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Hepatocellular uptake of ^{3}H-dihydromicrocystin-LR, a cyclic peptide toxin

John E. Eriksson [1], Lotte Grönberg [2], Siv Nygård [2], J. Peter Slotte [2] and Jussi A.O. Meriluoto [2]

[1] *Department of Biology and* [2] *Department of Biochemistry and Pharmacy, Åbo Akademi University, SF-20500 Turku (Finland)*

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The cellular uptake of microcystin-LR, a cyclic heptapeptide hepatotoxin from the cyanobacterium *Microcystis aeruginosa*, was studied by means of a radiolabelled derivative of the toxin, ^{3}H-dihydromicrocystin-LR. The uptake of ^{3}H-dihydromicrocystin-LR was shown to be specific for freshly isolated rat hepatocytes whereas the uptake in the human hepatocarcinoma cell line Hep G2 as well as the mouse fibroblast cell line NIH-3T3, and the human neuroblastoma cell line SH-SY5Y, was negligible. By means of a surface barostat technique it was shown that the membrane penetrating capacity (surface activity) of microcystin-LR was low, indicating that the toxin requires an active uptake mechanism. The hepatocellular uptake of microcystin-LR could be inhibited in the presence of bile acid transport inhibitors such as antamanide (5 μM), sulfobromophthalein (50 μM) and rifampicin (30 μM). The uptake was also reduced in a concentration dependent manner when the hepatocytes were incubated in the presence the bile salts cholate and taurocholate. A complete inhibition of the hepatocellular uptake was achieved by 100 μM of either bile salt. The overall results indicate that the uptake of microcystin-LR is through the multispecific transport system for bile acids. This mechanism of cell entry would explain the previously observed cell specificity and organotropism of microcystin-LR.

Introduction

Cyanobacteria or blue-green algae, commonly occurring in lakes and reservoirs, produce potent hepatotoxins termed microcystins. Microcystins constitute a group of cyclic heptapeptides which have been isolated from several different species of cyanobacteria [1–4]. Two of the seven amino acid residues in these peptides are variable [1,5–8]. The structure of the microcystin used in the present study is cyclo(-D-Ala-L-Leu-D-*erythro*-β-methyl-Asp-L-Arg-ADDA-D-Glu-*N*-methyldehydro-Ala) with leucine and arginine at the variable sites. ADDA is a hydrophobic β amino acid, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid. According to a previously proposed nomenclature, with one-letter suffixes to designate the variable portion [8], the toxin we used is termed microcystin-LR (MC-LR).

Previous studies have shown that microcystins are highly organ and cell specific. In experimental studies the in vivo damage is mainly restricted to the liver [4,9,10–12] and in cellular in vitro studies, only hepatocytes are affected by these toxins [13–15]. This cell specificity could be due to metabolic activation in the liver or due an uptake pathway shared with some physiological substrate specific for the liver. In a previous study with microcystin-YM (tyrosine and methionine as the variable residues) it was shown that the in vitro morphological effects on hepatocytes can be inhibited by low concentrations of bile acids or bile acid transport inhibitors [16]. This suggests that microcystins could be taken up through the transport system for bile acids. This multispecific transport system, consisting of several subsystems, is apart from being responsible for the uptake of bile acids also an entrance pathway for several xenobiotic substances (for review see Ref. 17).

Abbreviations: ADDA, 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyldeca-4,6-dienoic acid; DMEM, Dulbecco's minimal essential medium; DMC-LR, dihydromicrocystin-LR; 7-dm-MC-RR, 7-desmethyl-microcystin-RR; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; MC-LR, microcystin-LR; PBS, phosphate-buffered saline; Tes, *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid; Tricine, *N*-tris(hydroxymethyl)methylglycine; Tris, tris(hydroxymethyl)aminomethane.

Correspondence: J.E. Eriksson, Department of Biology, Åbo Akademi University, SF-20500 Turku, Finland.

In the present work we studied the MC-LR uptake in different cell types, by using a tritiated derivative of MC-LR, i.e., ^{3}H-dihydromicrocystin-LR (^{3}H-DMC-LR). In addition, in order to determine the membrane penetrating capacity of unlabelled MC-LR, the interaction of the toxin with mixed lipid monolayers was measured by means of a surface barostat technique. Low membrane penetrating capacity and highly cell specific uptake is reported as well as inhibition of ^{3}H-DMC-LR uptake by both bile acids and bile acid transport inhibitors.

Materials and Methods

Isolation of hepatocytes

Hepatocytes were isolated from male Wistar rats (200–250 g, fed ad libitum) by a two step collagenase perfusion of the liver as described elsewhere [18]. After isolation, 90–95% of the hepatocytes routinely excluded trypan blue. Experiments were carried out at 37 °C and at $1 \cdot 10^6$ cells/ml in a modified carbonate-free Krebs-Henseleit buffer [18] supplemented with 30 mM Hepes, 30 mM Tricine and 30 mM Tes.

