● PERSPECTIVE

Preclinical concepts and results with the $GABA_A$ antagonist S44819 in a mouse model of middle cerebral artery occlusion

Recent advancements in recanalizing therapies, *i.e.*, the combination of thrombolytic drugs with interventional thrombectomy, have considerably improved neurological outcome in ischemic stroke patients. Despite this progress, the large majority of stroke patients still exhibit neurological deficits in the long run, and ischemic stroke continues to be the most frequent cause of long-term disability. Hence, there is an unmet need for therapies that allow enhancing neurological recovery and brain plasticity in the post-acute stroke phase (Hermann and Chopp, 2012). Preclinical studies, *e.g.*, delivering growth factors (Reitmeir et al., 2011) or neural precursor cells (NPC) (Bacigaluppi et al., 2016), have shown that brain plasticity can be successfully stimulated in the post-acute stroke phase, resulting in functional neurological improvements. These findings raised the question whether it is possible to promote neurological recovery and brain plasticity in stroke patients.

Following ischemic stroke, neuronal excitability is tonically reduced in peri-infarct brain tissue *via* inhibitory influences of extrasynaptic $GABA_A$ receptors (Belelli et al., 2009; Hines et al., 2012). While tonic inhibition may protect ischemic tissue from injury in the acute stroke phase, in which tissue energy state is compromised, GABAergic tonic inhibition may impair neurological recovery in the subsequent post-acute stroke phase, in which neuronal activity is required for neuronal sprouting and rewiring. Indeed, the inhibition of $GABA_A$ receptors by the negative allosteric modulator $GABA_A$ L655708 enhanced neurological recovery in mice exposed to photothrombotic stroke and rats exposed to endothelin microinjection (Clarkson et al., 2010; Lake et al., 2015). This effect depended on the presence of the α5 subunit in $GABA_A$ receptors, as demonstrated in $GABA_A$ $\alpha5^{-/-}$ mice, in which L655708 failed to restore neurological deficits (Clarkson et al., 2010).

Using a potent and competitive $GABA_A$ receptor antagonist, S44819, which selectively binds to α5 subunit-containing $GABA_A$ receptors at their GABA binding site and has no affinity to the benzodiazepine site (Etherington et al., 2017), we recently showed promising efficacy in an animal model of ischemic stroke, *i.e.*, intraluminal middle cerebral artery occlusion (MCAO) in mice (Wang et al., 2018). $GABA_A$ α5 selectivity of S44819 was achieved by its binding to amino acid residues within the F-loop of the α5 subunit, which is involved in GABA binding (Etherington et al., 2017). In mouse hippocampal CA1 neurons, S44819 enhanced hippocampal long-term potentiation and blocked tonic currents mediated by extrasynaptic α5 subunit-containing $GABA_A$ receptors, but had no effect on synaptic $GABA_A$ receptor activity (Etherington et al., 2017). In rats and mice, S44819 improved memory in a variety of cognitive tests, when administered orally at doses between 0.3 and 3 mg/kg body weight (Gacsályi et al., 2017).

In the mouse MCAO model, we showed that S44819 at 10 mg/kg twice a day persistently improved motor-coordination and spatial memory in two consecutive studies, an explorative study followed by a confirmation study, in which the active drug or vehicle was orally delivered over 4 weeks starting 72 hours post-stroke and in which neurological recovery was evaluated over 42 days, *i.e.*, up to 14 days after completion of S44819 delivery (Wang et al., 2018). In this study, the improvement of motor-coordination and spatial memory was associated with reduced striatal atrophy, increased neuronal long-term survival, reduced peri-infarct astrogliosis, increased peri-infarct brain capillary formation and increased neural precursor cell proliferation and differentiation in the ipsilesional brain hemisphere. Contralesional pyramidal tract plasticity, evaluated by anterograde tract tracing, was not influenced by S44819. Concentrations of neurotrophic (brain derived neurotrophic factor, glial cell derived neurotrophic factor) and angiogenic (vascular endothelial growth factor, fibroblast growth factor) growth factors were elevated by S44819 in peri-infarct, but not contra-lesional brain tissue.

Considering its beneficial pharmacological profile and the absence of safety concern in animals–in particular no anxiogenic effects and no pro-convulsive effects were noted, S44819 underwent phase I dose escalation studies in healthy volunteers which revealed a good acceptability. This raises hopes that the compound should similarly be tolerated in human patients. Conceptionally important, S44819 was evaluated in a randomized double-blinded placebo-controlled phase I crossover trans-cranial magnetic stimulation (TMS) study in healthy young humans, in which single 100 mg doses of S44819 reduced active motor threshold, *i.e.*, the intensity needed to produce a motor-evoked potential of 0.5 mV, and the amplitude of the N45 potential, *i.e.*, a GABAergic component of the TMS-evoked electroencephalography response (Darmani et al., 2016). These observations demonstrated that S44819 reaches human cortex to increase cortical excitability.

