The genotoxicity of pyrrolizidine alkaloids is mediated by CYP3A in metabolically competent TK6 cell lines

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Abstract

Pyrrolizidine alkaloid (PA)-containing plants are among the most common poisonous plants affecting humans, livestock, and wildlife worldwide. A large number of PAs are known to induce genetic damage after metabolic activation. In the present study, using a panel of fourteen newly developed TK6 cell lines, each expressing a single human cytochrome P450 (CYP1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C18, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5, and 3A7), we identified specific CYPs responsible for bioactivating three PAs – lasiocarpine, riddelliine, and senkirkine. Among the fourteen cell lines, cells expressing CYP3A4 showed significant increases in PA-induced cytotoxicity, evidenced by decreased ATP production and cell viability, and increased caspase 3/7 activities. LC-MS/MS analysis revealed the formation of 1-hydroxymethyl-7-hydroxy-6,7-dihydropyrrolizine (DHP), the main reactive metabolite of PAs, in CYP3A4-expressing TK6 cells. DHP was also detected in CYP3A5- and 3A7-expressing cells after PA exposure, but to a much lesser extent. Subsequently, using a high-throughput micronucleus assay, we demonstrated that PAs induced concentration-dependent increases in micronuclei and G2/M phase cell cycle arrest in three CYP3A variant-expressing TK6 cell lines. Using Western blotting, we observed that PA-induced apoptosis, cell cycle changes, and DNA damage were primarily mediated by CYP3A4. Bayesian benchmark dose (BMD) modeling demonstrated that lasiocarpine, of the three PAs, was the most potent inducer of micronuclei, with a BMD of 0.019 μM at critical effect size of 50%. These results indicate that our TK6 cell system holds promise for genotoxicity screening of compounds requiring metabolic activation, identifying specific CYPs involved in bioactivation, and discriminating the genotoxic compounds that have different chemical structures.

Introduction

Pyrrolizidine alkaloid (PA)-containing plants are among the most common poisonous plants affecting humans, livestock, and wildlife worldwide. PAs were first reported more than five decades ago to induce liver tumors in rodents. Other organs and tissues of rodents, such as lung, kidney, brain, bladder, skin, and pancreas, were also found to be targets of PA-induced toxicity. Particularly, the retronecine-, heliotridine-, and otonecine-types of PAs are observed to be genotoxic in animal models. PAs require metabolic activation to exert their cytotoxicity, genotoxicity, and carcinogenicity. Metabolism of retronecine- and heliotridine-type PAs catalyzed by cytochrome P450 (CYP) enzymes forms the primary pyrrolic metabolites, dehydro-PAs (DHP esters), which then are hydrolyzed to form DHP (Figure 1). Otonecine-type PAs require an initial oxidative N-demethylation step, potentially catalyzed by CYPs, to produce DHP esters. These reactive intermediates can interact with cellular macromolecules, including proteins and DNA, and lead to hepatotoxic and genotoxic effects. Efforts have been made to identify the specific CYPs that are responsible for the bioactivation of PAs, and available data suggest that the CYP3A and 2B subfamilies mediate PA metabolic activation in rodents, but more data are needed to systematically evaluate the specific human CYPs account for the bioactivation of these genotoxic food contaminants.

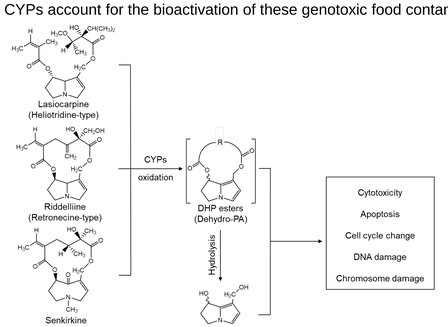


Figure 1. Proposed metabolic activation pathway of three pyrrolizidine alkaloids. Lasiocarpine, riddelliine, and senkirkine undergo metabolic activation to their reactive metabolites including DHP esters and DHP.

