BMS-204352: A Potassium Channel Opener
Developed for the Treatment of Stroke

Bo Skaaning Jensen

Section of Ion Channel Pharmacology, NeuroSearch A/S, Ballerup, Denmark

Key words: BK channel—Ca\(^{2+}\)-Activated K\(^+\) channel—Cerebral ischemia—hSlo channel—Maxi-K channel—Neuronal hyperexcitability.

ABSTRACT

During ischemic stroke, a fatal biochemical cascade that results in neuronal hyperexcitability is initiated when neurons at risk are exposed to excessive excitatory amino acids and pathologically high levels of intracellular calcium (Ca\(^{2+}\)). Therefore, neuroprotectants including NMDA-antagonists and blockers of voltage-gated Ca\(^{2+}\) channels have been proposed as novel strategies for stroke treatment. Since potassium channels are key players in the control of neuronal excitability, and activation of neuronal potassium channels decrease excitability and neurotransmitter release, a novel approach for targeting acute ischemic stroke has been to develop openers of neuronal potassium channels. Bristol-Myers Squibb is developing BMS-204352, a fluoro-oxindole potassium channel opener, as a potential neuroprotectant for the treatment of acute ischemic stroke. BMS-203252 is a potent and effective opener of two important subtypes of neuronal potassium channels, the calcium-activated, big-conductance potassium channels (K\(_{Ca}\) channels) and voltage-dependent, non-inactivating potassium channels known as KCNQ channels. BMS-204352 (0.3 mg/kg, i.v.) significantly reduced cortical infarct volume in a model of permanent occlusion of the middle cerebral artery (MCA) in spontaneous hypertensive rats (SHR), as compared to vehicle when administered 2 h post-occlusion. At doses from 1 \(\mu\)g/kg to 1 mg/kg i.v., BMS-204352 produced a significant reduction in cortical infarct volume in normotensive Wistar rats. In healthy humans, single and multiple i.v. doses of BMS-204352 (0.001 to 0.2 mg/kg) were safe, well-tolerated and without psychomotor function effects. Multiple doses of BMS-204352 (0.1–2 mg/kg i.v.) administered within 48 h after stroke onset were well tolerated in patients in Phase II studies, designed to evaluate safety, tolerability and pharmacokinetics. No clinically significant differences in organ toxicity or adverse effects were found, and total clearance and volume of distri-
bution were independent of dose. BMS-204352 failed to show superior efficacy in acute stroke patients compared to placebo in a Phase III study that included 1978 patients at 200 centers worldwide.

INTRODUCTION

Stroke is the third leading cause of death. In the USA, stroke is the most common cause of long-term disability and affects more than 700,000 people each year (1). Age and gender-standardized annual incidence rates of stroke in individuals between 45 and 85 years old are approximately 300 to 500 per 100,000 in most countries (30). Even milder forms of stroke have a significant impact on quality of life for affected patients, and are a serious socioeconomic problem in developed countries. An ischemic stroke occurs when a thrombus in a cerebral vessel interrupts blood flow to the brain, and, therefore, the focus of many stroke treatment strategies has been dissolution of the offending thrombus. Only a single form of therapy, thrombolysis, has currently proven effective in improving the outcome of acute stroke in a limited patient population (19), and the only approved stroke therapy, alteplase (tPA), is such a thrombolytic. The most common form of stroke, acute ischemic stroke, produces a core area of damaged tissue close to an occluded blood vessel, which is surrounded by a penumbra of tissue at risk because of proximity to the core and the low vascular perfusion. These exposed neurons die as a result of a neurotoxic biochemical cascade that is initiated by reduced energy stores, release of excitatory amino acids, and elevated intracellular Ca²⁺, resulting in excessive neuronal hyperexcitability. The neurons at risk eventually die due to the high intracellular Ca²⁺.

