Evaluation of in vitro immunotoxicity tests using transcriptomics

Chapter 9

Valorisation Addendum

Introduction

Chemicals with direct immunotoxic characteristics may cause a risk to human health, as humans can be exposed to these chemicals via food, drinking water, and the environment. These direct immunotoxic properties are evaluated in the safety assessment of new and existing chemicals and food additives (EPA 1998; Institóris et al. 1998), and during the preclinical phase of pharmaceutical development (EMA 2000; FDA 2002). Currently, immunotoxicity tests are mainly based on changes in organ weights in animal experiments, histopathology of those organs (spleen, thymus, bone marrow, lymph nodes) or by measuring serum parameters (FDA 2002). Disadvantages of animal tests are related to the high costs and long time needed as well as to ethical issues (Corsini and Roggen 2009). In addition, animal studies rely mostly on apical endpoints, do not sufficiently yield insights into the molecular mechanisms of direct immunotoxicity and often have limited prediction towards the human situation. Thus, animal free alternatives are urgently needed. The latter is a key element in a report from the National Research Council (NRC) of the U.S. National Academy of Science entitled "Toxicity testing in the 21st Century: A Vision and A Strategy"(2007). In this report, the NRC supports the shifts from animal testing to (preferably human-based) *in vitro* testing and from single endpoint studies to studies addressing toxicity pathways. This support for omics approaches in defining new endpoints was also shared by the European Centre for the Validation of Alternative Methods (ECVAM) (Bouvier d'Yvoire et al. 2012). The application of transcriptomics provides mechanistic insights into the mode of action of the chemical and can also yield biomarkers that represent a specific toxicological endpoint.

Innovation and social relevance of the research results

Recently, in vitro transcriptomics studies using human and rodent T cell lines and primary cells have led to a better insight into the mechanism of action of a limited set of immunotoxicants. The question which of the available models would be most suitable to screen compounds for immunotoxicity was however not yet answered. Therefore, the work described in the present thesis started with a comparative assessment of several models using three different model immunotoxicants (CsA, TBTO and DON). As it became apparent that the *in vitro* mouse models (CTLL-2 and EL-4 cell lines) as compared to the human Jurkat T cell line lacked some important features to detect the immunotoxicity of these immunotoxicants, a larger set of chemicals was studied by using the human Jurkat T cell line. These studies were performed by colleagues and resulted in the identification of a set of mechanism-based biomarkers that allowed to distinguish immunotoxicants from non immunotoxicants with a relatively high accuracy 85%. As some questions remained unanswered, such as: "How does this model perform when challenged with chemicals that were not tested before?" the set of biomarkers was further tested with a new set of compounds in this thesis. This additional pre-validation of the biomarkers resulted in excellent sensitivity (100%), specificity (80%) and accuracy (93%). In addition, the immunotoxicity of chemicals originating from classes that were not tested before could also be confirmed. It was concluded that the Jurkat T cell model in combination with the

biomarker gene set promises to be a useful module within a battery of tests to screen new and existing chemicals for immunotoxicity. Besides assessments on immunotoxicity of chemicals, one study was set up using known immunotoxicants in one microarray experiments. This allowed a direct comparison between the gene expression profiles of compounds with suspected similar modes of action.
Taken together, the results of this thesis showed that of the models assessed, the Jurkat T cell line gave the best prediction for immunotoxicity testing. Using this cell line in a toxicogenomics setting resulted in valuable mechanistic information and biomarkers for immunotoxicity were pre validated in a second experiment with another set of immunotoxicants and non-immunotoxicants. The research performed was in line of the framework of US NRC, US FDA and ECVAM contributing to the development of animal free screening models.

Concrete product
The set of biomarkers that were pre-validated in this thesis can be used in a high-throughput setting allowing screening of large sets of chemicals in a short time. In the present thesis a Fluidigm high-throughput PCR system was used, however, other possibilities exist. Biomarkers could also be tested using a Luminex setting, which has the advantage that the quantification of mRNA is done directly on lysates of cultured cells, so no RNA isolation and purification steps are required (Zheng et al. 2006). Though this makes the Luminex system more high throughput, the Fluidigm setting is fast, cheaper than the Luminex system, and has already proven itself in Jurkat immunotoxicity experiments.

Potential applications
An assay that is created on the basis of the outcome of this thesis can be used for several purposes. First, it can be used within drug development to screen for potential immunotoxic properties of newly developed pharmaceuticals. Second, within the framework of REACH, an assay could be used in the safety evaluation of existing chemicals and the presence of immunotoxicants in food, feed and water.

Implementation schedule
The present thesis proved the value of the identified biomarkers, however, some improvements are still to be investigated. To generate a more powerful validation, more non-immunotoxicants need to be used in this screening set up to balance the large number of known immunotoxicants with the low number of known non-immunotoxicants. A next step will be the submission of the results of the Jurkat screening assay to ECVAM for independent further validation. Because the cell line (Jurkat T cell line) and the primers in RT-PCR (Taqman) are both commercially available, this will shorten the time for validation by ECVAM. Parallel to this, similar exercises as done in this thesis could be performed for other immune cell lines, such as a macrophage cell line or a Natural Killer cell line. When compounds can be screened in three cell lines (T cell, NK and macrophage)

with the same set of biomarkers, this will increase the power of the assay, as immunotoxic chemicals might specifically affect NK cells or macrophages and do not affect T cells.

References

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