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ARE CELL-BASED PRODUCTS SAFE FOR CLINICAL APPLICATIONS?

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Abstract

Background: Mesenchymal stem cell (MSC)-derived conditioned media (CM) and extracellular vesicles (EVs) can effectively mimic the therapeutic effects of MSCs as demonstrated in preclinical animal models. Yet, to develop safe and effective cell-free therapies, a knowledge gap needs to be addressed pertaining to safety. In this study, we assessed the thrombogenicity of CM and EVs in an ex vivo setting.

Methods: Bone marrow-derived MSCs (passage 3) from 4 human donors were incubated in serum-free medium (SFM). After 24 hours, CM was collected and EVs isolated by ultracentrifugation. Protein content of CM and EVs was evaluated using ELISA and Pierce 660-nm protein assay. Thrombogenicity was evaluated using thromboelastography (TEG) by mixing whole blood from healthy donors with SFM, CM or EVs at 1:1 and 1:10 ratios (CM: blood volume, $n \geq 14$). Repeated measures mixed-model with Turkey's adjustment was performed using SAS 9.4. Tests were two-sided with significance set at $p<0.05$.

Results: Compared to SFM, CM accelerated the duration to initial clot formation (R) at a 1:1 ratio ($p<0.001$), but not at the 1:10 dilution; however, there was no effect on duration to max clot formation time (K) or clot strength (MA). Compared to EV-poor (EVP) fraction of CM, R was accelerated in the EVs at both 1:1 and 1:10 dilutions ($p=0.0075$ and $p=0.0356$, respectively) with no effect on K or MA. By comparing the variability of R values in response to CM from specific MSC donors, we found a significant donor effect ($p=0.0008$). Finally, EV lysate from donors with significantly reduced R had a higher content of tissue factor (TF) compared to their CM and non-thrombogenic donors.

Conclusion: Cell-free products, especially EVs, may be pro-coagulant; however, their thrombogenic potency is donor dependent. Our preliminary results imply that the presence of TF in EVs may be the key factor in this effect.