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The conjugation of microcystin-RR by human recombinant GSTs and hepatic cytosol

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HIGHLIGHTS

- Human GSTs and hepatic cytosols (HC) catalyzed microcystin-RR conjugation with GSH.
- Catalytic efficiency score for recombinant GSTs was T1-1 > A1-1 ≈ P1-1 > M1-1 > A3-3.
- MC-RR is slightly more efficiently detoxified than MC-LR by human recombinant GSTs.
- In HC the spontaneous reaction was favored (ratio 3:1) at physiological GSH levels.
- At low MC-RR and GSH content the enzymatic reaction was predominant in HC.

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ABSTRACT

Many cyanobacterial species can produce cyanotoxins, among which mycrocistins (MC) are a group of ≈100 congeners of hepatotoxic cyclic heptapeptides. MC-RR differs from MC-LR, the most studied congener only for one residue (arginine *vs* leucine), resulting in a ten-fold difference in the acute toxicity in mice. Although humans may be exposed to MC through several routes and kinetics appeared to be the major factor affecting congener-specific toxicity, little is known on MC metabolism. The accepted pathway for MC detoxication is GSH conjugation: here the MC-RR conjugation with GSH catalyzed by 5 recombinant human GSTs and human liver cytosol (HLC) has been characterized and appeared to be more efficient than MC-LR conjugation. The catalytic efficiency score is T1-1 > A1-1 ≈ P1-1 > M1-1 > A3-3 (0.161–0.056 pmol GSMC-RR (μg protein min μM)$^{-1}$). In HLC the spontaneous reaction is favored *vs* the enzymatic one (ratio 3:1) at physiological GSH content. However, at low MC-RR concentrations, representative of repeated oral exposure, and low GSH content (down to 0.05 mM), possibly associated to exposure to drugs or in patients affected by several pathologies, the relevance of the enzymatic reaction progressively increases, providing the predominant contribution to MC-RR detoxication.

1. Introduction

Cyanobacteria are a morphologically diverse group of photosynthetic prokaryotes that occupy a wide range of niches (Manganelli et al., 2012). Over the last decades, increased eutrophic conditions have favored cyanobacteria growth in water bodies up to elevated density, resulting in blooms and scum. As secondary metabolites they produce cyanotoxins, to which humans may be exposed through several routes: the oral one is by far the most frequent and quantitatively important, occurring by ingestion of contaminated drinking water, food (mainly aquatic organisms), dietary supplements (the so-called BGAS products) or water during recreational activities. The parenteral route of exposure has been demonstrated to cause death in patients when contaminated surface water was used for hemodialysis (Azevedo et al., 2002). Dermal and inhalation exposure have been considered up to now as minor routes, although recently an additional, non-waterborne, possible human exposure route for cyanotoxins has been proposed by inhalation of dusts containing cyanobacteria in the deserts (Metcalf et al., 2012).

Among cyanotoxins, microcystins (MC) are a group of more than 100 different structural variants of toxic cyclic heptapeptides (Kaasalainen et al., 2012). MC congeners differ from each other for two L-amino-acids in positions 2 and 4 (according to which they are named) and other changes such as methylation/desmethylation. The molecule is characterized by a common specific amino-acid (Adda), N-methyl-dehydroalanine, D-alanine, β-linked D-erythro-8-methylaspartic acid and γ-linked D-glutamic acid. The MC-RR

Abbreviations: MC, microcystins; GSTs, glutathione transferases; HLC, human liver cytosol; CDNB, 1-chloro-2,4-dinitrobenzene; MD, male donor; FD, female donor; ED, mix gender donor.

