Proteomic Analysis of Human-Derived Cell Culture Supplements

Jasmin Kemptner^{a,b} Karin Witzeneder^{a,b} Simone Hennerbichler^{a,b} Christian Gabriel^{a,b}

^a Red Cross Blood Transfusion Service of Upper Austria,

^b Austrian Cluster for Tissue Regeneration, Linz, Austria

Keywords

Cell therapy · Human platelet lysate · Human serum · Fetal calf serum · Proteomics

Summary

Objective: Development of cell therapy and advanced therapy medicinal products depends on in vitro expansion of human cells in fetal bovine serum (FBS) supplemented media. Human-derived supplements, such as human serum (huS) and human platelet lysate (hPL), represent suitable alternatives to FBS. Various studies demonstrated that the use of these human alternatives result in comparable or even improved proliferation and expansion ratios. **Methods:** Within this study three human supplement alternatives, huS, hPL_P (plasma containing hPL) and hPL_N (plasma replaced by saline), were compared by 2D gel electrophoresis, an important tool in proteomic analysis. 2D gel electrophoresis allows the determination of the protein number and the detection of protein changes (decreasing/increasing concentration). **Results and Conclusion:** The comparison of huS, hPL_P, and hPL_N gels resulted in clearly visible differences in protein pattern, protein number and concentration, particularly when comparing huS with hPL and hPL_P with hPL_N.

Introduction

Quite a high number of studies, either still in the starting blocks, ongoing or even completed, are dealing with the application of cell therapies and advanced therapy medicinal products [1]. One of the most important issues in all these studies is the high cell dose required for clinical applications. In vitro expansion of the cells is inevitable and mainly associ-

ated with the use of fetal bovine serum (FBS) as medium supplement. FBS is known for batch-to-batch variations and its cytotoxicity caused by uncharacterized xenogenic factors. Another big impact is the possibility of disease transmission due to prions, bacteria, or viruses [2]. The European Commission released a ‘Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents via Human and Veterinary Medicinal Products’ (EMA/410/01) that clearly states that first the use of material of non-animal origin should be preferred [3]. Hence, several research groups already tested human alternatives to the use of FBS, including human platelet lysate (hPL) and human serum (huS) [4]. Various studies dealt with the expansion of various cell types, testing the impact of different platelet activation methods and differing source materials (apheresis concentrates, outdated platelet pools, or buffy coats (BC)) on product quality [5, 6].

The application of proteomic tools, especially 2D gel electrophoresis, for the analysis of hPL and huS allows a fast visual examination of the corresponding protein composition [7]. Detected changes or differences in protein composition can be further analyzed by mass spectrometric methods for protein identification. Furthermore, 2D gel electrophoresis allows the detection of changes in protein concentration. Hence, increases or reductions in concentrations caused by changes in hPL or huS production can be determined. Additionally, the impact of these changes on in vitro expansion of various cells can be determined.

Material and Methods

For this study two types of hPL, hPL_P prepared from BC and kept in plasma anticoagulated with CPD and hPL_N where plasma is substituted by saline, were compared to huS in terms of protein composition. An additional paper within this issue by Witzeneder et al. [8] deals with the impact of the above mentioned human alternatives to FBS on the expansion of adipose tissue-derived stem cells (ASC) and dermal fibroblasts.

Fig. 1. Colloidal Coomassie Brilliant Blue stained 2D gels from **A** huS, **B** hPLP and **C** hPLN.

Human serum, hPL_P and hPL_N were prepared as described by Witzeneder et al. [8], and samples for 2D gel electrophoresis were taken after product finalization.

