Abstract

The human pterygium remains, even after three thousand years from its first description, an incompletely described disease, with a controversial surgical cure and a therapy that is more conservative than curative. Almost completely uninteresting for the ophthalmologists, more so for the researchers, the human pterygium represents a benign disease, but frequently relapsing after the surgical cure. The controversy linked to the therapy and prognosis derives from a very brief description of its microscopic immunohistochemical and molecular aspects. Despite the clinical use of antiangiogenic therapy, the angiogenesis of the pterygium is incompletely understood.

A specific target with implications in the formation of new vessels has not been found to this day. More so, lymphangiogenesis, the formation of new lymphatic vessels, is less known and less described in literature. The begining of the formation of new vessels in the human pterygium and the role in its pathogenesis are unknown. For this reason, the theme of the thesis is based on completing the angiogenic and lymphangiogenic profile of the human pterygium, which is characterised by microscopic, immunohistochemical and molecular parameters. Starting with the first hypothesis regarding the existence of a possible angiogenic factor, theory released by Wong in 1978, several aspects of angiogenesis in the human pterigyum have been widely discussed, especially for finding an antiangiogenic agent or an antivascular therapy used for the reduction of the relapses. Several factors involved in the normal angiogenesis as well as in tumour angiogenesis have been proved to be involved in the angiogenesis of the pterygium. In spite of the proof that sustains an intense angiogenic activity in the human pterigium, the formation of new blood vessels is not yet clear, the process of sprouting and intususception being most probably involved.

The newly formed vessels in the human pterygium have similar characteristics to the ones appearing in tumours. They are permeable and have a discontinuous vascular wall. Molecular and morphological differences between the lymphatic vessel of the conjunctiva and the ones in the pterygium can be found on Pub Med. Differences between the density levels and distribution of the lymphatic vessels in the normal conjunctiva and the pterygium have been revealed by the D2-40 immunostaining. The vessels in the normal conjunctiva have a different structure, being separated by other stromal components.

The rise of vascular density has been proved to be linked with the clinical parameters and the size of the pterygium. The role of the lymphatic vessels in the human pterygium is unknown. Data regarding the mechanism and the molecular base of development of the new lymphatic vessels is not available.

Optimization of the identification of new therapeutic targets in the multitude of markers that govern the angiogenesis process is the main motivation of this thesis. Presently, with the exception of VEGF, no other molecular marker has been intensely studied in the human pterygium.

The attempt to link the etiopathogenic factors, especially the ultraviolet rays, with the changes in the endothelial cells which underlie the start of the angiogenic process, represents a second motivation of this thesis. The lack of an experimental model used to dynamically study and test the antiangiogenic and/or antivascular agents, has lead us to the use of the chorioallantoid membrane of the embrionated egg and the hairless mice SKH 1 for the comparative study of the behaviour of the implanted pterygium. Between the two models, the validation of the optimal model used in the future as a testing support and evaluation of new therapies, is an important component of the present thesis.

From the clinical point of view, differences between the groups of male and female individuals have been identified. In the male group there has been identified a patient with one primary pterygium and two relapses. Another particularity in the male's group has been the unilaterality of the lesions, namely, the excised pterygium, be it primary or relapsing, has affected one eye, and the relapses appeared at the same eye considering the male subject with three relapses. The particularity of the female group consists of the fact that it has been the source of only primary pterygiums, and not one relapse. In contrast to the male group, there were five female patients with bilateral pterygium (3 with type I bilateral pterygium and 2 with type II bilateral pterygium).

Another important aspect refers to the incidence of pterygium in different age groups and depending on the sex of the patient. Therefore, in the case of the male patients, the age group of 31-40 years of age included 2 cases of primary pterygium, the 41-50 years of age group included 7 cases (5 primary pterygiums and 2 relapsing ones in the case of the same patient), the 51-60 years of age group included 2 cases of primary pterygium and the 71-80 group revealed 2 cases of primary pterygium. The vascularisation of the epithelium represents a pathologic entity rarely met but not

exceptional. The morphologic observations on the presence of intraepithelial blood vessels, have suggested an intense and continuous angiogenic process. Based on this hypothesis it has been considered useful the employment of immunocoloring for the description of the intraepithelial vessels in connection to their functional status. The identification of the activated status versus the non activated status has been made possible with the use of the immunocoloring with endogline (CD 105), present in the activated endothelial cells and absent in the mature, dormant ones. Endogline was positive in the intraepithelial and stromal vessels in the primary and relapsing pterygium, being absent in the conjunctival epithelium on the cvasinormal conjunctiva that is adjacent to the pterygium.