The use of neuroprotective agents, which is a somewhat different approach to stroke treatment, has generated at least as much activity as the investigation of thrombolytic therapies. Rather than dissolving the thrombus, neuroprotective agents use a variety of mechanisms in an attempt to save ischemic neurons from irreversible injury. Such neuroprotective agents target the neurons in the penumbra region of the infarct, since these are less likely to be irreversibly injured at early time-points after the ischemic insult than the neurons in the infarct core. Certain neuroprotective agents aim to prevent potentially detrimental events associated with the return of blood flow (7,14), since reperfusion itself may contribute to additional brain injury. These therapies are not expected to be associated with the risk of hemorrhage that is characteristic of thrombolytic agents, since neuroprotective agents do not directly affect clotting and blood flow. Nevertheless, neuroprotective compounds have failed in clinical trials despite promising preclinical data (8). The value of neuroprotective agents in human trials has been affected by dose-limiting side effects due to the mechanisms of action of such drugs. Some agents that were tested to completion have failed to demonstrate efficacy in humans. Neuroprotectants often target a specific class of neurotransmitter-gated ion channels that participate in the neurotoxic cascade. Among these are the NMDA (N-methyl-D-aspartate) excitatory amino acid receptors, which are voltage- and ligand-gated ion channels (6). Other strategies target only one causal factor, e.g., free radicals, in neuronal death.

A major proximal cause of the death of ischemic cells is the accumulation of pathological levels of intracellular Ca²⁺ (5,18,31). In eukaryotic cells, Ca²⁺ is a ubiquitous signaling molecule linked to a number of important cellular functions. Also in the mammalian brain, Ca²⁺ is the most important signaling molecule. In order to regulate and
control the intracellular levels of Ca\textsuperscript{2+}. A wide range of membrane proteins respond to changes in Ca\textsuperscript{2+}. Ion channels that react upon changes in intracellular calcium are of crucial importance for maintaining control of the membrane potential, as an increase in intracellular Ca\textsuperscript{2+} will affect the electrical potential across the plasma membrane. However, voltage-gated Ca\textsuperscript{2+}-channel antagonists would not limit Ca\textsuperscript{2+} entry through other voltage-gated mechanisms. Furthermore, such agents are predicted to have significant side effects, such as hypotension, since they do not specifically target Ca\textsuperscript{2+}-channels in ischemic neurons. In contrast, potassium channels play a key role in control of plasma membrane potential and modulation of cell excitability. Activation of potassium channels generally reduces cellular excitability, making potassium channels drug target candidates for the treatment of diseases related to hyperexcitability such as epilepsy, neuropathic pain, and neurodegeneration. Novel drug candidates have been targeting neuronal potassium channels to increase the activity of such potassium channels (20–22,29). Presently, two compounds, BMS-204352 (Bristol-Myers Squibb) and retigabine (Asta-Medica), in clinical trials for the treatment of stroke (13) and epilepsy (24), respectively, have been proposed to exert their protective action by activating potassium channels. An obvious candidate class of such important potassium channels is the Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels, which according to both structure, function and pharmacology can be divided into three subfamilies: big-conductance, voltage-dependent Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels (K\textsubscript{Ca} channels), intermediate-conductance, voltage-independent Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels, and small-conductance, voltage-independent Ca\textsuperscript{2+}-activated K\textsuperscript{+} channels. K\textsubscript{Ca} channels are blocked by scorpion toxins such as iberiotoxin, and are distributed widely throughout the central nervous system (CNS) (27). K\textsubscript{Ca} channels are found in most regions of the brain, including cortex, hippocampus and thalamus, and provide an intrinsic cellular mechanism for limiting Ca\textsuperscript{2+} influx into cells. Because K\textsubscript{Ca} channels are sensitive both to increases in intracellular Ca\textsuperscript{2+} as well as membrane depolarization and react by increasing K\textsuperscript{+} efflux from the neurons, they will rapidly hyperpolarize the plasma membrane and thereby reduce further voltage-dependent Ca\textsuperscript{2+} influx via both NMDA receptors and voltage-dependent Ca\textsuperscript{2+} channels. K\textsubscript{Ca} channels have been reported to regulate Ca\textsuperscript{2+}-dependent glutamate release and contribute to membrane repolarization following propagation of action potentials (15). As such, activation of K\textsubscript{Ca} channels has been suggested as a novel approach for the treatment of stroke (13). The fluoro-oxindole, BMS-204352, is a novel analog of the K\textsubscript{Ca} channel openers, NS004 and NS1619 (21,22) (see Fig. 1), and has been selected by Bristol-Myers Squibb for clinical development in the reduction of neuronal damage following acute ischemic stroke.

