

Ceramide 1 phosphate is a potent chemoattractant factor of endothelial colony forming cells and improve post ischemia tissue regeneration.

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Ceramide 1 phosphate (C1P) is a well-known chemotaxis inductor in macrophages and murine progenitor cells. In endothelial colony forming cells (ECFC), we have shown that C1P improved proliferation and tubule formation, although C1P chemotactic effects remain unknown. Considering that, C1P levels are elevated in ischemic tissues and that ECFC migration is a key step post-ischemia tissue revascularization, we here aimed to study whether C1P is an ECFC chemoattractant factor and improve their revascularization abilities *in vivo*.

Human cord blood-derived CD34+ cells were cultured in EGM2 and, after 14-18 days, ECFC colonies were obtained. ECFC were treated with C1P short chain analog C8-C1P. N=3-6, p<0.05, one-way ANOVA.

We found that C8-C1P is a potent chemoattractant factor for ECFC not only *per se*, but also combined with SDF1 (transwells, Figure 1A). C8-C1P-induced chemotaxis was completely suppressed by pharmacological inhibitors of ERK1/2 and AKT pathways (Figure 1). *In vivo*, we observed that C8-C1P not only has a potent vasculogenic effect by itself, but also potentiated plug vascularization mediated by ECFC (Geltrex plug implants, Figure 2). Moreover, in a murine model of hind limb ischemia, intramuscular injection of C8-C1P enhanced blood perfusion in the ischemic limb and slightly increased the revascularization mediated by untreated ECFC transplantation. Furthermore, administration of C1P-pretreated ECFC together with intramuscular C1P resulted in a significant improvement of leg reperfusion compared to each condition alone (Figure 2).

In conclusion, C8-C1P induce ECFC chemotaxis *in vitro*, through AKT and ERK1/2 activation, and *in vivo* in a hind limb ischemia model, where C8-C1P not only attract ECFC to the ischemic muscle, but also augmented ECFC revascularization abilities. Our results highlight the therapeutic potential of C1P to improve post ischemia tissue regeneration.

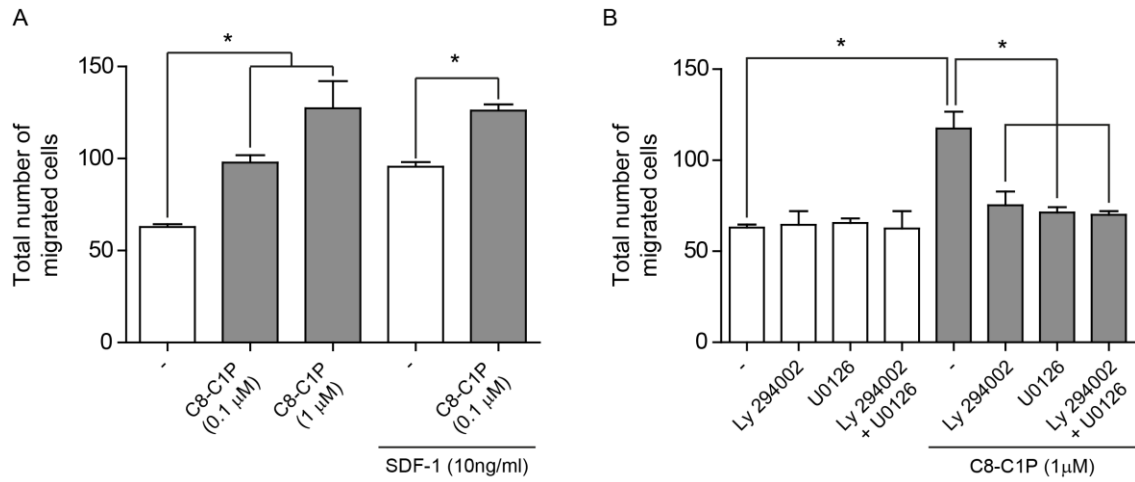


Fig.1. C1P driven chemotaxis was abrogated by AKT (Ly294002) and ERK1/2 (U0126) pathway inhibitors.

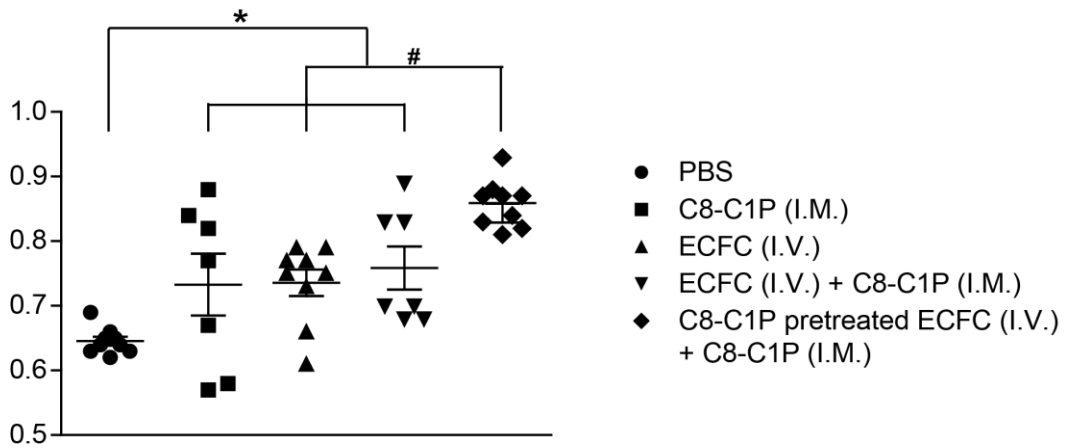


Fig.2. C1P attract ECFC to the ischemic muscle and augmented ECFC revascularization abilities *in vivo*.

Key words: Endothelial colony forming cell, tissue regeneration, angiogenesis, ceramide 1 phosphate.