chemistry proceedings

MDPI

Proceeding Paper

Proteogenomic Tools in the Assessment of Pharmacological Effects of Natural Compounds †

Radu Albulescu [1,2,*], Adrian Albulescu [1,3], Georgeta Caraene [1], Corina Bubueanu [1], Alice Grigore [4], Maria Petrescu [1] and Roxana-Mădălina Stoica [1,*]

[1] National Institute for Chemical-Pharmaceutical Research and Development-ICCF, 031299 Bucharest, Romania; rockadi2@gmail.com (A.A.); getabios@yahoo.com (G.C.); corina.bubueanu@yahoo.com (C.B.); maria.m.petrescu@gmail.com (M.P.)
[2] Titu Maiorescu University, 040441 Bucharest, Romania
[3] Stefan S. Nicolau Institute of Virology, 030394 Bucharest, Romania
[4] Ministry of Research, Innovation and Digitalization, 010342 Bucureşti, Romania; alicearmatu@yahoo.com
* Correspondence: radu_a1@yahoo.com (R.A.); roxym_stoica@yahoo.com (R.-M.S.)
† Presented at the 19th International Symposium "Priorities of Chemistry for a Sustainable Development", Bucharest, Romania, 11–13 October 2023.

Abstract: Proteogenomics is a recently developed omics application, adding enhancing the sensitivity of proteomics, and thus making possible the detection of proteome markers in very tiny amounts of samples. Even if the filed developed only in the last 10 years, the technology is very intensely applied in clinical diagnostics, and more recently, efforts are made to use it in non-clinical, in vitro and in vivo studies. The aim of this study was to investigate the applicability of single-plex and multiplex assays in the evaluation of proteome changes generated in vitro by the exposure to several standard compounds. The extracts demonstrated weak cytotoxic effects. The detection of cytokines Performing the same assays on tissue lysates permitted only the detection of low levels of cytokines.

Keywords: cytotoxicity; pro-quantum; IL-1; IL2-alpha; TNF-alpha; IFN-gamma

Citation: Albulescu, R.; Albulescu, A.; Caraene, G.; Bubueanu, C.; Grigore, A.; Petrescu, M.; Stoica, R.-M. Proteogenomic Tools in the Assessment of Pharmacological Effects of Natural Compounds. *Chem. Proc.* **2023**, *13*, 27. https://doi.org/10.3390/chemproc2023013027

Academic Editors: Mihaela Doni, Florin Oancea and Radu Claudiu Fierăscu

Published: 23 November 2023

1. Introduction

The detection of proteins in biological fluids is considered of major importance in assessing the health status. Different approaches have been developed so far in order to improve the predictive power of proteome analyses. A large set of techniques is available in the field, starting from "classical" separation techniques (electrophoresis, chromatography) and their technological updates like different variants of mass-spectrometric coupled technologies [1,2] or other enhancements based on affinity (micro-arrays [3], single-plex and multiplex antibody based detection [4,5], Biacore instruments [6] etc.). Proteogenomics is a recently developed omics application, by combining the specific protein detection with highly specific antibodies with the potential amplification of nucleic acids attached moieties, thus providing a means to amplify the signal. Even though the field has only developed in the last 10 years, the technology is very intensively applied in clinical diagnostics [7,8], and more recently, efforts are being made to use it in non-clinical, in vitro and in vivo studies [2–4]. Independent of the technology and platform used, the proteome approach shares a common limitation–the amount of protein detected is always lower than the effective amount in the sample, resulting in the need of larger sample volume and, eventually, complicated procedures to concentrate the proteins of interest (especially for the molecules present in very small amounts).

More recently, a breakthrough in the field occurred–the development of novel technologies for detection of proteins, where a low concentration protein is detected by the use of two target-specific antibodies, each coupled to a DNA oligonucleotide. Thus, if the target is present, the event is followed by the nucleotide sequences coming into proximity,

and therefore, the possibility of ligation and amplification of the signal in a PCR instrument. Originally, the technology was using "single-plex" assays (one pair of nucleotides labeled antibodies for a target protein), but in time, the technology evolved, and presently there are multiplex sets antibody pairs that allow the detection of 80 proteins or more in a sample [9]. These novel technologies made possible to overcome the original limitations, reducing considerably the amount of sample needed for such assays.

The aim of this study was to investigate the applicability of single-plex and multiplex assays in the evaluation of proteome changes generated in vitro by the exposure to several standard compounds.

2. Material and Methods

Compound to be tested was CBDA (canabindiolic acid), intended to be used as novel food and in some dermacosmetic products, provided by Absolute Essential Oils, Romania. The product was disolved in DMSO.