Based on these data, S44819 is evaluated since January 2017 in an international randomized placebo-controlled phase IIb trial, the Randomized Efficacy and Safety Trial with Oral S44819 after Recent Ischemic Cerebral Event (RESTORE - Brain), in which S44819 (150 mg or 300 mg, twice a day) or placebo is delivered over 90 days starting at 3 to 8 days post-stroke followed by a 15-day follow-up without the drug to evaluate consequences of drug washout. Inclusion criteria are ischemic strokes involving cerebral cortex in patients aged 18–85 years with a National Institutes of Health Stroke Scale score of 7–20 points. The primary endpoint is neurological recovery evaluated based on the modified Rankin scale. Secondary endpoints include the National Institutes of Health Stroke Scale score, Barthel index, Montreal Cognitive Assessment scale, and Trail-Making Tests. Results from RESTORE-Brain study are expected in the course of summer 2019.

The preclinical translation concept of S44819 has a number of strengths:

(1) Rigorous evaluation of drug effects in a relevant animal model of ischemic stroke, that is, intraluminal MCAO, in an explorative study followed by a confirmation study. Hence, this study fulfilled principles of a hypothesis-building study that was confirmed by a second independent data set.

(2) Stringent use of STAIR (Stroke Therapy Academic Industry Roundtable, 1999) and ARRIVE (Kilkenny et al., 2010) guidelines in the planning and conduction of animal experiments, including randomization and blinding, as well as in subsequent data reporting.

(3) Adequate statistical powering to detect drug effects with an alpha error of 5% and a beta error (1 – statistical power) of 20 % in each of both, the explorative and confirmation study.

(4) Solid behavioural assessments using comprehensive batteries of motor – coordination and cognitive tests that revealed neurological improvements which persisted after discontinua-

tion of drug delivery.

(5) Rigorous histochemical assessments revealing structural brain remodelling and plasticity that accompany motor–coordination and cognitive improvements.

(6) Phase I dose escalation studies complemented by a TMS study in healthy humans, which revealed that S44819 at single doses of 100 mg reduced the active motor threshold and amplitude of the N45 potential (Darmani et al., 2016), thus indicating effects of S44819 on cortical function at doses very similar to those clinically applied.

For evaluating effects of S44819, we employed a protocol very similar to protocols which we previously used for evaluating effects of growth factors (Reitmeir et al, 2011) and NPCs (Bacigaluppi et al., 2016) on post-ischemic neurological recovery, brain remodeling and plasticity. In these studies, treatment was also initiated at 3 days after MCAO, considering that ischemic injury is completed at this time-point, and motor-coordination and cognition were studied over 6 weeks, *i.e.*, up to 2 weeks after the completion of treatment, using comprehensive test batteries. Structural brain remodeling and plasticity were examined using histochemical studies. Interestingly, the time-window at which neurological recovery occurred following S44819 administration was faster than after growth factor (Reitmeir et al., 2011) or NPC (Bacigaluppi et al., 2016) delivery, suggesting both a rapid symptomatic and a delayed restorative effect. After growth factor and NPC delivery, neurological improvements gradually evolved over 4–6 weeks post-treatment onset. In contrast to S44819, growth factors and NPCs exhibited lesion-remote effects, that is, promotion of contralesional pyramidal tract plasticity (Reitmeir et al., 2011; Bacigaluppi et al., 2016). The effects of S44819 were confined to peri-lesional tissue, in line with its effect on peri-infarct tonic inhibition.

The currently conducted RESTORE–Brain trial is unique in that it was designed very closely to the animal study. Thus it fulfils an important principle of translation strategies, which is that studies in animals and humans should mirror each other if possible one to one. Considering the nature of a vascular occlusion that affects the middle cerebral artery territory, study inclusion in RESTORE–Brain requires cortical involvement of brain infarcts. The onset of S44819 treatment in humans (3–8 days post-stroke) closely replicates the treatment onset in the mouse study (72 hours post-stroke). The duration of S44819 treatment (28 days in mice, 90 days in humans) is subchronic in both cases. It needs to be considered that there is no consensus how post-stroke time-windows in mice should precisely be translated to human patients. These strengths in the study design raise hopes that the $GABA_A$ α5 antagonist S44819 might allow to improve neurological recovery in stroke patients.

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