Arteriogenesis: Revascularization of the Severely Ischemic Limb

Poster Presentation

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The incidence of severe limb-threatening ischemia (SLI) manifesting as forefoot rest pain, non-healing foot ulceration, and gangrene is on the rise due in part to the rising incidence of diabetes and to the aging of our population. A non-invasive cell based strategy that promotes infrageniculate neovascularization was investigated. The strategy combines the use of a programmed pneumatic compression device (PPCD) and granulocyte colony stimulating factor (G-CSF). The PPCD is approved by the FDA to help heal ischemic foot ulcers. The observed increase in arterial hemodynamics observed with PPCD use is in part attributed to arteriogenesis (the formation and widening of medium-sized blood vessels). However use is associated with variable outcomes, particularly in those most in need: elderly SLI patients with diabetes, renal failure, and/or congestive heart failure. We attribute this in part to the known reduced number and function of circulating reparative pro-angiogenic progenitor cells seen in this population. These cells would normally participate in arteriogenesis. G-CSF is a cytokine growth factor that is FDA approved for stem cell mobilization. It dramatically boosts the circulating number of reparative progenitor (stem) cells needed for neo-vascularization. G-CSF also induces endothelial cells to proliferate and migrate, and stimulates repair of mechanically wounded endothelial monolayers. Our hypothesis is that the boosting the circulating progenitor cell number with G-CSF will potentiate PPCD induced neo-vascularization.

We tested this hypothesis in three SLI patients consented for major amputation following IRB approval, and confirmation that no revascularization option was left. Each patient wore a PPCD for one hour at a time three times a day in the seated position until healing of severe ischemic ulceration and gangrene was achieved. G-CSF (Filgrastim, Amgen Inc) 10 mcg/kg was injected subcutaneously every three days for a total of 10 doses (FDA waiver for IND). Serial data included hemodynamic testing, tissue oxymetry and angiography. Wound size, ambulation distance, and pain assessment were recorded. Cytometry included serial hematology, circulating mononuclear cell count, with specific measure of CD34 cell count (endothelial progenitor cell).

Resolution of ischemic rest pain occurred in all three patients, two of whom required methadone and percoset on entry. All three became ambulatory. One patient healed a large ulcer over exposed Achilles tendon, with improvement of her ankle brachial index (ABI) to 1.05 from 0.43 on entry, and TcPO2 elevation from 1 mmHg to 35 mmHg (normal > 30 mmHg). Her toe and transmetatarsal (TM) pressures had been 0 mmH with non-pulsatile waveforms on entry. She eventually developed TM pulsatility. After healing her ankle ulcer she traumatized her 5^{th} toe which became cyanotic. The strategy

was repeated. She developed pulsatility in all 5 toes and healed the 5th toe. The second patient healed an opened transetatarsal amputation which became necrotic after a failed bypass. His AVI rose to 0.64 from 0 on entry. His TcPO2 rose from 3 mmHg on entry to 24 mmHg. The third patient healed an ulcer overlying exposed extensor tendons in her proximal foot. Her ABI rose from 0 to 0.56 mmHg. Her TcPO2 rose from 0 to 22 Hg. All three patients had dramatic neovascularization on follow-up angiogram. The cytometry data showed the White Blood Cell Count (WBC x 103 cells/ml) rose from 7 + 2 at the time of injection to 25 + 3 the day after, and fell to 16 + 3 by 48 hourse with each injection. The measured CD34+ cell count in the peripheral blood was 4.1 + 0.5 x 104 cells/ml one day after G-CSF dosing, and was not detectable before G-CSF dosing.

Healing of a dehisced TMA wound, an ankle wound with exposed Achilles tendon, a toe ulcer when the PPG waveforms are flat, or a dorsal foot wound in the setting of profound ischemia would be challenging even in the setting of good arterial perfusion. That healing occurred in the setting of severe ischemia supports efficacy of this approach. Healing correlated with hemodynamic and angiographic evidence of neovascularization. This occurred despite severe co-morbidities in each patient: coronary artery disease in two, renal insufficiency and diabetes in two, hypercoaguability in one and congestive heart failure in one. The unusual success seen in this small number of challenging patients is consistent with the extensive supportive in vitro and animal data derived over the past decade in the investigator's laboratory. This approach eliminates marrow harvest and ex vivo processing used in other cell therapy angiogenesis strategies. The investigators seek to expand the clinical experience in a controlled clinical trial, and to correlate clinical efficacy with biologic markers.