Integrated strategy for the assessment of kidney toxicity: the case of aristolochic acids

ABSTRACT

This PhD thesis aimed to provide additional evidence to demonstrate the potential of an integrated testing strategy using in vitro assays with physiologically based kinetic (PBK) modeling based-reverse dosimetry to predict in vivo toxicity without animal testing. Kidney toxicity was chosen as the toxicity endpoint and aristolochic acids (AAs) were selected as model chemicals. AAs are natural nephrotoxic, genotoxic and carcinogenic chemicals present in *Aristolochia* species. PBK models for rat, mouse and human were developed for aristolochic acid I (AAI) based on kinetic parameter values derived from in vitro incubations using relevant tissue fractions. Then, in vitro concentration-response curves for cytotoxicity of AAI were obtained in kidney cell lines and translated to in vivo dose-response curves for kidney toxicity using PBK modeling-based reverse dosimetry. The points of departure (PODs) obtained from these predicted in vivo dose-response curves generally fell within the range of PODs derived from in vivo literature data on kidney toxicity of AAI. The same PBK models were subsequently used to translate the in vitro concentration-response curves for AAI-DNA adduct formation to in vivo dose-response curves for kidney AAI-DNA adduct formation. The predicted in vivo AAI-DNA adduct formation in the rat, mouse and human kidney varied within an order of magnitude compared to the in vivo values reported in the literature. The PBK models were also used to predict the dose level that would be required in humans to obtain the level of DNA adducts in rats at the BMD10 (the benchmark dose causing a 10% extra risk above background level) value for AAI-induced tumor formation in the rat kidney. This analysis revealed that the dose level required to induce the level of DNA adduct formation that equals the DNA adduct level at the BMD10 were similar to AA doses estimated to be taken in Belgian patients that developed urinary tract cancer. Given that the exposure to AAI is often accompanied by the presence of AAII, in a next study the relative formation of DNA adducts by these two major AA congeners was investigated. The results revealed that the relative higher formation of AAI-DNA adducts as compared to AAII-DNA adducts observed in vitro was not reflected in vivo where the levels formed upon exposure to equal dose levels were relatively similar. PBK model based translation of the in vitro data to the in vivo situation revealed that PBK model based prediction of in vivo DNA adduct formation is feasible. However, predicted AAI-DNA adduct levels were higher than predicted AAII-DNA adduct levels, indicating that the difference between the in vitro and in vivo AAI-/AAII-DNA adduct ratios could only in part be explained by differences in in vivo kinetics of AAI compared to AAII. The discrepancy between the difference in DNA adduct formation of AAI and AAII in the in vitro and the in vivo situation is an issue that needs further investigation to also adequately predict the relative differences between the two AAs. In a final chapter this thesis aimed to investigate the possible risks associated with exposure to AAs based on AA levels measured in plant food supplements (PFS) and herbal products. This is of interest given the restrictions on the presence of AAs in food, installed in various countries including The Netherlands, after the incidences with induction of Aristolochic Acid

Nephropathy upon use of herbal weight loss preparations that accidentally contained AAs. The risk assessment of PFS and herbal products containing AAs purchased via online markets revealed that consumers can still be exposed to AA-containing PFS and herbal products and that the corresponding levels of exposure raise concern especially for people who frequently use the products. Altogether, this thesis presented further support for the use of combined in vitro-PBK modeling based alternative tools for risk assessment and revealed the continued risks posed by AAs present in PFS and herbal products.

Keyword: Kidney toxicity; Aristolochic acids; Physiologically based kinetic (PBK)