**CHEMISTRY**

Synthesis of BMS-204352 and other 3-aryl substituted oxindole compounds can be found in two patents US-05565483 (16) and US-05602169. In general, these compounds were synthesized by established procedures employing statins as intermediates. Since the original method produced racemic mixtures of compounds, Bristol-Myers Squibb developed a simple chiral process that provides the optically pure enantiomers of 3-fluoro-substituted 3-aryl oxindoles (described in ref. 23).
BMS-204352 activates KCa channels at nanomolar concentrations through a mechanism, which requires intracellular Ca\(^{2+}\) concentrations higher than 1 \(\mu\)M (13). EC\(_{50}\) is estimated as 300 to 400 nM at a Ca\(^{2+}\) concentration of 1 \(\mu\)M. Prolonged exposure of KCa channels to BMS-204352 does not result in desensitization and the effect is reversible (13).

BMS-204352 has no effect on a number of other potassium, calcium and chloride channels, as well as on many G-protein coupled receptors (13). It does, however, activate the neuronal KCNQ channels underlying the M-current (9,26), a widely distributed, non-inactivating K\(^{+}\) current that conducts significantly around the threshold for triggering the action potential, and, therefore, act as an efficient brake on neuronal excitability (17). EC\(_{50}\) for the effect on the KCNQ channels is close to 2 \(\mu\)M (see Table 1). It is therefore possible that the effect of BMS-204352 in cerebral ischemia besides activation of KCa channels involves activation of KCNQ channels and perhaps other mechanisms as well (9,26).

In vitro, BMS-204352 reduced the electrically stimulated release of \(^{3}\)H-glutamate from hippocampal tissue wedges, although the reduction is relatively small (25 to 30% reduction compared to control), albeit significant (13). In hippocampal slice preparations, BMS-204352 significantly reduced population excitatory postsynaptic potentials (pEPSPs) to 75% of control in area CA1 after stimulation of the Schaffer collateral/commissural fiber system. The effect was relatively small, but could be reversed by iberiotoxin (13).
In whole-animal experiments, BMS-204352 caused a small persistent decrease (by maximally 30% as compared to controls) in hippocampal field potential at i.v. doses ranging between 50 ng/kg and 1.0 mg/kg (13). BMS-204352 had no significant effect on mean arterial blood pressure or heart rate in anesthetized rats at a dose equal to the highest effective dose in the evoked potential model (1 mg/kg i.v.).

The effects of BMS-204352 in acute focal stroke have been evaluated in two different animal models (13). Spontaneous hypertensive rats (SHR) were treated with BMS-204352 (0.3 mg/kg i.v.) or vehicle two h after permanent MCA occlusion. A small, but significant reduction of 20% in cortical infarct volume was observed in the BMS-204352 treated group 5.5 and 24 h after occlusion. In this model, BMS-204352 produced significant reductions in cortical infarct volume when tested at doses between 0.01 and 0.3 mg/kg. In Wistar normotensive rats after permanent unilateral MCA occlusion, permanent ipsilateral common carotid artery (CCA) occlusion, and transient (1h) contralateral CCA occlusion, BMS-204352 (1 mg/kg, i.v.) produced similar levels of neuroprotection. BMS-204352 produced significant reductions in cortical infarct volume when administered at doses between 1 µg/kg and 1 mg/kg, but was ineffective at 3 mg/kg, indicating a U-shaped dose-response curve. Maximal neuroprotective efficacy could be observed following administration of 0.3 mg/kg BMS-204352 with reductions of typically 20 to 30%. BMS-204352 was equally effective when administered at one or two h following occlusion (13).

