

How to infuse heterologous human adult liver-derived progenitor cells safely?

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Background and Aims: Mesenchymal stem cell (MSC) infusions are currently evaluated in numerous clinical trials, but therapy-induced thrombi have been described in several patients. Most MSCs in fact express a procoagulant activity (PCA) linked to tissue factor (TF) expression, which is the fuse that ignites the coagulation cascade. The aim of this study was to optimize infusion protocols using Heterologous Human Adult Liver-derived Progenitor Cells (HHALPC) without inducing a thrombogenic risk after the infusion.

Methods: First cell dose escalation was studied using in healthy Wistar rat, with or without anticoagulants. Then the crucial role of TF in PCA was confirmed using flow chambers. Finally, we characterized the disseminated intravascular coagulation (DIC) induced by HHALPCs *in vitro* and investigated how to control the induced thrombotic and haemorrhagic risks in whole blood of healthy and cirrhotic patients.

Results: *In vivo* we showed that the thrombogenic risk induced by HHALPC infusions is dose dependent. Infusions of high cell doses such as 50×10^6 cells / kg induced DIC 1h after transplantation with a significant decrease in platelets ($p < 0.01$), fibrinogen ($p < 0.001$), and coagulation factors II, V and VIII ($p < 0.01$) compared to control rats infused only with PBS. Infusions of lower cell doses, such as 5×10^6 cells / kg did not activate the coagulation cascade. Adding anticoagulants during infusions of high cell doses, such as heparin (300 I.U./ 5×10^6 cells) or a combination of heparin (10 I.U./ 5×10^6 cells) and bivalirudin could control the thrombogenic risk. Using flowed whole-blood under shear *ex vivo*, we found that HHALPC promote fibrin clot formation in a TF-dependent way, inhibited by inactivate factor VII. By tubing loop model HHALPCs activated the coagulation cascade in a less explosive way in decompensated cirrhotic patient's blood, compared to healthy volunteers. HHALPCs only induced a significant decrease in platelets ($p < 0.01$) and fibrinogen ($p < 0.01$), but not in coagulation factors.

Conclusion: Low doses of MSCs (5×10^6 cells/kg) expressing TF do not induce a thrombogenic risk and could thus be used in future clinical trials treating acute decompensated cirrhotic patients, while monitoring platelet and fibrinogen levels. The thrombogenic risk induced by infusions of higher cell doses can be controlled by adding anticoagulants such as heparin and/or bivalirudin. These doses could therefore be used to treat metabolic patients.