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The Safety of an Adenosine A_1-Receptor Antagonist, Rolofylline, in Patients with Acute Heart Failure and Renal Impairment

Findings from PROTECT

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Abstract

Background: Adenosine exerts actions in multiple organ systems, and adenosine receptors are a therapeutic target in many development programmes.
Objective: The aim of this analysis was to evaluate the safety of rolofylline, an adenosine A_1-receptor antagonist, in patients with acute heart failure.

Methods: The effect of rolofylline was investigated in patients hospitalized for acute heart failure with impaired renal function. Intravenous rolofylline 30 mg or placebo was infused over 4 hours daily for up to 3 days. Adverse events (AEs) and serious AEs (SAEs) were recorded from baseline through 7 and 14 days, respectively, and clinical events were adjudicated through 60 days.
Results: Of 2033 patients enrolled, 2002 received study drug randomized 2 : 1 to rolofylline or placebo. Rolofylline and placebo were associated with a similar risk of pre-specified groups of AEs or SAEs, other than selected neurological events. Investigator-reported seizures occurred in 11 (0.8%) rolofylline-treated patients and zero patients receiving placebo ($p = 0.02$). Stroke occurred in 21 (1.6%) patients assigned to rolofylline compared with 3 (0.5%) placebo-treated patients through 60 days with a greater risk for stroke in the rolofylline group (hazard ratio 3.49; 95% CI 1.04, 11.71; $p = 0.043$). There was no temporal relation to rolofylline administration and no specific stroke subtype or clinical characteristics that predicted stroke in the rolofylline group.
Conclusions: Rolofylline treatment was associated with an increased seizure rate, an anticipated complication of A_1-receptor antagonists. An unanticipated, disproportionate increase in strokes in the rolofylline-treated patients emerged, although no clear temporal relation, aetiology, stroke subtype or interacting factor suggestive of a causal mechanism was identified. Further research into stroke as a potential complication of adenosine-modulating therapies is required. Additionally, this study underscores the value of longer follow-up durations for AEs, even for agents with short treatment periods, such as in acute heart failure.
Trial Registration: ClinicalTrials.gov identifiers NCT00328692 and NCT00354458.

Background

Adenosine, through the stimulation of the A_1-receptor, has multiple physiological effects (bradycardia, reduced lipolysis, sympatholysis, antinociception) and has been implicated in ischaemic preconditioning and reductions in glomerular filtration rate. Antagonists of the adenosine A_1-receptor are in development for a variety of diseases, including acute heart failure (AHF) with renal impairment and asthma, while A_1-receptor agonists are targeting atrial arrhythmias, diabetes mellitus, angina and neuropathic pain.[1] Preclinical studies with A_1-receptor antagonists suggest that these agents can increase renal blood flow and maintain or increase glomerular filtration rate through inhibition of tubulo-glomerular feedback, which could benefit patients with AHF. However, in the brain, adenosine A_1-antagonists can reduce the seizure threshold and may precipitate seizures in at-risk populations.[2] In the pilot study of PROTECT (**P**lacebo-controlled **R**andomised study of the selective A_1-adenosine receptor antagonist rolofylline for patients hospitalised with AHF and volume **O**verload to assess **T**reatment **E**ffect on **C**ongestion and renal func-**T**ion), in which 305 patients were randomized to placebo or three dosages of rolofylline, a selective adenosine A_1-antagonist, no seizures or other neurological events were observed, and a beneficial effect on serum creatinine was demonstrated.[3]

PROTECT (ClinicalTrials.gov identifiers NCT 00328692 and NCT00354458) examined the efficacy and safety of rolofylline in patients with renal dysfunction and volume overload requiring intravenous diuretics.[4] The results of PROTECT did

not demonstrate an improvement in the composite clinical efficacy endpoint.[5] However, rolofylline is the first adenosine A_1-antagonist tested in a large clinical trial, providing important insight into the potential safety of other adenosine modulating agents in development. This analysis investigated the safety of rolofylline based on the data from PROTECT.

Methods

The rationale, design and primary results of the PROTECT study have been published.[4,5] The primary objective of the study was to evaluate the effect of rolofylline in combination with intravenous loop diuretic therapy on heart failure signs and symptoms, renal function, morbidity and mortality, and safety in patients hospitalized with AHF and renal impairment.