BMS-204352 has also been evaluated in a rat model of traumatic brain injury (TBI), the lateral fluid percussion (FP) model of experimental brain injury in the rat (4). FP is widely regarded as a reproducible clinically relevant model of TBI. Anesthetized Sprague-Dawley rats were subjected to FP brain injury and treated with a bolus of BMS-204352 (0.03 mg/kg or 0.1 mg/kg) 10 minutes post-injury. Administration of 0.1 mg/kg BMS-204352 reduced the extent of cerebral edema in hippocampus (by 65%), thalamus (by 30%) and cortex (by 65–75%), and improved neurologic motor function at 1 and 2 weeks post-injury. The drug treatment appears only to shorten the time of recovery. At lower concentrations, BMS-204352 reduced cerebral edema only in thalamus. At neither dose, an effect on cognitive function or cortical tissue loss could be demonstrated (4).

### TABLE 1. Effect of MMS-204352 on neuronal K⁺ channels

<table>
<thead>
<tr>
<th>Target channel</th>
<th>BMS-204352 EC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kᵥ₅ channels, 50 nM Ca²⁺</td>
<td>&gt;5¹</td>
</tr>
<tr>
<td>Kᵥ₅ channels, 1 µM Ca²⁺</td>
<td>0.352¹</td>
</tr>
<tr>
<td>KCNQ4 channels</td>
<td>2.4²</td>
</tr>
<tr>
<td>KCNQ5 channels</td>
<td>2.4³</td>
</tr>
</tbody>
</table>

Data are from refs. 1–3,9,13,26. For BK-channels, BMS-204352 was applied to BK-channels in outside-out patches excised from HEK-293 cells in the presence of various calcium-concentrations, and EC₅₀ values were estimated from the shift in the half-maximal activation voltage induced by BMS-204352 (13). For KCNQ4 and KCNQ5 channels, HEK-293 cells stably expressing monomeric KCNQ4 or KCNQ5 channels were held at ~90 mV and the KCNQ currents were activated by depolarizing steps to ~30 mV. KCNQ currents elicited by increasing concentrations (0.1, 0.3, 1, 3, 10 µM) of BMS-204352, were measured at plateau level (~30 mV). EC₅₀ values were estimated from the resulting dose-current responses (9,26).
PRECLINICAL DEVELOPMENT

Metabolism

Currently, no definitive metabolism data are available. BMS-204352 has a high degree of lipophilicity (partition coefficient, $c \log P = 5.1$). Following i.v. administration, BMS-204352 quickly entered the rat brain at high levels ($T_{\text{max}} = 15 \text{ min}; C_{\text{max}} > 10 \mu g/g in brain; 5 \text{ mg/kg i.v. bolus dose}$) with a brain:plasma ratio of 9.6. In rats the plasma half-life was estimated at 1.6 h, and the brain half-life 1.9 h. Total clearance and volume of distribution of BMS-204352 is dose-independent. Following single or multiple i.v. dosing regimens, terminal $T_{1/2}$ is 20 h in healthy human volunteers as well as in patients suffering from acute stroke (28).

Safety Profile

In anesthetized rats or dogs as well as in conscious dogs heart rate and mean arterial blood pressure (MABP) were unaffected by BMS-203252 at doses up to 1 mg/kg i.v. There was no significant effect on MABP or blood gases in rats undergoing permanent MCAO. A transient decrease in blood pressure in dogs was observed at 20 mg/kg, but not at 10 mg/kg (13).

