Neuroprotective effects of inhibition of α5β1 integrin following experimental stroke: A dual centre pre-clinical study

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Blood-brain barrier (BBB) dysfunction after ischemic stroke exacerbates brain damage by contributing to edema and inflammation. The β1 integrin receptor family may contribute to this dysfunction via alteration of BBB-forming tight junction proteins. We hypothesize that inhibition of the β1 integrin receptor subtype α5β1, which is acutely expressed in infarct and peri-infarct vasculature after experimental stroke, reduces BBB permeability, improves functional recovery and reduces infarct volume. Using randomized and blinding protocol, transient middle cerebral artery occlusion (MCAO) was carried out in mice (60 min; n=8) and rats (90 min; n=15) in two independent laboratories. ATN-161 (α5β1 inhibitor; 1 mg/kg) was administered IV immediately upon reperfusion and on post-stroke day 1 and 2. Infarct volume was determined by cresyl violet (mice) and T2 weighted MRI (rat) at day 3 post MCAO. Steady state contrast enhanced MRI was used to assess BBB breakdown in rats at day 3. ATN-161 resulted in a significant reduction in infarct volume in both mice and rats when measured at post-stroke day 3 ($p<0.001$). Behavioral tests (open field, rotorod, sticky label and 28 point neuroscore), demonstrated significantly improved functional recovery in both mice and rats following treatment with ATN-161. BBB permeability was decreased upon ATN-161 treatment in vivo as determined by reduced IgG and claudin-5 immunostaining in mice and reduced extent of Gadolinium enhanced MRI signal change in rats.

Finally, in vitro studies where stroke was simulated using oxygen and glucose deprivation or TNF- α, ATN-161 (10 μM) treatment demonstrated decreased barrier permeability as measured by trans- endothelial cell electrical resistance, FITC-dextran permeability, and claudin-5 immunocytochemistry. Collectively, our results demonstrate that post-stroke inhibition of α5β1 integrin with the small peptide ATN-161 profoundly reduces infarct volume, improves functional outcome and decreases BBB permeability in both mice and rats using two different ischemic stroke models. Therefore, inhibition of α5β1 by ATN-161 could represent a novel stroke therapeutic target worthy of further investigation.