Kokkinidou Despoina

METHODS FOR THE ISOLATION OF MONOCYTES POPULATION FOR IN VIVO STUDIES OF INFLAMMATORY RESPONSE IN STENTS.

Coronary artery disease is considered one of the principal causes of death, affecting a significant fraction of the human population and accounting for almost half of the total number of heart-related fatalities in the developed world. Despite efforts to treat the condition using coronary implants (stents), complications often arise in treated patients, chief among them being the problem of restenosis. One of the possible causes of restenosis may be the mechanical wear and corrosion that stents undergo following implantation in the vasculature of patients.

To investigate the possible connection between stent corrosion and vessel restenosis, innovative in vivo imaging techniques are used to evaluate the induced inflammation in the vessel. Specific markers of the inflammation process include the accumulation of activated monocytes (macrophages) at the site of implantation of the stent. The objective of this study is the development of in vitro techniques for the separation and isolation of monocytes from blood as well as their labelling with the appropriate dyes so that they can be monitored in experimental mice.

The isolation technique comprising the optimum combination of all factors and which was ultimately judged as the ideal method to be pursued experimentally, is the combination of magnetic separation of monocytes through negative selection from whole blood and the use of centrifugation using the specific density polysaccharide solution Histopaque-1077. The identification and characterization of the isolated monocyte population was based on the use of the fluorescent antibodies CD115 and CD11b as well as on flow cytometry analysis of cell scattering characteristics. The labelling of the isolated cells in order to monitor the monocytes in vivo was successfully performed using the fluorescent membrane dye Vybrant DiD.

The isolated monocyte population was initially quantified using a hemocytometer, and following labelling with fluorescent antibodies, the cells were analysed using flow cytometry technology in order to extract quantitative information and percentages of successfully characterized cells. Labelling the cells with the selected fluorescent markers has resulted in the positive labelling of up to 99.7% of the

isolated cell population, demonstrating that they are indeed the target population of monocytes.

In conclusion, utilizing the protocol developed for the isolation of the monocyte population, the percentage of cells successfully isolated from murine blood is high enough to allow further in vivo investigations and applications, while the fluorescent markers studied will allow successful monitoring of the cells in mouse models.