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A Novel Selective αvβ3 Antagonist Inhibits Oxygen-Induced Retinal Neovascularization in Mice

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Angiogenesis plays a critical role in the loss of vision of several retinal proliferative diseases including age-related macular degeneration (AMD), proliferative diabetic retinopathy (PDR) and retinopathy of prematurity. Integrins, the superfamily of cell-surface receptors that mediate cell-cell and cell-matrix interactions, are heterodimeric receptors composed of α and β subunits and integrins mediate the interaction between cells and extracellular matrix (ECM). It has been reported that αvβ3 and αvβ5 integrins are upregulated in neovascular membranes from patients with PDR and αvβ3 integrin is increased in patients with wet AMD. Specific ECM proteins which can activate integrin signaling are also observed in retina. Vitronectin is the important ECM in eyes and its related binding receptor of αvβ3 integrin plays a pivotal role in the regulation of angiogenesis. Blockade of integrin αvβ3 is recently considered as a potential strategy of anti-angiogenesis therapies in retina. Here we investigated the effect of a novel disintegrin of ARLDDL, a genetically modified mutant from snake venom of rhodostomin, on the retinal angiogenesis. Compared with monoclonal antibody, this protein drug has more binding sites with αvβ3 integrin and has much higher affinity with target integrin. ARLDDL is conjugated with human serum albumin (HSA) or modified human serum albumin (HSA-(C34S)) to prolong the *in vivo* half-life and hopefully to reduce immunogenicity in human being. In addition, to avoid the side effects such as bleeding, the variants of ARLDDL have selective integrin αvβ3 genetic modification antagonistic activity and substantially reduced αIIβ3 or α5β1 antagonistic activity as compared with its wild-type protein of Rhodostomin.

In this study, the data given are means ± S.E.M. The significance of difference between the experimental group and control was assessed by one-way analysis of variance (ANOVA) and 2-tailed Student's t-test. The difference is significant if the *p* value is less than 0.05. Male ICR mice were used for oxygen-induced retinopathy (OIR) mice model. It was found that αvβ3-associated ECM, vitronectin, was up-regulated in several area including inner nuclear layer, inner plexiform layer, ganglion cell layer and nerve fiber layer in the retina of mice following OIR by IHC staining. Intravitreal injection of ARLDDL variants markedly inhibited retinal neovascularization. HSA-(C34S)-ARLDDL inhibited the migration of human umbilical vein endothelial cell induced by vascular endothelial growth factor toward vitronectin- or fibronectin- coated transwells (89.2 ± 1.6 % and 59.5 ± 11.1 %, respectively, with 1 μM HSA(C34S)-ARLDDL). ARLDDL variants also significantly inhibited the formation of blood vessels in matrigel angiogenesis model in B6 mice which were anaesthetised by trichloroacetaldehy (400mg/kg) during surgery. In conclusion, our results show that the derivatives of rhodostomin, ARLDDL, which is highly specific to αvβ3 integrin, significantly and potently inhibited endothelial cells mediated angiogenesis. These results indicate that the interaction between αvβ3 and ECM plays an important role in retinal angiogenesis and variants of ARLDDL, the highly selective and long-acting αvβ3 integrin antagonists, are potential therapeutic candidates for the treatment of retinal pathological neovascularization such as age-related macular degeneration, proliferative diabetic retinopathy and retinopathy of prematurity.