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Characterization of Microcystin-Induced Toxicity on Primary Human Hepatocytes

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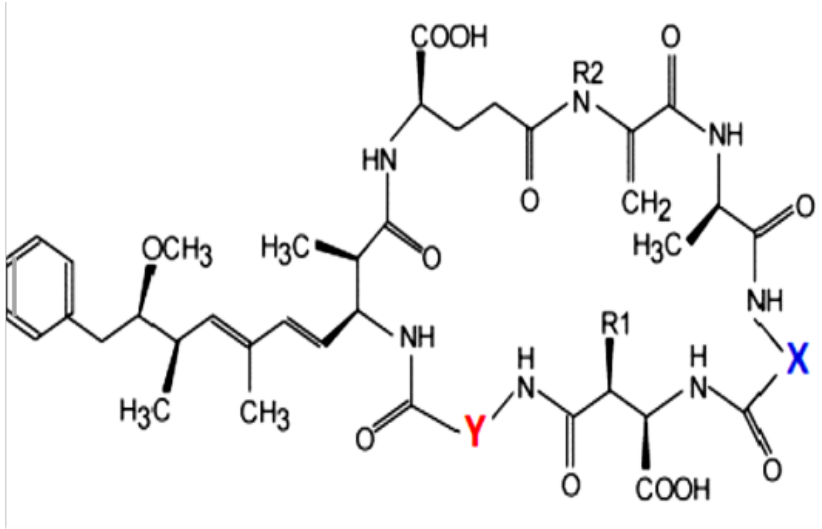
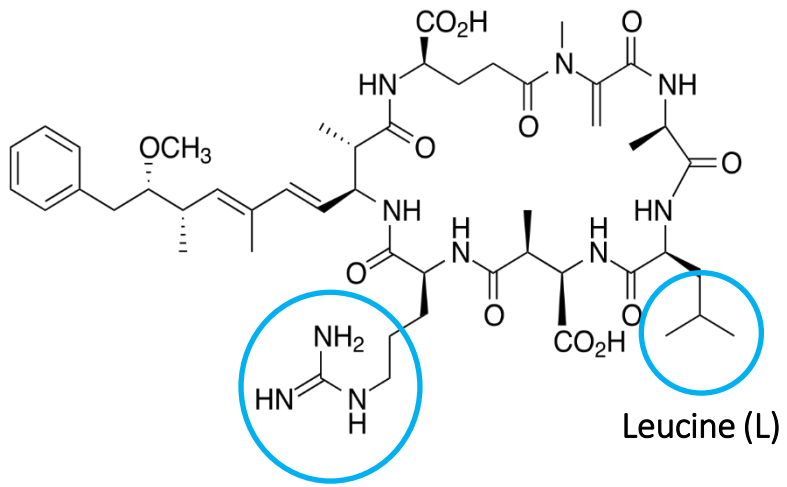
Microcystin



- Naturally forming toxins from cyanobacteria.
  -Often found in lakes and water reservoirs
- Most common group of cyanotoxins
  -Over 200 known congeners
- Known hepatotoxin
- Most are hydrophilic and require active transport.
  -Organic anion transporting proteins (OATP)

Amino Acid Composition of Microcystin Congeners

Microcystin LR



The amino acid composition determines hydrophobicity and is thought to determine the toxicokinetic and toxicodynamic profiles of each congener.

Microcystin Congener	Amino Acid Summary
LW	leucine, tryptophan
LF	leucine, phenylalanine
LY	leucine, tyrosine
LA	leucine, alanine
WR	tryptophan, arginine
LR	leucine, arginine
YR	tyrosine, arginine
RR	arginine, arginine