Materials and Methods

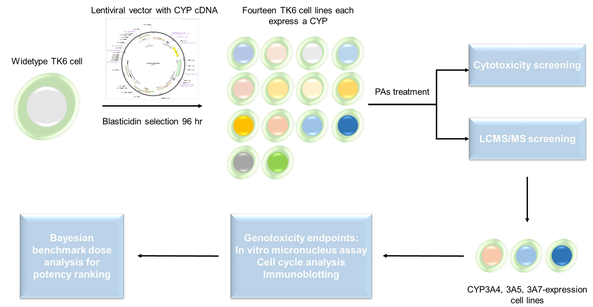


Figure 2. Schematic pipeline of the study

Results

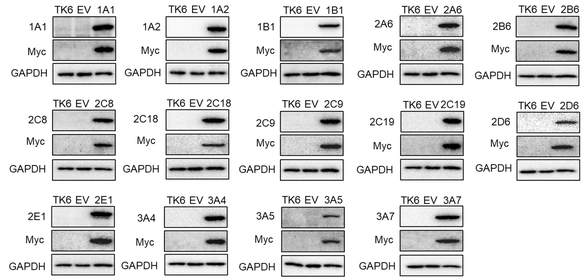


Figure 3. Characterization of CYP protein expression in stably transduced TK6 cells.

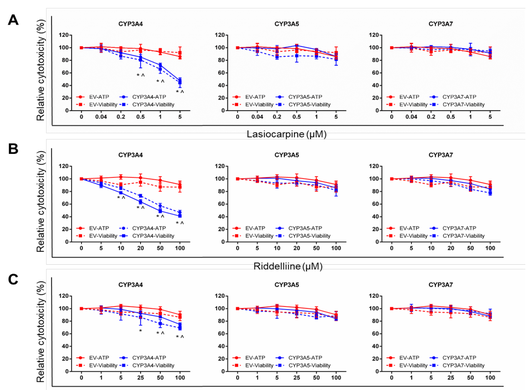
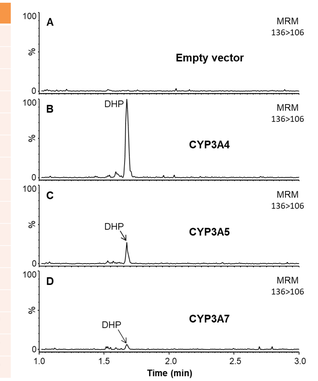


Figure 4. Cytotoxicity of lasiocarpine (A), riddelliine (B), and senkirkine (C) in TK6 cells transduced with empty vector (EV), CYP3A4, 3A5, and 3A7. Cells were treated at the indicated concentrations for 24 h. * (ATP level) and ^ (cell viability) indicate for a treatment group versus the corresponding EV cells at the same concentration.

Results

Table 1. Levels of DHP formation in TK6 cells transduced with various CYPs after 24-h exposure to 1 μM lasiocarpine, 10 μM riddelliine, and 50 μM senkirkine*

Transduced TK6 cells	DHP Formation (nmol/mg protein)		
	Lasiocarpine	Riddelliine	Senkirkine
EV	< LOD	< LOD	< LOD
CYP1A1	< LOD	< LOD	< LOD
CYP1A2	< LOD	< LOD	< LOD
CYP1B1	< LOD	< LOD	< LOD
CYP2A6	< LOD	< LOD	< LOD
CYP2B6	< LOD	< LOD	< LOD
CYP2C8	< LOD	< LOD	< LOD
CYP2C9	< LOD	< LOD	< LOD
CYP2C18	< LOD	< LOD	< LOD
CYP2C19	< LOD	< LOD	< LOD
CYP2D6	< LOD	< LOD	< LOD
CYP2E1	< LOD	< LOD	< LOD
CYP3A4	5.76 ± 1.09	4.72 ± 0.17	5.08 ± 0.59
CYP3A5	0.59 ± 0.14	0.22 ± 0.15	0.27 ± 0.04
CYP3A7	0.08 ± 0.04	0.67 ± 0.28	0.22 ± 0.06



*DHP released into the medium were quantified by LC–MS/MS and are expressed as nmol/mg protein. EV, TK6 cells transduced with empty vector; LOD, lower limit of detection.

