleeding events was comparable in the placebo and the combined atopaxar group (p = 0.58). The incidence of bleedings according to TIMI criteria did also show no statistically significant difference between the placebo and combined atopaxar groups (p = 0.77) and no dose-dependent trend (p = 0.63) (35).

In the LANCELOT-CAD trial, overall bleeding events according to CURE criteria were enhanced in the atopaxar group compared to placebo (p = 0.03). A trend towards more bleeding events at higher doses of atopaxar could also be observed (p for trend = 0.01). However, no differences in the incidence of CURE major bleedings have been reported. Also while applying TIMI criteria, a trend towards higher incidences of bleeding, mainly due to increased TIMI minimal bleeding in the atopaxar treatment arm with no significant differences in TIMI major and minor bleedings compared to the placebo group could be observed, with the most bleeding events in the 200 mg atopaxar group (p for trend across doses = 0.072) (36).

In the J-LANCELOT trial, in CAD patients there were only two major and minor bleedings according to CURE criteria reported, no dose-dependent trend has been observed. Bleedings according to TIMI criteria showed a higher incidence in the atopaxar treatment arm with a dose-dependent trend (p = 0.086), but no statistically significant differences compared to placebo group could be observed (p = 0.22). No TIMI major bleedings have been reported, only one TIMI minor bleeding was reported in the 100 mg group. In patients in the ACS group, there were two major life-threatening bleeding events in the placebo group reported and one minor bleeding event in the 200 mg atopaxar group according to CURE bleeding criteria. According to TIMI criteria, a slightly higher rate of bleedings in the 100 mg and 200 mg atopaxar treatment groups could be observed, but no significant differences between placebo group and atopaxar treatment arm (p = 0.61) and no dose-dependent differences (p = 0.27) (37). The bleeding events of the three studies are summarized in Figure 1.

Efficacy

In the LANCELOT-ACS trial, for secondary end point evaluation, patients also received a continuous 12-lead ECG (Holter). Horizontal or downsloping ST-Segment depression (>0.1 mV) or ST-segment elevation (> 1 mV) was defined as recurrent ischaemia. The oral intake of atopaxar showed significantly reduced incidence in recurrent ischaemic events while continuous ECG-monitoring by 34% (p = 0.02) in the first 48 hours. No significant differences between
the combined atopaxar and placebo arms (p = 0.20) were observed with regard to cardiovascular death, myocardial infarction, stroke or recurrent ischaemia with numerically lower incidents in the combined atopaxar group compared to placebo group (p = 0.20). Also no dose-dependent trend could be observed (p = 0.26) (35).

In the LANCELOT-CAD trial, ischaemic cardiovascular events showed no statistically significant difference between the combined atopaxar group in comparison to placebo group (p = 0.20) (36).

In the J-LANCELOT trial, the secondary endpoint also comprised a composite of cardiovascular death, myocardial infarction, stroke or recurrent ischaemia. Here, in ACS patients, no statistically significant differences could be observed between the combined atopaxar group and placebo group (p = 0.73). In CAD patients, the occurrence of recurrent ischaemia was numerically lower in the combined atopaxar group than placebo group, however not statistically significant (p = 0.066) (37).

**Adverse effects**

In the LANCELOT-ACS trial, there were no statistically significant differences in liver function abnormalities regarding the combined atopaxar and placebo groups. In the 200-mg atopaxar dosing group, a more frequently elevation of alanine aminotransferase (>3 times upper limit of normal (ULN)) was recorded at the beginning but resolved before study ending and no cases of Hy’s law (alanine aminotransferase >3 times above the upper limit of normal and total bilirubin >2 times above the upper limit of normal) have been reported. After relative QTcF (Fridéricia’s formula) prolongation due to ACS, a smaller decrease in the 100 mg and 200 mg atopaxar groups resulted in greater QTcF interval mean decrease in the placebo group (p = 0.04). However, no cases of syncope or arrhythmias have been reported (35).

In the LANCELOT-CAD trial, in patients treated with atopaxar a more frequent, mainly transient elevation of alanine aminotransferase >3 times the ULN and >5 times the ULN, especially at higher doses could be observed. No case of liver failure or Hy’s law has been reported. Also a slightly increase in QTcF interval in the atopaxar treatment arm could be observed (36).

In the J-LANCELOT trial, a statistically significant dose-dependent increase in liver function abnormalities and QTcF prolongation in the atopaxar group could be observed (37).

**Conclusion**

Atopaxar is a novel oral PAR-1 antagonist.

An extensive phase II clinical development program has shown that the additional administration of atopaxar in patients with ACS or CAD on top of the current antiplatelet therapy was generally well tolerated. Interestingly, there was no statistically significant increase in major bleeding events according to CURE- and TIMI-criteria, however patients treated with atopaxar tended to have more minor and minimal bleeding complications. Although not powered for primary efficacy end points, the data suggest that the additional administration of atopaxar to dual antiplatelet therapy might be beneficial in preventing recurrent ischaemic events. However, in a phase III trial (TRACER), the additional administration of vorapaxar to standard antiplatelet therapy in patients suffering from ACS resulted in a significantly enhanced incidence of bleeding including intracranial haemorrhage, without showing a significant benefit in primary efficacy end points (40). Therefore, a careful selection of patients at high risk for ischaemia and, i.e. the exclusion of patients with impaired liver and kidney function, as well as patients at high risk for bleeding events seems to be crucial for establishing an additional antithrombotic medication on top of the current stand therapy.

Regarding the current therapy with aspirin and thienopyridines, PAR-1 receptor blockade may become an option as additional (on top) therapy or as an alternative for selected patients, which has to be tested in the future. Moreover, the widespread appearance of PAR-1 receptors in the cardiovascular system and the effects of atopaxar beyond platelet activation impairment may establish further clinical applications of atopaxar in the fields of vascular remodeling and heart failure.

The first data of atopaxar administration to dual antiplatelet therapy delivered promising results. Further (phase III) studies are needed to evaluate the clinical efficacy and safety of atopaxar.

**Conflict of interest**

The authors declare, that there is no conflict of interest.

**References**