Cultivation of cell lines

Mouse NIH-3T3 fibroblasts, human SH-SY5Y neuroblastoma cells [19,20] and human Hep G2 hepatoma cells [21] were cultured in Dulbecco's Minimal Essential Medium (DMEM) supplemented with 10% foetal calf serum, 0.1 mM pyruvate, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37 °C in a humidified 95% air/5% CO_2 atmosphere.

Isolation of microcystin-LR

MC-LR and 7-desmethyl-microcystin-RR (7-dm-MC-RR) were isolated from the cyanobacteria *Microcystis aeruginosa* and *Oscillatoria agardhii* (strain CYA-129), respectively. The freeze-dried material was kindly provided by Olav Skulberg, Norwegian Institute for Water Research, Oslo. Extraction and purification of the toxin was performed as described elsewhere [11,22,23]. The structure of MC-LR has been verified by employing fast atomic bombardment mass spectrometry (FAB/MS) and nuclear magnetic resonance (NMR) [4].

^{3}H labelling of microcystin-LR

3.5 mg MC-LR in 1.6 ml 70% isopropanol/30% water was reduced with 0.5 mg NaB^3H_4 (specific activity 250 mCi/mg, 10 Ci/mmol). Reaction was carried out at room temperature for 22 h under continuous shaking. The reaction products were separated in the HPLC system used for isolation of MC-LR [11,22,23]. Two strongly radioactive reaction products eluting in the vicinity of the native toxin peak were collected. These two compounds coeluted with FAB/MS identified epimers of dihydromicrocystin-LR (DMC-LR), i.e., MC-LR with *N*-methyldehydroalanine reduced to *N*-methylalanine (Refs. 24, 25, and Meriluoto, J.A.O., Nygård, S.E., Dahlem, A.M. and Eriksson, J.E., unpublished results). The labelled compounds were eluted at 11.0 min and 13.0 min, the native toxin at 11.3 min (Meriluoto, J.A.O., Nygård, S.E., Dahlem, A.M. and Eriksson, J.E., unpublished results).

Biological characterization of the DMC-LR epimers showed that the reduction with NaB^3H_4 caused an approximately 3-fold decrease in the lethality compared with unlabelled MC-LR. The epimers were liver specific in their action, inducing similar lesions as the unlabelled toxin. In addition the organotropism of the epimers was reflected in their tissue distribution. Both epimers were rapidly concentrated in the liver. Other tissues and organs contained only minor amounts of the epimers (Refs. 24, 25, and Meriluoto, J.A.O., Nygård, S.E., Dahlem, A.M. and Eriksson, J.E., unpublished results). The specific activities of the toxic fractions were 170 and 310 mCi/mmol, respectively (Meriluoto, J.A.O., Nygård, S.E., Dahlem, A.M. and Eriksson, J.E., unpublished results). Fraction 1 (11.0 min, 170 mCi/mmol) was used in the present study.

Surface barostat measurements

The membrane penetrating capacity of MC-LR and 7-dm-MC-RR was studied by using a lipid monolayer consisting of egg phosphatidylcholine (EPC) and cholesterol (CHL), both lipids with a chemical purity > 99%. The monolayer experiments were performed on a KSV 5000 surface barostat (KSV Instruments, Helsinki, Finland). The adsorption was determined in a zero-order trough [26] consisting of a 30 ml temperature controlled reaction compartment (25.5 cm^2), a 7.1 cm^2 compartment for surface pressure determination (with a platinum Wilhelmy plate), and a 68.1 cm^2 lipid reservoir. The compartments were connected with glass bridges, giving a total trough area of 101.6 cm^2.

Toxin penetration into the lipid monolayer was determined at 37 °C by spreading 125 μl of a lipid solution, EPC/CHL (molar ratio, 7 : 3) in hexane/ethanol (9 : 1, v/v), on a buffer surface (20 mM Tris-HCl, 145 mM NaCl (pH 7)) in the zero-order trough. The organic solvent was allowed to evaporate for 5 min, after which the lipids were compressed to a monolayer with a pre-determined surface pressure (barrier speed 6 mm^2/s). Constant surface pressure was maintained during the experiment by computer controlled compensatory barrier movement. When the monolayer had stabilized at the chosen surface pressure (about 5 min) MC-LR or 7-dm-MC-RR was added to the subphase (final concn. 1 μM) of the magnetically stirred (150 rpm) reaction compartment. Toxin penetration into the monolayer was registered as an increase of the monolayer area.

Toxin penetration was also determined by measuring the change in surface pressure after toxin addition at constant surface area. These experiments were done in magnetically stirred teflon cups (10.8 cm^2, 20 ml). Lipid solution was spread on the buffer surface until the chosen surface pressure was obtained. When the monolayer had stabilized, MC-LR or 7-dm-MC-RR were added (final concn. 1 μM) to the subphase, and the change in surface pressure was registered after 30 min.