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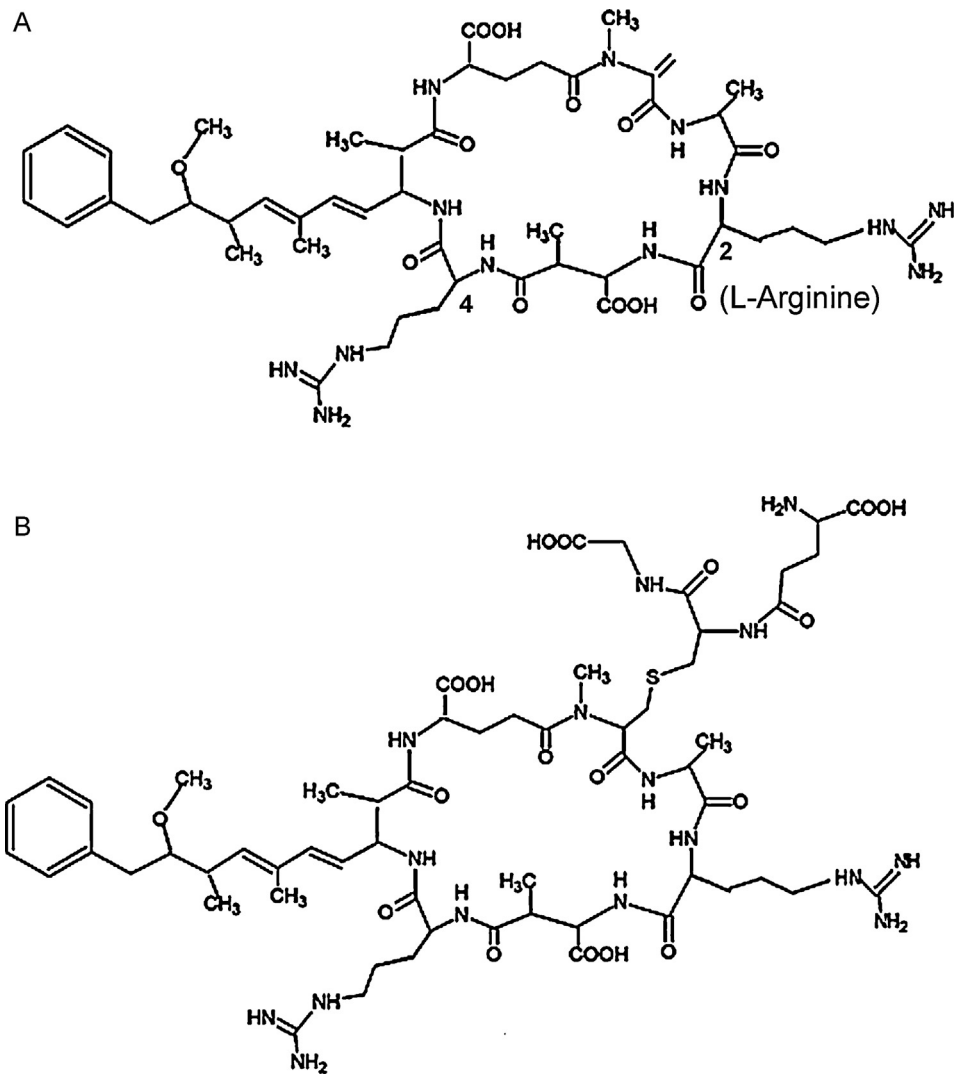

Fig. 1. Chemical structure of microcystin-RR (MC-RR) (A) and its GSH conjugate (GS-MCRR) (B).

congener, whose structure is shown in Fig. 1, contains arginine (R) in both position 2 and 4.

The MC variants are characterized by different acute hepatotoxicity: ip LD_{50} in mice are spread in a wide range of values (from 50 up to 1200 μg/kg) (Funari and Testai, 2008). In addition, neurotoxic effects have been reported for some specific MCs, such as MC-LF and MC-LW (Feurstein et al., 2011), and more recently also for MC-LR (Li et al., 2012a,b). MC-RR and MC-LR differs only for the aminoacid in position 2 (arginine *vs* leucine), but this minimal structural change results in a ten-fold difference in the acute toxicity (i.p. LD_{50} in mice: 500 and 50 μg/kg, respectively) (Funari and Testai, 2008).

The mechanism of action of MC hepatotoxicity is associated with specific inhibition of protein serine/threonine phosphatases (PP1 and PP2A), which alters phosphorylation of cellular proteins involved in signal transduction, resulting in a cascade of events from lipid peroxidation and oxidative stress to apoptosis (Botha et al., 2004; Gehringer, 2004). Furthermore, MC-LR has been described as tumor promoter (Nishiwaki-Matsushima et al., 1992) and, on this basis, classified as a 2B carcinogen by the International Agency for Research on Cancer (IARC, 2006). Finally, a role for cyanobacteria in the etiology of amyotrophic lateral sclerosis (ALS) and Alzheimer's disease has been proposed, suggesting that MC neurotoxicity might be linked to neurodegeneration (Li et al., 2012a; Metcalf and Codd, 2009; Stipa et al., 2006).

Despite difference in acute toxicity among variants, the inhibitory capacity of single MC congeners on PP1 and PP2A *in vitro* is comparable, with IC_{50} values in the nanomolar range (Hoeger et al., 2007; Monks et al., 2007; Fischer et al., 2010). Minimal structural changes among variants have been reported to result in three- to four-fold differences in the uptake, tissue distribution, and excretion (Meriluoto et al., 1990), thus underlying the importance of individual kinetic profiles in explaining the differences in toxicity among variants. Indeed, a congener selective transport by the hepatic organic anion transporters OATP1B1 and 1B3 into primary human hepatocytes has been shown, with MC-LW and -LF eliciting cytotoxic effects at lower equimolar concentrations than MC-LR and MC-RR (Fischer et al., 2010). In addition, HEK293 cells overexpressing OATP1B1 or 1B3 elicited a higher sensitivity to MC-LR than to MC-RR, all together suggesting a lower uptake of MC-RR in the liver (Fischer et al., 2010; Monks et al., 2007).

Beside the absorption/distribution processes, biotransformation can be the additional crucial step in defining the toxicokinetic profile of individual congeners. Despite this, data on human biotransformation are still very scant. The accepted pathway for MC detoxication is GSH conjugation (Pflugmacher et al., 1998). The GSH