Toxicity

BMS-204352 is well tolerated in rats and dogs. No drug-related cytotoxic effect of BMS-204352 on target organ changes have been observed, neither in rats at doses up to 10 mg/kg/day for 1 month, nor in dogs at doses up to 20 mg/kg/day for 10 days. In either species, excellent safety margins could be established after systemic exposure to BMS-204352 at levels relevant to the proposed therapeutic dose in humans. BMS-203252 had no effect in genotoxic tests in in vitro or in vivo assays, and is not teratogenic in rats or rabbits (11). BMS-204352 had no effect on reproductive function or early embryonic development in rats. In guinea pigs, BMS-204352 produced no antigenic response (11).

CLINICAL DEVELOPMENT

Phase I

Healthy volunteers received single i.v. doses (0.001–0.4 mg/kg) or multiple doses (0.001–0.2 mg/kg/day for seven days) of BMS-204352 in three randomized, double-blind, placebo-controlled clinical trials to investigate its safety, tolerability and pharmacokinetics (12,25). At single or multiple doses up to 0.2 mg/kg BMS-204352 was well tolerated, safe, and not associated with any effect on either cardiovascular parameters or psychomotor function. At doses at 0.3 or 0.4 mg/kg BMS-204352 caused vehicle-associated postural hypotension that leveled at 3 to 6 h and disappeared at 12 to 24 h after treatment (12,25). Total clearance and volume of distribution were dose-independent. The terminal $t_{1/2}$ was 20 h after a rapid initial drop in blood levels of BMS-204352 during the first several h (12,25).
Phase II

The safety and pharmacological profile of BMS-204352 (0.1, 1, or 2 mg/kg i.v.) over a period of 72 h were examined in a multicenter, double-blind study in acute stroke patients (10). Multiple doses of 0.1, 1, or 2 mg i.v. were used. The dosing was initiated within 48 h of the onset of stroke symptoms, and at all doses the drug was well tolerated. No clinically significant differences in adverse events were observed between control subjects and those who received BMS-204352. Volume of distribution and total clearance were shown to be independent of the dose, thus confirming results obtained in phase I clinical trials (12,25).

Phase III

The phase III trials, POST-010 and POST-011, were designed to investigate the safety and efficacy of 2 doses of BMS-204352 versus placebo in patients with acute stroke (2,3). The studies included 1978 patients at 200 centers worldwide. Patients included in the trials had a historical Rankin scale score ≤1, a baseline NIH stroke scale of 6 to 20 and a modified Rankin scale score ≥2. Patients received BMS-204352 at 1.0, 0.1 mg or placebo within 6 h of stroke symptom onset. Four i.v. doses were given at 24 h intervals over 72 h. The primary outcome measure was a change from baseline to week 12 on the NIH stroke scale. The secondary outcome included the modified Rankin score and responses on the modified Rankin scale and the Barthel Index, respectively, measured at week 12. BMS-204352 failed to show superior efficacy compared to placebo (2,3). Bristol Myers Squibb still considers BMS-204352 in full development against ischemic stroke (BMS: Annual Report Near-term pipeline, 3/1–2002. http://www.bms.com/annual/2000ar/annual_site/pipeline/data/pipe.html) although no information on the continued trials is currently available.

CONCLUSIONS

BMS-204352 represents a novel first-in-class compound that has been evaluated in acute ischemic stroke. BMS-204352 is an activator of several subtypes of neuronal potassium channels (9,13,26) and from a mechanistic point of view, these types of drugs appear to be an ideal therapeutic approach, as they will decrease neuronal excitability and excitatory transmitter release. Unfortunately, the enthusiasm with this approach is not supported by the clinical outcome of the Phase III trials, which might be due to the relative small reduction in infarct volume also seen in animals at the therapeutic doses. The future will show whether the lack of efficacy of BMS-204352 in the Phase III trials adds this compound to the increasing number of neuroprotective agents that have been evaluated for acute ischemic stroke and have failed.

REFERENCES


