An inter-species comparison of the triclopyr in vitro and in vivo toxicokinetic properties, for risk assessment purpose.

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Abstract

Triclopyr is a synthetic auxin, a broad-leaf herbicide, used in pasture and Rice. Hence, external human exposure is possible via dietary and non-dietary route. Herein, we present a multi-faceted approach, utilizing newly generated data from in vitro systems and existing internal exposure data from in vivo performed in mammalian toxicology species such as humans, and as such in silico predictions of systemic exposure to recalcitrant oral gavage and dietary exposure profiles in various species, including humans. The oral absorption of triclopyr was complete in mammals as well as in human volunteers and, in vitro, in a CaCo-2 cells experiment. In vitro comparative metabolism using liver microsomes confirmed lack of metabolism in humans, similar to other species. Excretion mechanisms, previously investigated in in vivo renal clearance study in dog, are unique to that species, while a more rapid renal elimination in rat and human would correlate with known lower renal levels compared to dog. Comparative in vitro plasma protein binding confirmed that the plasma free fraction available for glomerular filtration is higher in dogs compared to rat and humans. Renal clearance was additionally investigated in primary proximal tubule cells to determine direction, magnitude, and transporters involved in mammalian tubular excretion/secretion fluxes.

Overall the results of these studies will be utilized to confirm that the triclopyr toxicokinetic profile of rats is more similar to humans, while dogs present a very species specific excretion system of organic acids. These data can be key to interpret toxicity features in rats. In addition, since the point of departure for triclopyr risk assessment are based on rat studies, the similarities between these two species greatly reduce uncertainties (and, possibly, safety factors) for the use in human health risk assessment.

Background

➢ Hazard identification
   • Triclopyr reference doses for risk assessment are based on rat toxicity findings
   • Dog NOELs are equal or higher than rats; a decrease in phosphosulfophthalein (PSP) excretion occurred at lower doses, was attributed to physiological, non-adverse, pharmacokinetic differences.
   • Rat 71.1
   • Dog 96.8
   • Human 86.5

➢ Timchalk (1997): Sub-linearity in Dogs urinary excretion at high doses are hypothesised to be due to a combination of:
   • Protein binding →<2% free at lower plasma concentrations, >6% at higher doses
   • Increased amount available from plasma for glomerular filtration
   • Balance between active secretion into the tubules/resorption from the tubules
   • Seccretion can get saturated at high doses, resulting in net reabsorption.

Strategy

To reinforce evidence that the Mode of action of toxicity observed in the beagle dogs is not in relevant to humans either for quantitative or qualitative differences in key events.

Human data are limited to a single robust human volunteer pharmacokinetic study (Carmichael, 1989). We can further reduce uncertainties investigating inter-species differences on the kidney clearance of triclopyr

➢ by using innovative in vitro tools now available?
   • Plasma Protein binding
   • Active secretion/resorption at the tubular level
   • by using the Boobis (2008) decision tree for human relevance of a non-cancer MoA.

Methods

➢ In vitro Protein Binding
   • Test system: Rapid Equilibrium Dialysis (RED) plates with Plasma (dog, rat, humans) collected on Na Citrate or Na heparin pH 7.4, PBSt, ph 7.4
   • 6 concentration of 14C-triclopyr evaluated (n=3) via LCS counting – warfarin as parallel control.
   • Incubation/equilibration time: 4 h, 37°C, 300 rpm.

➢ In vitro permeability studies
   • Test system: highly differentiated, primary dog, (human) and rat proximal tubule cell monolayers.
   • According to Brown CD et al., 2008
   • TEER assessed pre-treatment
   • Positive control PAH 10 µM
   • Mannitol used to estimate paracellular flux
   • Probenecid used as OAT4/URAT1 inhibitor

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➢ Proximal tubule in vitro model recapitulate species difference between rats and dogs (human ongoing)
   • Dogs showed a net resorption of triclopyr
   • Rats showed a net secretion of triclopyr
   • In both species, secretion is inhibited by probenecid at basolateral membrane (inhibition of OATs), consistent with Timchalk’s hypothesis, 1997

Results

➢ Species difference in In vitro Protein binding data experimentally demonstrated in vitro:
   • The Dogs shows saturation of protein binding at high pharmacological concentrations, consistent with in vivo data from Timchalk, 1997
   • Rats showed a net secretion at higher dose compared to dog

Interim conclusion

➢ The new dataset support TimChalk 1997 hypothesis on dog specific urinary clearance mechanisms (sub-linear excretion):
   • Confirmed species difference in saturation of protein binding at high concentration
     - Higher glomerular filtration likely in dog
   • Net resorption at high concentration confirmed
     - Lower secretion likely in dog at high doses

Future direction

➢ Generate and assess human proximal tubule flux data
➢ Confirm the non-human relevance framework for dog findings for risk assessment purpose

References


Timchalk C, Nolan NJ. Pharmacokinetics of triclopyr (3,5,6-trichloro-2-pyridinonic acid) in the beagle dog and rhesus monkey perspective on the reduced capacity of dogs to excrete this organic acid relative to the rat, monkey, and human. Toxicol Appl Pharmacol. 1997 Jan;144(2):268-78.