Patient Population and Study Design

In brief,[5] patients aged ≥18 years with AHF confirmed by a B-type natriuretic peptide (BNP) ≥500 pg/mL or N-terminal pro-BNP (NT-pro-BNP) ≥2000 pg/mL, dyspnoea at rest or with minimal exertion accompanied by fluid overload, and renal impairment (estimated creatinine clearance 20–80 mL/min) who could be entered into the study within 24 hours of presentation and required intravenous furosemide of at least 40 mg/day were enrolled. After written informed consent was obtained, patients who met all entry criteria were randomized 2 : 1 to daily 4-hour infusions of either rolofylline 30 mg or placebo in a double-blind fashion administered for up to 3 days. Patients were evaluated prior to study drug and daily through day 7 or discharge and at day 14 for signs and symptoms of heart failure, and contacted via phone at 60 days for assessment of morbidity and mortality and at 180 days for mortality.

Safety Data Collection and Evaluation

Safety and tolerability were assessed by verbal questioning of the patient, clinical observation by the study staff, physical examinations, monitoring of vital signs and laboratory assessments. Non-serious adverse events (AEs) and serious AEs (SAEs) were recorded for each patient after enrolment from baseline through day 7 and day 14, respectively. A blinded Clinical Events Committee (CEC) reviewed and adjudicated all hospitalizations and deaths through day 60. For reporting purposes, the CEC-identified event terms were analysed. An unblinded Data Monitoring Committee (DMC) also continuously monitored the study to ensure safety of the patients.

Seizure Minimization Plan

A risk mitigation plan was instituted for the phase 3 PROTECT study, including extending the duration of the infusion from 2 to 4 hours to reduce peak blood concentrations, excluding patients deemed at high risk for seizures (table I), including those patients at intermediate risk but treating them with oral benzodiazepines (lorazepam or clonazepam 1.0 mg, orally) 30 minutes before study drug, and enhancing procedures for monitoring of seizures. Investigators were specifically encouraged to report all seizure and seizure-like activity and a blinded Neurological Event Team (NET) reviewed and assessed all of these events.

Stroke Classification

After PROTECT started enrolment, the DMC recommended further assessment of stroke events. A blinded Stroke Classification Subcommittee, consisting of two neurologists with expertise in stroke and vascular neurology (JPM, VI) and the CEC Chairperson (JRT) reviewed and classified all potential stroke events that occurred from randomization to day 60 as either fatal or non-fatal, and as one of the following subtypes: ischaemic/thrombotic, ischaemic/embolic, haemorrhagic, procedure-related stroke, stroke of unknown mechanism, transient ischaemic attack (TIA) and neither stroke nor TIA. Fatal stroke was defined as a stroke that led directly or indirectly to death within 30 days. The categories were drawn from the NINDS (National Institute of Neurological Disorders and Stroke) Stroke Data Bank[6] and the Trial of Acute Stroke Therapy.[7]

Table I. Categorization of seizure risk

High risk (excluded from study)	Intermediate risk (eligible with seizure prophylaxis)
History of seizure (except febrile seizure)	History of stroke (≥2 years)
History of or current brain tumour of any aetiology	History of brain surgery (≥2 years)
History of clinical stroke (<2 years)	History of encephalitis/meningitis (≥2 years)
Brain surgery (<2 years)	History of closed head injury with LOC >30 minutes (≥2 years)
Encephalitis/meningitis (<2 years)	Closed head injury with LOC <30 minutes
History of closed head injury with LOC >30 minutes (<2 years)	History of closed head injury with LOC >30 minutes
History of penetrating head trauma	Serum sodium <128 mmol/L within 24 hours prior to randomization
History of or risk for alcohol withdrawal seizures	Concomitant medications known to decrease seizure threshold (theophylline, neuroleptics and other antipsychotics, antidepressants, cyclosporine, prescription and over-the-counter antihistamines/decongestants, and stimulants used for decongestion or weight loss)
Advanced Alzheimer's disease	Patients the investigator has determined to be at increased risk for seizure because of any unstable medical condition resulting in widely fluctuating oxygen, glucose or electrolyte levels, or other factors
Advanced multiple sclerosis	Investigator discretion

LOC = loss of consciousness.