Uptake studies

The uptake of ^{3}H-DMC-LR or the ^{14}C-labelled bile acids cholate or taurocholate in freshly isolated hepatocytes was measured by using a filtration technique. Hepatocytes were preincubated at 37 °C for 5 min before addition of ^{3}H-DMC-LR or the ^{14}C-labelled bile acids. Samples of 100 μl of the hepatocyte suspensions were taken at time intervals and filtered on Whatman GF/C filters, moistened with the incubation buffer solution, and followed immediately by 3 ml of the same buffer solution. Filtration was carried out by using mild vacuum (< 25 mmHg below atmospheric pressure). Filters were placed in scintillation vials and the radioactivity was counted by adding Aqualuma (Lumac, Landgraaf, The Netherlands). An internal standard ratio was used to correct for quenching. The filters did not cause any significant quenching as they became transparent after addition of the scintillation cocktail.

Since unlabelled DMC-LR was not available in sufficient amounts, most experiments with hepatocytes were performed with mixtures of 0.5 μM ^{3}H-DMC-LR and varying concentrations of MC-LR. Results were expressed as calculated total uptake of MC-LR. As DMC-LR and MC-LR are chemically and toxicologically closely related (Refs. 24, 25, and Meriluoto, J.A.O., Nygård, S.E., Dahlem, A.M. and Eriksson, J.E., unpublished results) this procedure is not expected to affect the results to any greater extent.

The uptake of ^{3}H-DMC-LR in established cell lines was carried out at the culture conditions described above. Short term experiments were performed in DMEM without any additions. Longer incubations were carried out in DMEM modified as described above. After addition of the ^{3}H-DMC-LR, cell culture dishes were sampled at time intervals and cells were rinsed five times with phosphate-buffered saline (PBS), pH 7.4. After removal of PBS, the cells were scraped off the dish and suspended in 0.2% (v/v) Triton-X 100 in PBS. This suspension was used for liquid scintillation counting and protein determinations. Protein was determined using the Bio-Rad assay (Richmond, CA). ^{3}H-Inulin was used as a impermeable reference compound in these studies. The uptake in the established cell lines was very low. In order to obtain higher CPM values, the ^{3}H-DMC-LR was not diluted with unlabelled MC-LR in the experiments with these cells.

Chemicals

Radiochemicals were purchased from Amersham (Buckinghamshire, U.K.). ^{3}H-Inulin had a specific activity of 2.5 Ci/mmol. ^{14}C-Cholic acid and ^{14}C-taurocholic acid both had a specific activity of 56 mCi/mmol. Antamanide was a generous gift from Prof. Theodor Wieland (Max-Planck-Institut für Medizinische Forschung, Heidelberg, F.R.G.). All other organic chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Results

Toxin penetration into a mixed lipid monolayer

The penetrating capacity of 1 μM MC-LR into the EPC/CHL lipid monolayer was determined at various initial pressures. 7-dm-MC-RR was used for comparison, since this toxin is chemically somewhat different from MC-LR and is also less toxic than MC-LR. It was observed that only 7-dm-MC-RR showed a clear surface pressure dependent membrane penetrating capacity, which reached a maximum at or around 20 mN/m (Fig. 1). The penetrating capacity of MC-LR was only to a low degree surface-pressure dependent (Fig. 1). It appeared that 7-dm-MC-RR had somewhat greater penetrating capacity than MC-LR, giving maximally a 7% increase in the monolayer area at a constant surface pressure of 20 mN/m (Fig. 1).

The membrane penetrating capacity of the toxins was also analyzed in monolayers at constant area. At 1 μM 7-dm-MC-RR increased the surface pressure from an initial 10 or 20 mN/m with about 0.5 mN/m within 30 min after toxin addition (curves not shown). MC-LR did not cause any significant increase in surface pressures from the initial values 10 and 20 mN/m (data not shown).

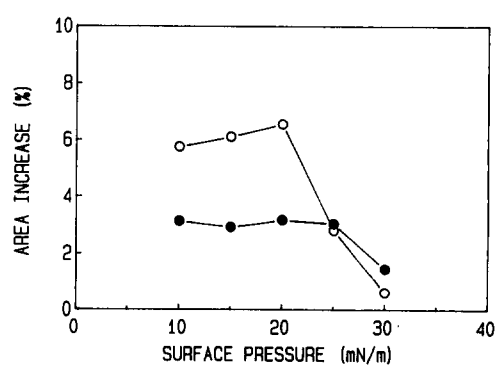

Fig. 1. The penetration of MC-LR and 7-dm-MC-RR into an EPC/CHL monolayer. 1 μM of toxin was added to the subphase of an EPC/CHL monolayer (molar ratio 7:3) at different surface pressures, and toxin penetration was registered as a percentage increase of the monolayer area 30 min after addition of MC-LR or 7-dm-MC-RR, respectively. The results are means of two determinations, on a subphase buffer consisting of 20 mM Tris-HCl and 145 mM NaCl (pH 7.0) at 37 °C. MC-LR (●); 7-dm-MC-RR (○).