Epi-Derm tissue cultures and reagents (culture medium, wash buffer, lysis buffer, pozitive and negative control compounds, MTT assay kit-purchased from Mattek, Slovakia. After control and preincubation, cultures were exposed to the extracts, according to the manufacturer's protocol, using a CO_2 incubator, in standard conditions (5% CO_2, 37 °C, 90% humidity. On the day of receipt, EpiDerm™ tissues are conditioned by incubation to release transport-stress related compounds and debris overnight. After pre-incubation, tissues are topically exposed to the chemicals test for 60 min. Preferably, three tissues are used per chemical test (TC) and for the positive control (PC) and negative control (NC). Tissues were rinsed and blotted to remove traces of test compound, and introduced in fresh medium. After a 24 h. incubation period, the medium is collected for analysis of cytokines (Note: This step is optional, since no improvement in assay performance was noted by using IL-1α or other cytokines as complementary endpoint). Samples are cultivated other 18 h. Afterwards, the MTT assay is performed by transferring the tissues to 24-well plates containing MTT medium (1 mg/mL). After a 3 h. MTT incubation, the blue formazan salt formed by cellular mitochondria is extracted with 2.0 mL/tissue of isopropanol (extractant solution, part # MTT-100-EXT) and the optical density of the extracted formazan is determined using a spectrophotometer at 570 nm. Relative cell viability is calculated for each tissue as % of the mean of the negative control tissues. The irritative potential is predicted if cell viability is under 50%.

After completion of the exposure, and post-cultivation, the MTT assay was performed to measure the cytotoxic effects, while supernatants and cell lysates were used for the assessment of proinflammatory cytokines–IL-1 beta and TNF-alpha using specific Proquantum immunoassay kits and protocols. Briefly, tissue lysis was achieved by exposing the tissue to lysis buffer (SDS solution), while supernatants were collected during the experiments in different moments of the experiments. Bots supernatants and lysates were stored in liquid nitrogen until used. For the cytokine assays, 10 microliters of each sample were transferred to a corresponding well of a 96 well plate, and the mixure of antibodies added, followed, after coupling, by addition of the of the Taq-Man mixture of reagents.

3. Results

The extracts demonstrated only weak cytotoxic effects (classified as "non-toxic"). The detection of cytokines could be achieved starting from a minute level of supernatants and/or cell lysate–10 microliters for each analysis, and allowing the quantification of minimal amounts of IL-1 (from 5–9.3 nanoG/mL), and TNF-alpha (range 8–6 nanoG/mL) and IFN-gamma (range 3–5 nG/mL) depending on the cultures treated with plant extracts.

Initial effects with regard to cytotoxicity are illustrated in Table 1.

Table 1. Results of cytoxicity evaluation for CBDA.

Product	Mean OD	St.dev.	P [3]	P [4]	Relative Vi-ability (%)	Conclusion
PBS, [2]	0.454	0.0271	-	-	100	
EtOH [2]	0.439	0.0183	-	-	99.3	
SDS, [1]	0.057	0.0063	-	-	1.92	
CBDA 26 mg/mL	0.174	0.00661	0.001	0.001	45.5	Irritant (I), Non-Corrosive (NC)
CBDA 5.2 mg/mL	0.314	0.0023	0.002	0.003	75.48	Non-Irritant (I)

[1]: positive control; [2]: negative control; [3]: student t test, 2 tails, homoscedastic distribution, compared to negative control, [4]: student t test, 2 tails, homoscedastic distribution, compared to positive control.

According to the classification criteria, we conclude that the product CBDA pure compound is irritant (I) but non-corrosive (NC), at the maximum possible concentration in ethanol of 26 mg/mL, and respectively non-irritant (NI) at the concentration of 5.2 mg/mL. În consequence, at working concentrations below 5.2 mg/mL, the product can be safely used, according to the specific recommendation of use of the product.

Effects of CBDA on the expression of cytokines (IL-2, IFN-gamma, TNF-alpha ajd IL-1 alpha) are presented in Table 2.

Table 2. Results of effects of CBDA on production of some cytokines.

Sample	Cytokine Conc. (ng/mL)			
	IL-2	IFN-Gamma	TNF-Alpha	IL-1alpha
Control	N/D	N/D	N/D	N/D
CBDA 26 mg/mL(SN)	$1.6 \times 10^2 \pm 0.7$	$2.1 \times 10^2 \pm 7$	$3.1 \times 10^2 \pm 19$	$4 \times 10^2 \pm 52$
CBDA 5.2 mg/mL (SN)	$2.1 \times 10^2 \pm 12$	$3.9 \times 10^2 \pm 12$	$7.8 \times 10^2 \pm 27$	$5.4 \times 10^2 \pm 71$
Lysate CBDA 26 mg/mL	N/D	N/D	N/D	$0.7 \times 10^2 \pm 2.1$
Lysate CBDA 5.2 mg/mL	N/D	N/D	N/D	$1.3 \times 10^2 \pm 3.9$
Positive control (SN)	$1.7 \times 10^4 \pm 8 \times 10^2$	$4.9 \times 10^3 \pm 5 \times 10^2$	$6.7 \times 10^3 \pm 6 \times 10^2$	$1.2 \times 10^4 \pm 1.3 \times 10^3$
Positive control (Lysates)	$2.9 \times 10^3 \pm 2 \times 10^2$	$0.8 \times 10^3 \pm 93$	$1.2 \times 10^3 \pm 1.1 \times 10^2$	$2 \times 10^4 \pm 0.4 \times 10^3$