Statistics

Selected patient characteristics are described by mean ± SD. Seizures and strokes were evaluated on the basis of CEC- or NET-adjudicated events. AEs and SAEs were grouped according to pre-specified categories of Medical Dictionary for Regulatory Activities (MedDRA®) terms (see Statistical Analysis Plan, Supplemental Digital Content, http://links.adisonline.com/DSZ/A63), including the categories of supraventricular tachyarrhythmias, atrial fibrillation/atrial flutter, ventricular tachyarrhythmias, bradyarrhythmias or conduction disturbances, major adverse ischaemic cardiac-related events (myocardial ischaemia, acute coronary syndrome, myocardial infarction or cardiac-related death), and major adverse ischaemic cardiovascular (above cardiac events as well as TIA, stroke and stroke-related death) events. Events are described as the number of patients affected and percentage of the specific treatment group, while the risk differences and corresponding 95% CI were calculated using the method of Miettinen and Nurminen.[8] To capture the overall safety burden (i.e. events within a class that occur repeatedly in the observation period), treatment groups were compared on the total number of specific events within a class using Poisson regression. The time-to-first event for various outcomes by class was analysed using Kaplan-Meier estimates and Cox models, with a term for treatment as an explanatory variable. Treatment-emergent stroke events were further analysed by time-to-event analysis of stroke rate between subgroups of selected baseline covariates (history of diabetes, hypertension, hyperlipidaemia, smoking, atrial fibrillation/atrial flutter, age >75 years) with analysis for interaction effects. The possible interaction between atrial fibrillation/flutter and the occurrence of stroke was analysed using the method of Miettinen and Nurminen.[8] Analyses were conducted with SAS® (Statistical Analysis Software, version 9.1; 2002–2003 by SAS Institute Inc., Cary, NC, USA). p-Values are exploratory in these analyses.

Results

Overall Safety

The PROTECT study randomly assigned 2033 patients to placebo (677) or rolofylline 30 mg (1356). Ten patients (1.5%) assigned to placebo and 21 (1.5%) assigned to rolofylline did not receive study drug and were not included in the safety analysis. One patient randomized to placebo received rolofylline in error and is included in the latter group for the safety analysis. Only

one patient was lost to follow-up for the 60-day assessment. A summary of patient characteristics is presented in table II.

At least one AE was reported in 409 (61.4%) patients of the 666 patients in the placebo group and 840 (62.9%) of the 1336 patients in the rolofylline group, leading to study drug discontinuation in 21 (3.2%) placebo patients and 62 (4.6%) rolofylline patients. Ninety-eight (14.7%) patients in the placebo group experienced 135 SAEs and 185 (13.8%) patients in the rolofylline group experienced 257 SAEs (see SDC, online supplement table 1). SAEs led to study drug discontinuation in 1.5% of patients in each group, and SAEs with a fatal outcome occurred in 5.3% of patients in the placebo group and 3.7% of patients in the rolofylline group. Most SAEs were cardiac events (60 [9.0%] in placebo and 96 [7.2%] in rolofylline-treated patients) – primarily worsening heart failure. Three (0.6%) placebo patients and 7 (0.5%) rolofylline patients experienced cardiac arrest or life-threatening ventricular dysrhythmias.

Table II. Selected demographic characteristics of patients enrolled in PROTECT

Patient characteristics	Placebo (n = 677)	Rolofylline 30 mg (n = 1356)
Baseline characteristics		
Age [y; mean (SD)]	70.2 (11.5)	70.2 (11.7)
Male (%)	67	67
Race White (%)	96	95
Weight [kg; mean (SD)]	81.9 (19.8)	82.1 (19.4)
Screening BP [mean (SD)]	124/74 (18/12)	124/74 (18/12)
Screening CrCl [mL/min; mean (SD)]	49.6 (17.2)	49.0 (16.9)
Serum Cr [mg/dL; mean (SD)]	1.50 (0.59)	1.52 (0.56)
LVEF within 6 months[a]	32 ± 0.13	32 ± 0.13
Medical History (%)		
Hypertension	77.8	80.2
Ischaemic heart disease	68.5	70.5
Hyperlipidaemia	52.4	51.7
Atrial fibrillation/flutter	57.0	53.5
Diabetes mellitus	45.8	45.2
Intermediate seizure risk	9.7	9.7
Brain surgery (≥2 y)	0	0.1
Closed head injury with LOC >30 min (≥2 y)	0.1	0.5
Encephalitis/meningitis (≥2 y)	0.3	0.1
Stroke ≥2 y	9.3	8.8
Medications[b] (%)		
ACE inhibitor or ARB	74.4	76.3
β-blocker	75.7	76.5
Aldosterone inhibitor	42.4	44.5
Digoxin	29.6	27.3

a Mean ± SD in patients with data available within 6 months of admission (n = 975).

b In the 2 weeks prior to admission.