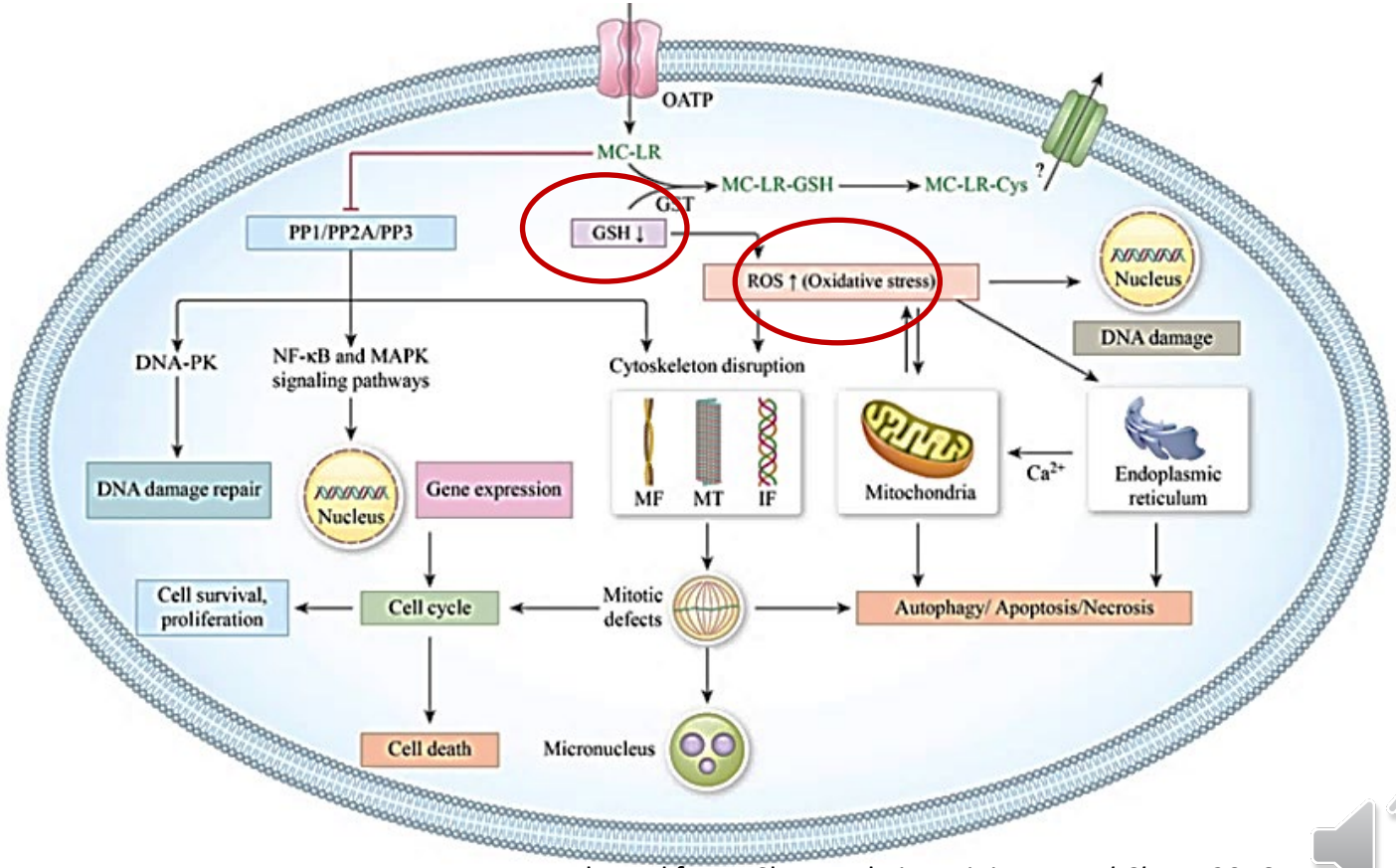
Microcystin Hydrophobicity and Proposed Molecular Mechanisms of Toxicity

Relative Hydrophobicity of Amino Acid Sidechains

Hydrophobicity

LF ≈ LW > LY > LA > WR = LR > YR > RR

More Hydrophobic **Less Hydrophobic**



Adapted from: Chen and Xie, *Mini Rev Med Chem*; 2016

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Objectives

1. Compare the effects of hydrophobic and hydrophilic microcystin congeners on human hepatocytes.

2. Determine which molecular mechanisms are responsible for microcystin-induced hepatotoxicity.

Methods

Chemicals: Microcystin congeners (LA, LF, LR, LW, LY, RR, WR, YR) were purchased from Enzo Life Sciences, Inc. (Farmingdale, NY, USA)