As can be observed, the exposure to CBDA is inducing some effects upon the potential of secretion of the four cytokines. The effects appear to be dependent on concentration of CBDA, however, the secretory levels are in the range of a very low pro-inflammatory response, even in the maximal dose. When examining the intracellular level of the same cytokines, only a very low level of IL-1 alpha could be detected, while the other cytokines could not be detected. In the same time, the positive controls are presenting a measurable level of the four cytokines, both in the supernatants (SN) and in the lysates.

4. Discussion

Performing the PRoQuantum immunoassay on tissue lysates and supernatants of the EPi-Derm inserts made it possible the detection and quantification of several cytokines produced by exposure to the samples. The presence of cytokines in supernatants (collected rapidly after the exposure to the samples) was significant, and was an indicator of some mild inflammatory effects of the samples on the dermis, although the overall classification was „non-irritant". Performing the same assays on tissue lysates only allowed the detection of low levels of IL-1, most likely due to the initial low levels of intracellular molecules and also, we speculate, the low persistence of inflammation in the exposed tissue (the products were classified as non-irritant), contrary to the positive control.

Author Contributions: Concept, R.A.; writing-original draft preparation, R.-M.S.; writing and editing, R.A., A.A., G.C., A.G., C.B., M.P. and R.-M.S.; performed the experiments, R.A., A.A. and G.C.; analyzed the data, R.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Romanian Ministry of Research, Innovation and Digitalization, by grants PN 23 28 05 01 and PN 19 41 05 01.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Boys, E.L.; Liu, J.; Robinson, P.J.; Reddel, R.R. Clinical Applications of Mass Spectrometry-Based Proteomics in Cancer: Where Are We? *Proteomics* **2023**, *23*, e2200238. [CrossRef] [PubMed]
2. Jia, K.; Zhao, X.; Dang, X. Mass Spectrometry-Based Itraq Analysis of Serum Markers in Patients with Pancreatic Cancer. *Oncol. Lett.* **2020**, *19*, 4106–4114. [CrossRef] [PubMed]
3. Chen, G.; Yang, L.; Liu, G.; Zhu, Y.; Yang, F.; Dong, X.; Xu, F.; Zhu, F.; Cao, C.; Zhong, D.; et al. Research Progress in Protein Microarrays: Focussing on Cancer Research. *Proteom. Clin. Appl.* **2023**, *17*, e2200036. [CrossRef] [PubMed]
4. Cook, D.B.; McLucas, B.C.; Montoya, L.A.; Brotski, C.M.; Das, S.; Miholits, M.; Sebata, T.H. Multiplexing Protein and Gene Level Measurements on a Single Luminex Platform. *Methods* **2019**, *158*, 27–32. [CrossRef] [PubMed]
5. Serna, G.; Ruiz-Pace, F.; Cecchi, F.; Fasani, R.; Jimenez, J.; Thyparambil, S.; Landolfi, S.; Elez, E.; Vivancos, A.; Hembrough, T.; et al. Targeted Multiplex Proteomics for Molecular Prescreening and Biomarker Discovery in Metastatic Colorectal Cancer. *Sci. Rep.* **2019**, *9*, 13568. [CrossRef] [PubMed]
6. Das, S.; Devireddy, R.; Gartia, M.R. Surface Plasmon Resonance (Spr) Sensor for Cancer Biomarker Detection. *Biosensors* **2023**, *13*, 396. [CrossRef] [PubMed]
7. Dayon, L.; Cominetti, O.; Affolter, M. Proteomics of Human Biological Fluids for Biomarker Discoveries: Technical Advances and Recent Applications. *Expert Rev. Proteom.* **2022**, *19*, 131–151. [CrossRef] [PubMed]
8. Petrera, A.; von Toerne, C.; Behler, J.; Huth, C.; Thorand, B.; Hilgendorff, A.; Hauck, S.M. Multiplatform Approach for Plasma Proteomics: Complementarity of Olink Proximity Extension Assay Technology to Mass Spectrometry-Based Protein Profiling. *J. Proteome Res.* **2021**, *20*, 751–762. [CrossRef] [PubMed]
9. Liu, K.Y.P.; Lu, X.J.D.; Zhu, Y.S.; Le, N.; Kim, H.; Poh, C.F. Plasma-Derived Inflammatory Proteins Predict Oral Squamous Cell Carcinoma. *Front. Oncol.* **2018**, *8*, 585. [CrossRef] [PubMed]