ARB = angiotensin receptor blocker; **BP** = blood pressure; **Cr** = creatinine; **CrCl** = creatinine clearance; **LOC** = loss of consciousness; **LVEF** = left ventricular ejection fraction.

Cardiovascular Events

There were no differences in the rate of any of the pre-specified cardiovascular adverse event categories analysed by adverse event rate by patient (table III; see SDC, online supplement table 2) or by number of AEs per 10 000 patient days (see SDC, online supplement table 3) during days 1–14 or in time-to-first event for days 1–60 (see SDC, online supplement table 4). Specifically, there were no significant rolofylline-related increases in hypotension, arrhythmias or conduction disturbances. Additionally, there was no difference in the rate of the pre-specified composites of major ischaemic cardiovascular (myocardial ischaemia, myocardial infarction, TIA, stroke and cardiovascular death) or major cardiac ischaemic vascular-related (myocardial ischaemia, fatal and non-fatal myocardial infarction, and sudden cardiac death) events.

Neurological Events

Seizures

Seizures were evaluated up to 14 days after study drug administration. Approximately 10% of the patients were categorized as intermediate seizure risk by the investigators, predominantly due to a prior history of stroke (table II). No patient assigned to placebo experienced a seizure, while investigator-reported seizures were noted in 11 patients (0.82%) treated with rolofylline ($p = 0.019$). The NET determined that these 11 events (table IV) included three generalized tonic-clonic seizures, six focal seizures with secondary generalization and two non-seizure events (patients A and B), resulting in nine

Table III. Selected investigator-reported AEs through day 14

AEs	Rolofylline (n[a] = 1336) [n (%)]	Placebo (n = 666) [n (%)]	Difference in AE rate [% (95% CI)]	p-Value[b]
Seizure	11 (0.82)	0 (0.00)	0.82 (0.24, 1.47)	0.02
Supraventricular tachyarrhythmias	27 (2.02)	11 (1.65)	0.37 (–1.05, 1.54)	0.57
Atrial fibrillation/flutter	19 (1.42)	9 (1.35)	0.07 (–1.22, 1.10)	0.90
Ventricular tachyarrhythmias	54 (4.04)	35 (5.26)	–1.21 (–3.39, 0.67)	0.22
Bradyarrhythmias or conduction disturbances	25 (1.87)	8 (1.20)	0.67 (–0.61, 1.75)	0.27
Major ischaemic cardiovascular events	45 (3.37)	17 (2.55)	0.82 (–0.89, 2.30)	0.32
Major ischaemic cardiac vascular-related events	30 (2.25)	14 (2.10)	0.14 (–1.40, 1.42)	0.84

a N = patients with AE through day 14.

b p-Values calculated based on the Miettinen-Nurminen test.[8] There was no difference in results when the total number of events per 10 000 person-days was evaluated (see SDC, online supplement table 3; http://links.adisonline.com/DSZ/A63).

AEs = adverse events.

adjudicated seizures in the rolofylline group (patients 1–9; p = 0.033). The three generalized seizures occurred during or immediately after the infusion of the study drug without any apparent lateralizing features. Two of these patients had single-seizure episodes and one patient had two seizure episodes 20 minutes apart. Brain imaging studies did not show acute pathology: one was normal, another had subcortical lucencies on computed tomography (CT) suggestive of old lacunar infarctions, while another had an old (apparently silent) right parietal infarction demonstrated by CT and magnetic resonance imaging. Of the six focal seizures, three occurred within 7 hours and three occurred between 2 and 7 days of administration of the study drug. The three patients with early focal seizures had cortical brain lesions on imaging studies with topography concordant with the lateralized seizure semiology. One had a left parietal lucency in CT involving cortical and subcortical areas, suggestive of subacute on chronic infarction, another had an acute ischaemic frontal infarction in addition to multiple old cortical-subcortical ischaemic lesions, and the third had multiple haemorrhagic strokes suggestive of cardiac embolism, although no history of atrial fibrillation. This patient died and post-mortem examination revealed a recent subendocardial myocardial infarction. Of the three patients with late focal seizures, one had an old ischaemic stroke, one had subacute subdural haematomas and acute renal failure, while another had an acute left temporal intraparenchymal haematoma.

