Protein profiling by 2-DE/MS of control and tumoral cell lines following pharmacological treatment.

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Two-dimensional gel electrophoresis in conjunction with MALDI-TOF mass spectrometry has been used to assess protein alterations in control and tumoral cell lines before and after pharmacological treatment. We have conducted a series of measurements in which Cisplatin, 5-aza-2'-deoxycytidine (Decitabine) and Trichostatin A were tested on pancreatic ductal adenocarcinoma and cervix squamous cell carcinoma. Statistical analyses of the generated 2-D maps have revealed that a number of proteins have experienced up- or down-regulation, while a limited number have been turned on or off. A number of the altered proteins have been identified by MALDI-TOF mass spectrometry. Based on these measurements and considering recently published data on this argument the following questions are partially addressed:

- Known biological functions of some of the altered proteins.
- Does this type of data has a functional relevance?
- Is this a good approach for finding new biomarkers for certain type of cancer?
- How does it compare with other approaches such as SELDI and DNA micro-arrays?

Proteomics – ein neues wichtiges Gebiet in der pharmazeutischen Analytik

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Nach der grossen Anstrengung der letzten Jahre zur Entschlüsselung der Genome des Menschen und vieler anderer höherer und niederer Organismen ist heute deutlich, dass die wirkliche Herausforderung und eine deutlich höhere Komplexität auf der Ebene der Proteine existiert. Krankheiten sind letztlich immer bestimmt durch Änderungen auf der Proteinebene, durch Unterschiede in der Höhe des (zellspezifischen) Expressionslevels und in Proteinmodifikationen, und die meisten modernen Pharmazeutika haben Proteine als Targets. Zusätzlich rücken weitere Fragen wie Proteinlokalisation und insbesondere Protein-Protein-Interaktionen in das Zentrum des Interesses, alle diese Fragen nach der Funktion eines Proteins sind nicht mittels der Gensequenz vorhersagbar.
Proteomics - die Untersuchung des Ensembles aller Proteine in einer Zelle, einem Zellkompartiment oder einem funktionellen Multiproteinkomplex unter definierten Bedingungen - setzt zunächst ein neues Instrumentarium voraus, das in den letzten 10 Jahren entwickelt worden ist. Entscheidend war dabei die Kombination von gelelektrophoretischen und chromatographischen Trennverfahren mit der Massenspektrometrie. Die Proteomics-Werkzeuge zur Proteinidentifizierung und –charakterisierung, die zu einem wichtigen Werkzeug der Arzneimittelentwicklung, in der Targetidentifizierung und –validierung, sowie für die Toxikologie geworden sind, werden im Vortrag vorgestellt, Stärken und aktuelle Begrenzungen werden diskutiert.

Similar Biological Medicinal Products- Current Regulatory Situation in Europe

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Issues related to the development of "biogeneric" products were, and still are, discussed intensively within pharmaceutical industry and regulatory bodies as patents for several large biopharmaceutical products will expire in the next years. It is clear that due to the complex nature of biological molecules a generic approach for the development of such medicinal products cannot be applied. Recently two CPMP guidelines on the comparability of medicinal products containing biotechnology-derived proteins came into operation, which cover also the situation of biological products claiming similarity yet being produced by different and unrelated manufacturers. However, it is clearly stated that owing to the lack of specific information about the manufacturing process of the originator company this situation represents the most complicated case and requires an extensive comparability exercise including pre-clinical and/or clinical trials. Due to the large diversity of biological products little specific guidance is provided. In Directive 2003/63/EC published on 27 June 2003 the regulatory term for this class of products was defined to be "similar biological medicinal products". Finally, Directive 2004/27/EC, published in May 2004, amending Directive 2001/83 provided the regulatory pathway for biosimilar products. According to the new Article 10(4) introduced by this guideline which deals with biosimilar products the results of appropriate pre-clinical or clinical tests have to be submitted for the approval. The type and amount of data that have to be provided shall be determined on a case-by-case basis in accordance with relevant guidelines and the competent regulatory authorities.
Even though legislation is now in place in Europe which define "similar biological medicinal products" and a regulatory pathway for their approval specific regulatory requirements have to be determined for each product on a case-by-case basis in accordance with advise provided by the competent regulatory authorities.

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QUANTITATION OF HUMAN ANTI-D IMMUNOGLOBULIN - DOES AN ACCEPTED ACCURATE ASSIGNED POTENCY VALUE EXIST?