The simultaneous reduction of high abundant plasma proteins and the enhancement of low abundant proteins were performed with ProteoMiner large-capacity kits (Bio-Rad, Vienna, Austria). 1 ml of hPL or huS was applied to the equilibrated ProteoMiner columns. Incubation and washing procedure were performed according to the instruction manual. Protein elution was performed three times with 100 µl elution solution instead of two times. Subsequently an acetone precipitation was carried out to remove interfering contaminations. The protein eluate was divided in two 150 µl aliquots. 450 µl ice cold acetone (Herba Chemosan, Austria) were added, and the mixture was incubated over night at -20 °C. Precipitated proteins were pelleted by centrifugation (13,000 × g, 10 min, 4 °C). Subsequently 2D separation was performed. Therefore, the protein pellets were solubilized in rehydration buffer (7 mol/l urea (Sigma, Vienna, Austria), 2 mol/l thiourea (Sigma), 2% CHAPS (Merck, Darmstadt, Germany), 2% IPG buffer pH 3–10 (GE Healthcare, Munich, Germany), 0.002% bromophenol blue (AppliChem, Darmstadt, Germany), 7 mg dithiotreitol (Sigma)) for subsequent isoelectric focussing. Commercial IPG Strips (24 cm, pH 3–10; GE Healthcare) were rehydrated overnight with the sample solutions. First dimension of 2D gel electrophoresis was performed on Ettan™ IPGphor 3 (GE Healthcare). For subsequent SDS-PAGE the IPG strips were equilibrated with 1% dithiotreitol in equilibration buffer (6 mol/l urea, 75 mmol/l Tris HCl pH 8.8 (Merck), 29.3% glycerol (Sigma), 2% sodium dodecyl sulphate (Sigma), 0.002% bromophenol blue) for 15 min followed by 2.5% iodoacetamide (Sigma) in equilibration buffer for additional 15 min. SDS-PAGE was performed with lab-cast 10% polyacrylamide gels on Ettan™ DALTsix connected to EPS 601 power supply and MultiTemp III (GE Healthcare). A colloidal Coomassie Brilliant Blue staining was performed. Gels were fixed (30% EtOH (Merck), 2% phosphoric acid (Merck) in water) for 30 min, washed (2% phosphoric acid in water) for 20 min, and stained (0.12% CBB G250 (Sigma), 20% EtOH, 10% ammonium sulphate (Merck), 10% phosphoric acid in water) for 2.5 h. Destaining was performed in water until the background was clear (14–16 h). Gel images were obtained by VersaDoc 4000MP (Bio-Rad) and analyzed with PDQuest Advanced 2D analysis software (Version 8.0.1, May 2006).

Results

The obtained gel images of huS, hPL_P, and hPL_N are depicted in figure 1. Analysis with PDQuest Advanced software

resulted in the detection of 180 protein spots for hPL_P, 103 spots for hPL_N, and 167 spots for huS. Comparison of hPL_P and hPL_N demonstrates a reduction of protein spot number from 180 (hPL_P) to 103 (hPL_N). The additional washing with saline accounts for the lower spot number in hPL_N, since plasma proteins were removed, whereas the higher spot number of hPL_P can be explained by the presence of platelet as well as of plasma proteins. Comparing the protein profile of hPL_P to those of hPL_N and huS, corresponding protein spots can be detected. The round boxes in figure 1 show protein spots detectable in hPL_P and huS, whereas the square boxes indicate those protein spots which are consistent in hPL_P and hPL_N.

Discussion

The presented gel electrophoresis data were originally obtained to track changes in protein profiles of hPL produced according to varying protocols. In the course of this study the identification of increasing or decreasing proteins spots was used in order to determine potential key components influencing cell culture. The mass spectrometric identification of interesting protein spots, such as spots decreasing or increasing in concentration, is still ongoing as many spots were detected and considered as interesting. Additional gel electrophoretic experiments, such as differential gel electrophoresis (DIGE), and liquid chromatography-coupled mass spectrometric experiments will be necessary to achieve a more comprehensive protein profile of both hPL and huS. Those methods are more capable of analyzing low abundant proteins, and probably the real active component(s) of hPL and huS will be found amongst those.

Disclosure Statement

The authors did not provide a conflict of interest statement.

References

- 1 www.clinicaltrials.gov/ct2/results?term=cell+therapy.
- 2 Tekkotte C, Gunasingh GP, Cherian KM, Sankaranarayanan K: 'Humanized' stem cell culture techniques: the animal serum controversy. *Stem Cells Int* 2011;2011:504723.
- 3 Note for Guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMA/410/01 rev.3). Official Journal of the European Union 2011/C 73/01 www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003675.pdf.
- 4 Bieback K, Hecker A, Kocaomer A, Lannert H, Schallmoser K, Strunk D, Kluter H: Human alternatives to fetal bovine serum for the expansion of mesenchymal stromal cells from bone marrow. *Stem Cells* 2009;27:2331–2341.
- 5 Blande IS, Bassaneze V, Lavini-Ramos C, Fae KC, Kalil J, Miyakawa AA, Schettter IT, Krieger JE: Adipose tissue mesenchymal stem cell expansion in animal serum-free medium supplemented with autologous human platelet lysate. *Transfusion* 2009;49:2680–2685.
- 6 Rauch C, Feifel E, Amann EM, Spötl HP, Schenach H, Pfaller W, Gstraunthaler G: Alternatives to the use of fetal bovine serum: human platelet lysates as a serum substitute in cell culture media. *ALTEX* 2011;28:305–316.
- 7 Liunbruno G, D'Alessandro A, Grazzini G, Zolla L: Blood-related proteomics. *J Proteomics* 2010;73:483–507.
- 8 Witzeneder K, Lindenmair A, Gabriel C, Höller K, Theiß D, Redl H, Hennerbichler S: Human-derived alternatives to fetal bovine serum in cell culture. *Transfus Med Hemother* 2013;40(6):417–423.