Benzodiazepine pre-treatment was administered to 222 rolofylline-treated patients and only one of these patients developed seizures. Applying the same criteria *post hoc*, 9 of the 11 patients with investigator-reported seizures were determined to have at least intermediate seizure risk, while one was discovered to be at high risk (table IV). Of the 1114 rolofylline-treated patients who did not receive benzodiazepine prophylaxis, eight patients (0.72%) developed NET-confirmed seizures, compared with one rolofylline-treated patient (0.45%) who received prophylaxis (p = 0.66). The number of events were too few to truly evaluate the potential efficacy of benzodiazepine prophylaxis in preventing rolofylline-related seizures. However, given that patients receiving prophylaxis had a much greater seizure risk, yet still had similar or lower seizure rates compared with those without prophylaxis, it is likely that some rolofylline-related seizures were prevented by benzodiazepine administration. A total of 339 patients received protocol-specified benzodiazepines, 61% of whom had at least one adverse event, compared with 63% of the 1663 patients without prophylaxis. There was no evidence of an adverse interaction with rolofylline and there were few specific AE terms with a higher incidence in the benzodiazepine-treated patients.

Stroke

During 60 days of follow-up, one event was adjudicated as a TIA and 24 events as strokes (table V). Among the 24 strokes, 17 occurred within the

first 14 days (2 placebo [0.30%], 15 rolofylline [1.12%]; difference in rate 0.822 [95% CI –0.42, 1.60; Miettinen and Nurminen method (M-N) p = 0.059]), one of which was also followed by a seizure (see SDC, online supplement table 7). Another seven strokes occurred from day 15 to day 60 (one placebo, six rolofylline). There was a significant difference in the time-to-stroke event through 60 days for the rolofylline-treated group (hazard ratio 3.49; 95% CI 1.04, 11.71; p = 0.043; figure 1). All stroke events were fully documented with imaging. The two early strokes in the placebo group were ischaemic; one classified as cardioembolic and the other cryptogenic. There were nine early ischaemic strokes in the rolofylline-treated patients (four cardioembolic, five cryptogenic), five early haemorrhagic strokes and one spinal cord infarct. None of the early strokes in either group were classified as thrombotic. Most notable was the high fatality rate associated with these early strokes, with both of the placebo-treated patients dying as a result of their strokes and almost half of the rolofylline treated patients' strokes proving fatal (7 of 15; 47%). The dispro-

Table IV. Characteristics of patients randomized to rolofylline with seizure events

Patient no.	Age (y)	Sex	No. of doses of rolofylline received	Time of event post last 4-h infusion	Investigator determined seizure risk	Received prophylaxis	Underlying pathology	*Post hoc* seizure risk	NET assessment
1	73	Female	1	2½ h into infusion	Low	No	Respiratory failure; history of prior seizure[a]	High	Generalized seizure
2	76	Male	3	20 min/40 min	Low	No	None	Low	Generalized seizure
3	59	Male	3	2.5 h	Low	No	Prior stroke	Intermediate	Generalized seizure
4	72	Female	1	1½ h into infusion	Low	No	Prior stroke	Intermediate	Focal seizure
5	76	Male	2	7 h	Low	No	Cocaine abuse, prior stroke, shower of emboli	Intermediate	Focal seizure
6	72	Male	1	7 h/13 h	Low	No	Haemorrhagic stroke	Intermediate	Focal seizure
7	70	Female	2	3 days	Low	No	Acute renal failure; subdural haematoma	Intermediate	Focal seizures secondary to subdural haematoma
8	87	Female	3	5 days	Intermediate	Yes	Prior stroke	Intermediate	Focal seizure
9[b]	55	Male	3	7 days	Low	No	Intracerebral haemorrhage	Intermediate	Focal seizures secondary to haematoma
A	86	Male	3	Immediately at end of infusion	Low	No	TIA/atrial fibrillation	Intermediate	TIA
B	78	Female	3	2 days	Low	No	Transient cerebral hypoxia	Intermediate	Transient cerebral hypoxia

a Prior history of seizure unknown at time of enrolment and treatment.

b Simultaneous development of intracerebral haematoma and seizures.