The density of intraepithelial vessels positive for CD 105 has been higher in the relapsing pterygiums compared to primary pterygiums. In the conjunctive tissue of the normal conjunctiva positive vessels for CD 105 have not been observed. The density of the intraepithelial vessels positive for CD 105 has risen directly proportionate with the thickness of the pterygium epithelium. Besides the positive reaction to the CD 105, the active state of the intraepithelial angiogenesis in the human pterygium has also been demonstrated by the existence of the CD 105 positive endothelial cells with a heterogeneous organisation, all the stages of development of the blood vessels, starting with isolated endothelial cells, endothelial cords, vessels with a split lumen and vessels with a well-formed, permeable lumen have been revealed. Therefore, in regard to the angiogenic process it can be said that the intraepithelial angiogenesis in pterygium has a mixed mechanism of sprouting and intususception. RNA scope is a useful method of quantification of VEGF. The expression of VEGF mRNA is restricted at the epithelial compartment of the primary and relapsing pterygium. VEGF mRNA is distributed heterogeneously in the epithelial compartment. The relapsing pterygium reveals an excess expression of VEGF mRNA compared to the primary pterygium. The basal cells of the pterygium are an important source of VEGF. VEGFR 3 is expressed in the epithelial and conjunctival compartment of the primary pterygium. In the stromal compartment, VEGFR 3 is expressed at the level of the endothelial cells of the lymphatic vessels and of the postcapillary venules. Our data sustain the existence of a subtype of activated VEGFR 3 positive macrophage cells, capable of inducing and sustaining the lymphangiogenesis process within the pterygium stroma. The role of VEGFR 3 in the

mechanism of lymphatic intususception is obvious and needs further investigation. Mast cells within the pterygium are VEGFR 3 positive.

Lymphangiogenesis is present the human pterygium, it appears early and it develops through two distinct mechanisms: sprouting and intususception. The D2-40 positive lymphatic vessels present an active remodelating phenomenon and continuous organisation due to the presence of lymphatic endothelial proliferation detected using the immunoreaction with Ki67. There are two major paths recognised as been implicated in the p53 dependant DNA repairing in the UV exposed cells: repairing through the nucleotid excision and repairing through the bases excision (BER). The wild type of p53 cells present a normal BER, while the mutant p53 cells or the null cells for p53 present defects of BER. Based on these previous discoveries, the persistent expression of timine dimers, together with the absence of p53 expression in the stromal components in the majority of our cases, suggests that the absence of the protective function of p53 against UV rays induces DNA destruction in the endothelial cells and in the fibroblasts from the fibrovascular compartment of the human pterygium. Recent reported discoveries regarding the presence of ciclobutane pirimidine in the 270 codone of the p53 gene which was subjected to a reduced level of repairing compared to adjacent position, have demonstrated the existence of cell mutations in cutaneous tumoral models in mice. Based on these findings, we have speculated that a similar mechanism may be involved in the persistence of timine dimers followed by the inducing of mutagenic modifications in the stromal cells of the human pterygium. These epithelial and stromal cell mutations could be responsible for the increased number of relapses and for the resistance against conventional therapies. The chorioallantoid membrane represents and optimal model for the study of angiogenic and lymphangiogenic aspects, but also of other particular aspects of the human pterygium, like the cells with progenitor characteristics. The implantation of human pterygium on chorioallantoid membrane may be considered, after validation in the present study, as being useful for the study of stroma-implant interaction, for the testing of new therapeutic agents and for the dynamic follow-up of their effects, as a preclinical stage of the study, before its use in the clinical trials.

The presence of intraepithelial activated vessels, the over expression of PROX 1 in the lymphatic endothelium, the DNA alteration correlated with the p53 expression, as well as the execution and validation of the experimental model on the

chorioallantoid membrane as a model for testing of future antiangiogenic and antivascular therapies, represent the original elements of the present thesis.

Key Words: ANGIOGENESIS, LYMPHANGIOGENESIS, HUMAN PTERYGIUM, TARGETED THERAPY