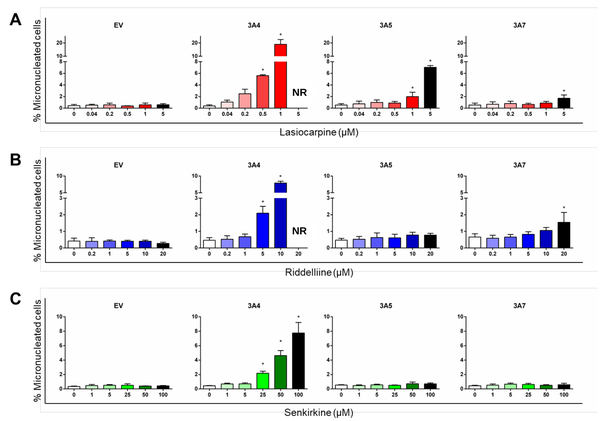


Figure 5. Induction of micronuclei by lasiocarpine (A), riddelliine (B), and senkirkine (C) in CYP-expressing TK6 cells using a high-throughput micronucleus assay. The stopping gate was set to record 10,000 intact nuclei. Data were expressed as means ± SD from at least three independent experiments. * indicates P < 0.05 comparing treated groups to DMSO control. NR: not reported due to > 55% cytotoxicity in at least one independent experiment.

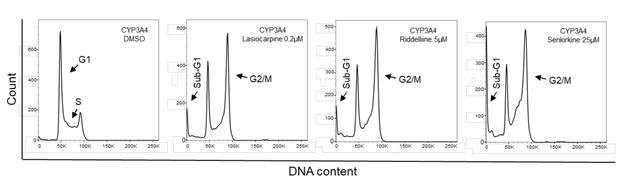


Figure 6. PAs induced cell cycle changes in TK6 cells expressing CYP3A4

Results

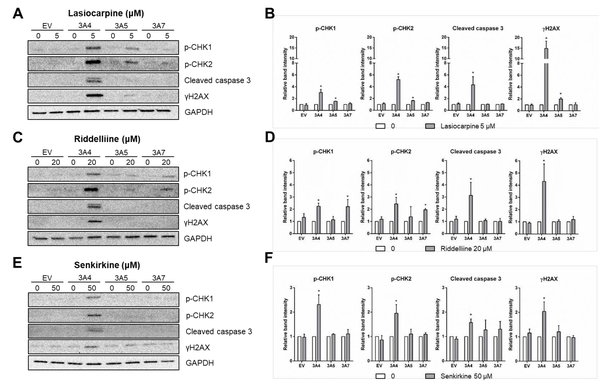


Figure 7. Western blotting confirmed DNA damage, cell cycle changes, and apoptosis induced by PAs. Cells were exposed to 5 μM lasiocarpine (A and B), 20 μM riddelliine (C and D), or 50 μM senkirkine (E and F) for 24 h. Protein expression were quantified and expressed as the means ± SD from three independent experiments. Intensities of bands were normalized to the amount of GAPDH. * indicates P < 0.05 between the treatment group and corresponding vehicle control.

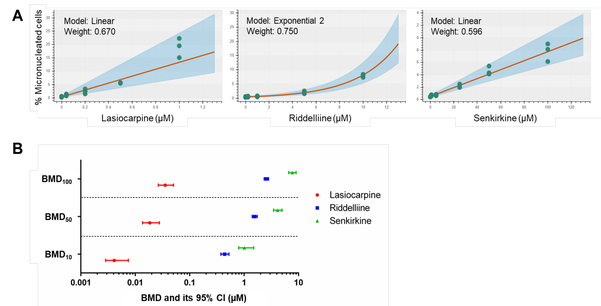


Figure 8. Bayesian benchmark dose (BBMD) modeling evaluating the potency of PAs. The micronucleus (MN) data in CYP3A4-expressing TK6 cells exposed to lasiocarpine, riddelliine, or senkirkine were used for the BMD modeling. (A) The model with the highest posterior weight (i.e., the best fitting curve) for each PA is presented. (B) The BMD (BMD50 and BMD100) estimates producing a 50% or 100% increase above the controls. The bar represents the lower (BMDL) and upper (BMDU) 95% confidence interval (CI) for each BMD value.

Conclusion

- **A series of 14 CYP-expressing TK6 cell lines were created for genotoxicity testing.**
- **The cytotoxicity and genotoxicity of three Pyrrolizidine Alkaloids (PA) were measured using these metabolically competent TK6 cell lines.**
- **PA-induced micronucleus formation, apoptosis, cell cycle changes, and DNA damage were primarily mediated by CYP3A4.**
- **To a lesser extent, CYP3A5 and 3A7 were also involved in PA bioactivation.**
- **PAs with different structures showed different genotoxic potential after metabolic activation.**