NET = Neurological Event Team; **TIA** = transient ischaemic attack.

Table V. Adjudicated stroke events in PROTECT

Stroke type	Days 1–14		Days 15–60		Overall	
	Placebo total[a] (fatal)	Rolofylline total (fatal)	Placebo total (fatal)	Rolofylline total (fatal)	Placebo total (fatal) [n = 666]	Rolofylline total (fatal) [n = 1336]
Ischaemic stroke	2 (2)	9 (4)	1 (0)	4 (2)	3 (2)	13 (6)
Cryptogenic	1 (1)	5 (3)	1 (0)	0	2 (1)	5 (3)
Cardioembolic	1 (1)	4 (1)	0	2 (1)	1 (1)	6 (2)
Thrombotic, large artery	0	0	0	1 (1)	0	1 (1)
Thrombotic, small artery	0	0	0	1 (0)	0	1 (1)
Haemorrhagic stroke	0	5 (3)	0	1 (1)	0	6 (4)
Stroke, unknown mechanism	0	1 (0)[b]	0	1 (0)	0	2 (0)
Transient ischaemic attack	0	1 (0)	0	0	0	1 (0)
Total strokes[c]	**2**	**15**	**1**	**6**	**3**	**21**

a Absolute numbers of events are given. Randomization was 2 : 1 rolofylline : placebo.

b Spinal cord infarct.

c Excluding transient ischaemic attacks.

portionate number of stroke events continued to be evident for stroke developing later in the course of the study (days 15–60), with one non-fatal ischaemic stroke in the placebo group compared with six strokes in the rolofylline-treated patients. These later strokes also had a high fatality rate. There was no evidence that the effect of rolofylline on the stroke changed over the 60-day period compared with placebo ($p = 0.61$, using time as a continuous variable), or that the effect of rolofylline on the stroke is greater earlier (≤14 days) than later (>14 days) compared with placebo ($p = 0.86$). While the PROTECT patient population had characteristics that presented a high risk for stroke (e.g. older age, male sex, hypertension, atrial fibrillation, ischaemic heart disease, diabetes, hyperlipidaemia, prior stroke and reduced ejection fraction; table II), none predicted stroke in univariate analyses (see SDC, online supplement table 8), including no interaction with atrial fibrillation. Inclusion of these variables as covariates demonstrated no interaction effect on the rates by treatment group, suggesting that none of these factors contributed independently to the difference in stroke rates between the rolofylline- and placebo-treated groups. Amongst patients assigned to rolofylline, stroke occurred in 1.80% with atrial fibrillation and in 1.31% without atrial fibrillation (difference in rate 0.49 [95% CI −1.0, 1.9]; M-N $p = 0.47$), and, amongst those assigned to placebo, 0.53% and 0.35%, respectively (difference in rate 0.18 [95% CI −1.48, 1.61]; M-N $p = 0.73$). Logistic regression analysis demonstrated no significant interaction between atrial fibrillation and the effect of treatment on stroke ($p = 0.94$). Thus, there was no temporal association with the administration of rolofylline, the stroke subtype or any underlying risk factor for stroke in the rolofylline-treated patients to suggest a causal mechanism between rolofylline and the observed disproportionate increase in stroke rate.

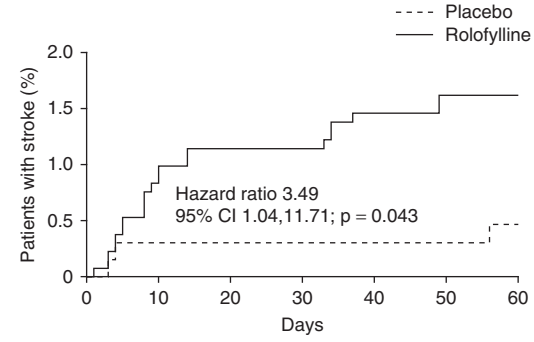

Fig. 1. Kaplan-Meier curve of time from study drug to stroke event by treatment group.