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Human anti-D immunoglobulin is a liquid or freeze-dried preparation containing immunoglobulins, mainly immunoglobulin G. It contains specific antibodies against erythrocyte D-antigen. Anti-D immunoglobulin product is fractionated from donors "immunised" with D-antigen and contains only traces of anti-D in respect to "common" immunoglobulins. In Rh prophylaxis Anti-D is administered pre- and postnatal. Despite its widespread use and efficacy, the mechanism of action of this therapy is unproven.
The Pharm. Eur. propose three different methods to assign the potency, whereas method A the agglutination assay is the method of choice.
Method B (Elisa) and C (flow cytometry) may only be used for potency determination if a satisfactory correlation with the results obtained by Method A has been established for the particular product.
The agglutination assay carried out using an AutoAnalyzer is described the validation data are presented and compared to data using the flow cytometry and a modified agglutination assay.
The capability and the limitations of both the agglutination and the flow cytometry assay and the different principles of measurement in regard to international standardisation and inter laboratory trials to assign the potency are discussed.
Hopefully the question will be answered: "Does an accepted accurate assigned potency value exist?".

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MASS SPECTROMETRY IN COMPLEX MATRICES: THE POTENTIAL OF ESI, APCI, AND MALDI

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Mass spectrometry comprises a family of methods capable of analyzing compounds qualitatively, and particularly in combination with separation techniques, also quantitatively. Its high specificity and sensitivity makes it an ideal tool for analysis in complex matrices. However, matrix effects such as signal suppression may hamper its applicability and make quantitative assays challenging. The susceptibility to these deteriorating effects depends on the ionisation technique used. Electrospray ionisation (ESI) is sensitive to the presence of salts, surfactants and other compounds competing with the analyte for the charge. Desalting, solid phase extraction and/or chromatographic separation are frequently required. For plasma samples, several techniques of protein precipitation are in use. Atmospheric Pressure Chemical Ionisation (APCI) is mainly used as an alternative to ESI for small, less polar analytes. MALDI-TOF MS is mostly used as a qualitative offline technique and tends to be more robust to the presence of salts and other compounds accompanying the analyte. Tandem and multiple stage MS increase specificity and can improve the signal-to-noise ratio.

Examples from our own work illustrate the use of MS techniques for analysis of samples in complex matrices. The ceramide pattern in total lipid extracts from the human Stratum Corneum was analyzed using either an offline combination of thin-layer chromatography and reversed-phase LC/ESI-MS [1] or an online approach based on normal-phase LC/APCI-MS [2]. Cholesterol oxidation products were analyzed in food using LC/APCI-MS [3]. Peptides from gastro-analogous pepsin digests of food proteins such as β-casein were analyzed using MALDI-TOF MS and LC/ESI-MS [4].

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APPLICATION OF FLOW FIELD FLOW FRACTIONATION ANALYTICS FOR CHARACTERISATION OF PHARMACEUTICAL PROTEIN FORMULATIONS

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Protein drugs show a number of physical instabilities like denaturation, aggregation, turbidity and precipitation. Full analytical characterization of these phenomena is not yet satisfactory. Spectroscopic studies allow insight into structural perturbations, but without preceding separation steps, small amounts of denatured material cannot be detected in the presence of an excess of native drug. State of the art separation systems like SEC are limited to dissolved drug and its soluble aggregates; precipitates or turbid formulations cannot be analyzed without loosing information. Methods like SDS-PAGE require pre treatment of samples and allow only semi quantitative evaluation. Even more complex situations evolve when particulate formulations have to be assessed for the presence of protein aggregates. Flow field flow fractionation (FFF), preferably used with multi angle light scattering detection (MALS) is a valuable tool to analyse physical instabilities of protein drugs. The principle of the method, the separation of molecules and particles according to their size in a laminar liquid flow, allows detection of native drug in the presence of its soluble and insoluble aggregates or other particulate matter [1]. The method and its parameters are introduced in detail and based on examples with BSA, EPO, GCSF, IFN-alpha and MABs the potential of FFF in protein analytics is outlined. It is apparent, that FFF detects higher amounts of high molecular weight aggregates than SEC. It is therefore hypothesized, that FFF data may be closer to the real situation in turbid pharmaceutical formulations. A case study with silicon oil droplets is reported, showing the separation of drug molecules and their aggregates in the presence of colloidal contaminants [2].

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