Basi biochimiche dell'azione dei farmaci

“PREVENTION OF CORNEAL GRAFT REJECTION”:
Immunosuppressive and anti-apoptotic gene therapy.

Pisa, 11-11-2014
Caterina Serratore
Corneal transplantation or Keratoplasty: LAMELLAR o PENETRATING

As in other transplanted tissues, corneal allograft rejection is dependent on alloreactive T-cell activation.

Several studies have identified risk factors for rejection, such as corneal neovascularisation, presence of herpetic eye disease, and repeat corneal grafting.
“Clinical presentation, risk factors and treatment outcomes of first allograft rejection after penetrating keratoplasty in early and late postoperative period” 26,711-717 Eye (2012)
“Prolonging survival of corneal transplantation by Selective Sphingosine-1-Phosphate Receptor 1 Agonist”

In the past years, various immunomodulatory strategies have been used in experimental corneal transplantation, such as manipulation of co-stimulatory molecule function including Cyclosporine A (CsA) and FTY720.

Plos One, September 12, 2014
MECHANISM OF ACTION OF CsA

Mechanism of action of cyclosporine or tacrolimus (FK506)

Expert Reviews in Molecular Medicine © 2000 Cambridge University Press
FTY720 demonstrated substantial efficacy in prolonging survival of the grafts through regulating the S1P1.

FTY720 is phosphorylated to FTY720p by sphingosine Kinase 2. FTY720-P induces long-term down regulation of S1P1 on lymphocytes, and thereby inhibits the migration of lymphocytes toward S1P.


FTY720 acts as a full agonist on sphingosine-1-phosphate (S1P) receptors (S1P1, S1P3, S1P5). S1P1 selective agonist may be a more effective drug for prevention of rejection in corneal transplantation.

Plos One, September 12, 2014
Experiment animals

A total of 216 BALB/c mice were used in the study. Firstly, 120 BALB/c mice were used to determine the optimal dose of selective S1P1 and non-selective FTY720. Then, 96 mice were performed corneal transplantation and divided into four groups:

- **Control group**
- **CsA treated group**
- **FTY720 treated group**
- **Selective S1P1 agonist treated group.**
14 days post transplantation

Control  Cyclosporin A  FTY720  S1P1 selective agonist

Plos One, September 12, 2014
Both S1P1 selective agonist and FTY720 promoted the graft survival in a dose-dependent manner.

*Plos One, September 12, 2014*
Protein levels of the pro-inflammatory cytokines IL-2, IFN-γ, anti-inflammatory cytokines IL-10 and TGF-b1 in the serum of the four group mice.

The levels of TGF-β1 in the S1P1 selective agonist treatment group was higher than that in the control group. So, S1P1 agonist may also as a regulator of TGF-b1.

Plos One, September 12, 2014
Compared with the control group, the expression of IL-2 and IFN-γ had no significantly changes after treatment by S1P1 or FTY720.
Corneal Neovascularization and the utility of topical VEGF inhibition:
Ranibizumab (Lucentis) Vs Bevacizumab (Avastin)

Corneal NV is a potential complication of numerous bacterial, parasitic, and viral infections.

“Corneal neovascularization occurs when the balance between angiogenic and antiangiogenic factors is tipped toward angiogenic molecules. Vascular endothelial growth factor (VEGF), one of the most important mediators of angiogenesis, is upregulated during neovascularization”.

“Corneal Neovascularization: An Anti-VEGF Therapy Review”.
VEGF binds to two members of a receptor tyrosine kinase family, VEGFR-1 and VEGFR-2, also known as Flt-1 and KDR, respectively. VEGF binding to VEGFR-2 induces the dimerization and autophosphorylation of receptors by intracellular kinase domains, which leads to a mitogenic and proliferative signal.
VEGF is a molecule that binds to certain cell to stimulate new blood vessel formation. When VEGF is bound to the drug, it cannot stimulate the formation and growth of new blood vessels.

“Safety and Efficacy of Bevacizumab in High-Risk Corneal Transplant Survival”
ClinicalTrials.gov
Bevacizumab is a humanized monoclonal antibody that binds to isoforms of VEGF-A. Corneal NV diminishes the integrity of epithelial tight junctions, thereby permitting macromolecules such as bevacizumab to penetrate through the corneal epithelial barrier.

Topical bevacizumab is an effective treatment for corneal NV; however, there is some variability in the clinical response to topical bevacizumab treatment.

“Corneal Neovascularization and the utility of topical VEGF inhibition: Ranibizumab Vs Bevacizumab”  
Ocul Surf. Author manuscript. Published in final edited form Apr 2012; 10(2): 67-83
Immunoreactivity to bevacizumab was limited to the superficial epithelial layers of normal corneas (A), whereas immunoreactivity to bevacizumab was found in all layers of most neovascularized corneas (B).
Ranibizumab is a recombinant humanized monoclonal antibody fragment that binds and inhibits VEGF-A isoforms.

Ranibizumab has a molecular weight of 48kD, theoretically allowing for better corneal penetration; additionally, ranibizumab has been affinity-matured to optimize its VEGF-A binding potential. Topical ranibizumab was efficacious earlier in the course of treatment than topical bevacizumab.
Anti-apoptotic gene therapy prolongs survival of corneal endothelial cells during storage

The apoptosis is a principal reason for rendering donor tissue unsuitable for transplantation.

The goal of this study was to transduce human donor corneas in vitro using a lentiviral vector to perform gene transfer of baculoviral p35 or mammalian Bcl-xL to corneal endothelium. The results show enhanced survival and prolonged retention of physiological EC morphology in cells expressing either p35 or Bcl-xL.

Gene Therapy 18, 778-787 (August 2011)
T A Fuchsluger, U Jurkunas, A Kazlauskas and R Dana