Discussion

In patients with AHF with renal impairment, rolofylline 30 mg infused over 4 hours for up to 3 days was associated with a similar incidence of AEs and did not appear to increase cardiovascular AEs overall compared with standard therapy. However, consistent with its mechanism of action, rolofylline-treated patients were reported to have significantly more seizures than placebo-treated patients. A disproportionate increase in stroke in the rolofylline-treated patients also emerged. These strokes were adjudicated to be of diverse types and no consistent temporal relationship or underlying risk factors were identified to suggest a possible mechanism related to rolofylline administration.

A major limitation of the development of new therapies for AHF has been the cardiovascular adverse effects of the agents, especially hypotension, myocardial ischaemia, and arrhythmias.[9-11] The important role of adenosine in inflammation, coronary vascular tone and atrioventricular conduction raised the concern that rolofylline would have adverse cardiovascular effects. In PROTECT, there were no increases in cardiovascular AEs, including hypotension, myocardial ischaemia or infarction, supraventricular or ventricular dysrhythmias, and bradyarrhythmias or conduction disturbances, addressing this potential concern.

Adenosine is a potent inhibitor of neuronal activity and acts as an endogenous anticonvulsant. The effects of adenosine are mediated by activation of high affinity (A_1, A_{2A}) and low affinity (A_{2B} and A_3) membrane receptors that couple to G proteins to influence intracellular signalling pathways.[12] The inhibitory effects of adenosine are achieved in large part by activation of high-affinity presynaptic A_1-receptors that are linked to G proteins, which in turn activate G protein-dependent inwardly rectifying K+ channels and phospholipase C, and inhibit Ca2+ channels and adenylyl cyclase. As a consequence, the release of various neurotransmitters, in particular glutamate, is inhibited. Pharmacologically, adenosine and its A_1-receptor selective analogues are effective in seizure suppression[13] and neuroprotection.[14] Adenosine A_1-receptor antagonists, including theophylline and aminophylline, have been associated with seizures, and in three early phase 1/2 studies, 3 patients of 172 treated with ≥30 mg rolofylline experienced seizure on rolofylline in the presence of an established seizure substrate (marked fluctuations in serum glucose in the context of pseudoephedrine, a known proconvulsant; undiagnosed meningioma and undisclosed history of seizure).[3,15] While no seizures were observed in the placebo group, 11 patients who received rolofylline treatment had investigator-reported seizures (0.8%). Drug-related seizures are usually generalized, as was the case in three patients. However, the remaining patients had partial seizures with or without secondary generalization, suggesting that an underlying acute or chronic focal lesion also played a role in seizure generation. Medication-induced seizures typically occur in patients with a predisposition to seizures, commonly a pre-existing hemispheric lesion involving the cerebral cortex. Indeed, all patients with partial seizures had focal ischaemic or haemorrhagic lesions with a location concordant with seizure semiology. In seven patients, the seizures occurred during or within 7 hours of administration of their last dose of study drug, probably with pharmacologically active serum rolofylline concentrations at the time of the seizure. There also appeared to be an unexpected excess of delayed seizures in the rolofylline-treated patients; four patients had partial seizures between 2 and 7 days after their last drug administration, at a time when serum concentrations were low or negligible.[16] While it is possible that the drug remains in the brain for a longer time than its pharmacokinetic characteristics would suggest, some of these patients had suffered recent intracranial haemorrhages, a condition that is well known to be associated with acute seizures and that may have been the main trigger responsible for their seizures. Nonetheless, there was an apparent excess of temporally-related and mechanistically consistent seizures in rolofylline-treated patients in the PROTECT study, despite dosing adjustments, pre-dosing risk stratification and benzodiazepine prophylaxis.