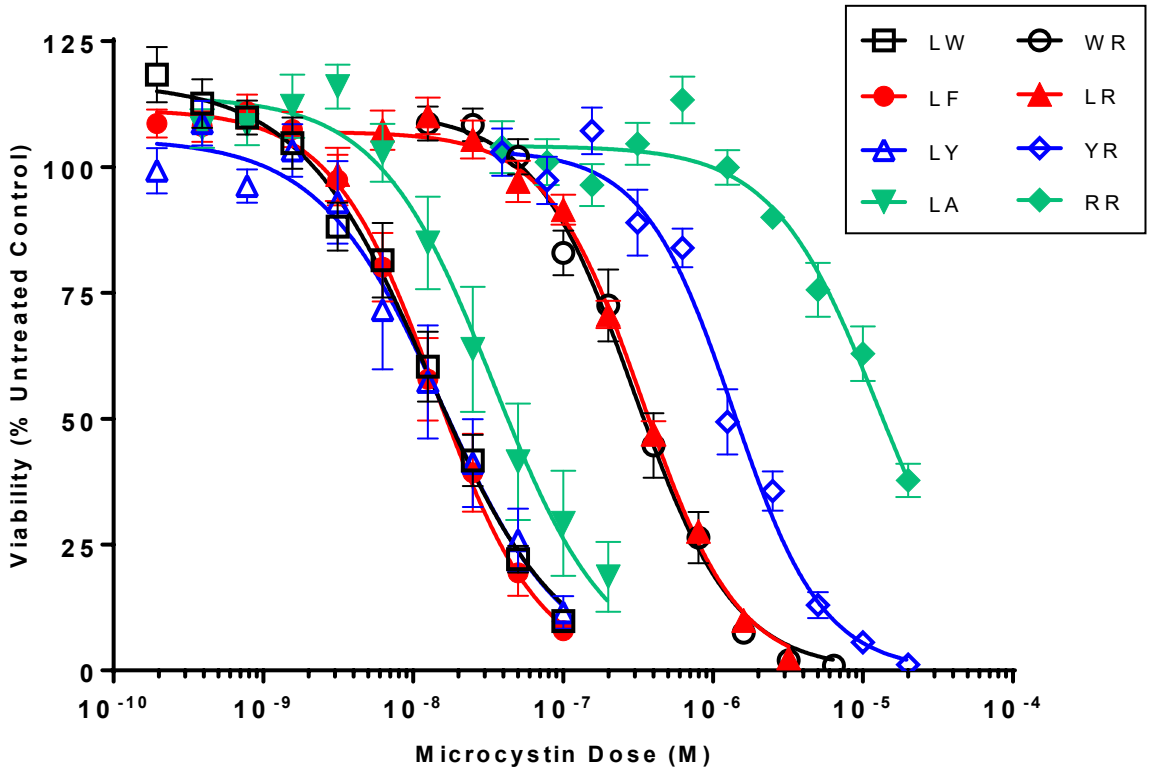
Hepatocytes: Primary human hepatocytes (Lonza; Walkersville, MD, USA) were plated in 96-well collagen I-coated plates.

Cell Viability Assay: CellTiter-Glo 2.0 (Promega; Madison, WI, USA).
Hepatocytes were treated with a range of concentrations (50 pM to 20 µM) of each congener for 24 hours.

Reactive Oxygen Species (ROS) Assay: ROS-Glo H_20_2 Assay (Promega).
Hepatocytes were treated with microcystin congeners at respective EC50 concentrations (determined by viability assay) for 2 or 4 hours.

Glutathione (GSH) Assay: GSH/GSSG-Glo Assay (Promega).
Hepatocytes were treated with microcystin congeners at respective EC50 concentrations (determined by viability assay) for 2 or 4 hours.

Toxicity of Hydrophobic and Hydrophilic Microcystin Congeners on Primary Human Hepatocytes



MC Congener	EC50 (M)
LW	1.28×10^{-8}
LF	1.41×10^{-8}
LY	1.55×10^{-8}
LA	3.43×10^{-8}
WR	2.96×10^{-7}
LR	3.39×10^{-7}
YR	1.36×10^{-6}
RR	1.27×10^{-5}

*No effect on ROS or GSH

Viability and Hydrophobicity of Microcystin Congeners

Microcystin Congener	Amino Acid Summary	Viability (EC50)	Relative Hydrophobicity of Amino Acid Sidechains
LW	leucine, tryptophan	1	1
LF	leucine, phenylalanine	1	1
LY	leucine, tyrosine	1	3
LA	leucine, alanine	4	4
WR	tryptophan, arginine	5	5
LR	leucine, arginine	5	5
YR	tyrosine, arginine	7	7
RR	arginine, arginine	8	8

(Monera *et al.*; *J Pept Sci;* 1995)

Viability (EC50)

LW ≈ LF ≈ LY > LA > WR ≈ LR > YR > RR

More Toxic — Less Toxic

Hydrophobicity (Amino Acids)

LF ≈ LW > LY > LA > WR = LR > YR > RR

More Hydrophobic — Less Hydrophobic

Conclusions

- MCLW is the most potent congener and MCRR is the least potent in human hepatocytes. The rank order for cytotoxicity is LW>LF>LY>LA>WR>LR>YR>RR.
- Cell viability correlate with congener hydrophobicity.
- GSH and ROS levels did not significantly change when treated with microcystins.
- These results suggest that active transport plays an important role in the observed differences in toxicity between congeners.

Conclusions (cont'd)

- Concentrations of microcystins used in these studies are similar to those found in the serum (<0.16–28.8 ng/mL) of documented human exposures (Hilborn ED et al., *Environ Toxicol*., 2007)

- Using primary human hepatocytes and microcystin concentrations that are relevant to human exposures, the results of this study provide direction for future research.

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Thank you

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