In PROTECT, there was a disproportionate, near-significant increase in strokes in the rolofylline-treated patients, with an almost 4-fold

higher rate of patients experiencing a stroke in the first 14 days compared with the placebo-treated patients, and a significant difference in the time-to-stroke event through 60 days. Although the actual number of neurological events adjudicated as stroke was quite low in PROTECT, their rate of occurrence was high in the short timeframe of the trial, with the majority occurring within the first 14 days. Most of the data reported or inferred for expected per-day stroke rates, ischaemic or haemorrhagic, is for *recurrent* stroke. In this setting, the highest documented recurrent stroke rates in clinical trials (where the ischaemic stroke subtype was characterized) was 2.6% for high-grade atheromatous stenosis within 7 days of the initial stroke.[17] None of these were evident in the current study, and a minimum 8% per year in a setting of atrial fibrillation, with the event rates roughly evenly divided over the time period.[18] Even in a setting of prior ischaemic stroke not associated with atrial fibrillation, annual *recurrent* rates have averaged 8%, with comparable rates among the ischaemic stroke subtypes.[19] In VERITAS (Value of Endothelin Receptor Inhibition with Tezosentan in Acute heart failure Studies), a 1435-patient study of the effects of the endothelin receptor antagonist tezosentan in patients with AHF, only 0.1% and 0.7% of patients in the placebo group suffered a stroke during the first 7 and 30 days, respectively.[20] While stroke rates appear to be slightly elevated in patients admitted for AHF, the rate of over 1% for the first 14 days in PROTECT remains conspicuously high. Considering the very low daily event rate for strokes of any kind, strokes in the current trial occurred at a far higher rate, occurred earlier and appeared more deadly than expected or previously documented, supporting the possibility that these results represent a clinically important difference in stroke rates.

Unlike rolofylline-induced seizures, which were an anticipated adverse effect based on the biology of the adenosine A_1-receptors, it is difficult to identify a mechanism by which rolofylline might cause stroke. Of note, in a 50-patient study of another A_1-receptor antagonist, BG9928,[21] there was one stroke event in a patient, but it occurred 11 days after the last administration of BG9928 in a patient with a previous history of stroke, cerebral artery occlusion, carotid endarterectomy and coronary artery disease. There was no evidence for an increased stroke rate at times more proximal to the drug administration, and analysis of the limited number of events suggested that the risk was proportional throughout the 60 days of follow-up. There is no obvious mechanism by which rolofylline should increase the stroke rate weeks after treatment. The PROTECT patient population was at high risk for ischaemic stroke events,[22] as demonstrated by the 3 of 16 ischaemic strokes occurring in the placebo group, and the extraordinary baseline rate of risk factors for stroke, most notably atrial fibrillation (54%). However, there was no difference in risk factors between the treatment groups who developed stroke nor was there a significant treatment interaction with rolofylline. There was no evidence of increased vascular, cardiac or cardiovascular events, arguing against a generalized prothrombotic or vascular injury effect of rolofylline, and no increased incidence of atrial fibrillation/flutter, hypertension or other factor that may have selectively increased stroke risk in the rolofylline group. The strokes in rolofylline-treated patients did not appear to have a consistent subtype, with cardioembolic, thrombotic and haemorrhagic all represented. However, the study was underpowered to evaluate the potential interaction of these factors and the effect of rolofylline on stroke. The number of stroke events is small and these apparent differences in stroke rates may be solely due to chance. Despite these considerations, it remains difficult to ignore a potential role for the adenosine antagonist tested in this trial. Few data exist concerning the risk/benefit for adenosine in a setting of stroke or risk for stroke, ischaemic or haemorrhagic. The relationship between brain ischaemia and adenosine infusion was investigated in an animal model of transient cerebral ischaemia, where adenosine infusions through a microdialysis probe into the striatum improved 'neurological outcome,' although the changes did not reach statistical significance.[23] Recent studies suggest that adenosine (as cyclic adenosine monophosphate) may play a role in atherosclerosis[24] and,

as adenosine itself, may be a potential neuroprotectant in chronic neurological disease, but few data have appeared for A_1-receptor antagonists in stroke, *per se*.[25,26]

Conclusions

Although there were no differences in the general AEs between rolofylline and placebo, based on the data from the current clinical trial, it remains unclear if A_1-receptor antagonists should be considered a risk factor for stroke. The trial was not powered to evaluate specific rare safety concerns and these findings may be a result of type 2 error with the multiple comparisons and relatively low number of events. Development of adenosine A_1-antagonists modified to prevent crossing of the blood-brain barrier may be possible and may be less likely to induce seizure. Further research into stroke as a potential complication of adenosine-modulating therapies is required. Additionally, this study underscores the value of longer follow-up durations for AEs, even for agents with short treatment periods, such as in AHF.

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