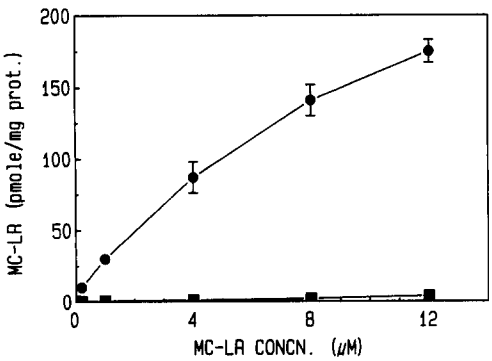

Fig. 2. Dose-dependent calculated total uptake of MC-LR in freshly isolated rat hepatocytes and uptake of ^{3}H-DMC-LR in Hep G2 cells. The hepatocytes were exposed to 0.5 μM of ^{3}H-DMC-LR diluted with unlabelled MC-LR to yield the indicated final concentrations. In the experiments with Hep-G2 cells the ^{3}H-DMC-LR was used as such without dilution with unlabelled MC-LR. The uptake shown is after 20 min incubation at 37°C. See text for further details. Values are means ± SD ($N = 3$). ●, isolated hepatocytes; ■, Hep G2 cells.

Comparison of ^{3}H-DMC-LR uptake in different cell types

The 3T3, SH-SY5Y and Hep G2 cells did take up only negligible amounts of ^{3}H-DMC-LR (Fig. 2). In Fig. 2 only the trace for the Hep G2 cells is shown, but similar values were obtained with the other two cells types. Also in long term experiments (up to 24 h) the uptake in all three cell types was less than 12 pmol/mg cell protein (Table I). This uptake did not exceed that of ^{3}H-inulin which was used as a non-penetrating reference compound (results not shown). The small amounts of ^{3}H-DMC-LR found in these cell types represent partly cell-adsorbed toxin not released by rinsing with PBS and partly uptake due to pinocytosis.

In contrast, freshly isolated hepatocytes showed a rapid uptake of ^{3}H-DMC-LR (Fig. 2, Fig. 3A, B). Before the uptake started there was an almost instantaneous 'adsorption' to the hepatocytes. This adsorption alone was at corresponding concentrations considerably

TABLE I

The uptake of ^{3}H-DMC-LR in different cell types

Hepatocytes received a mixture of 0.5 μM ^{3}H-DMC-LR and 3.5 μM unlabelled MC-LR. In this case the values represent the calculated total uptake of MC-LR. Other cell types received 4 μM of ^{3}H-DMC-LR. Values are mean values ± S.D. ($N = 3$). n.d., no data.

Time (h)	Uptake of ^{3}H-DMC-LR (pmol/mg cell protein)			
	hepatocytes	3T3 cells	Hep G2 cells	SH-SY5Y
0.5	90.0 ± 11.0	2.5 ± 0.3	1.5 ± 0.2	0.7 ± 0.2
1	n.d.	4.7 ± 0.2	5.8 ± 1.3	1.0 ± 0.5
4	n.d.	6.2 ± 1.1	6.0 ± 0.5	1.3 ± 0.3
12	n.d.	8.0 ± 0.6	7.8 ± 0.9	2.2 ± 0.5
24	n.d.	11.5 ± 1.2	11.0 ± 1.5	2.5 ± 0.4

higher than the total uptake in the non-hepatic and hepatoma cells. The calculated total uptake of MC-LR was linear within 3–4 min. The maximal velocity of the calculated total uptake (V_{max}) was 31 pmole · min^{-1} · mg cell protein^{-1}. K_m as determined from the Lineweaver-Burk plot (Fig. 3B) was 8 μM.

Effects of bile acid transport inhibitors on the ^{3}H-DMC-LR uptake

Organic compounds known to inhibit the hepatic uptake of bile acids were used to prevent the uptake of ^{3}H-DMC-LR. The addition of 5 μM of the cyclic decapeptide antamanide [27], 30 μM rifampicin or 50 μM sulfobromophthalein, respectively, 30 s before the addition of 0.5 μM ^{3}H-DMC-LR together with 3.5 μM MC-LR, completely inhibited the ^{3}H-MC-LR uptake (Table II). In addition, hepatocytes pre-incubated with the above mentioned transport inhibitors were not susceptible to the effects of MC-LR, i.e., no alteration in cell morphology could be seen when cells were examined under the microscope (results not shown).

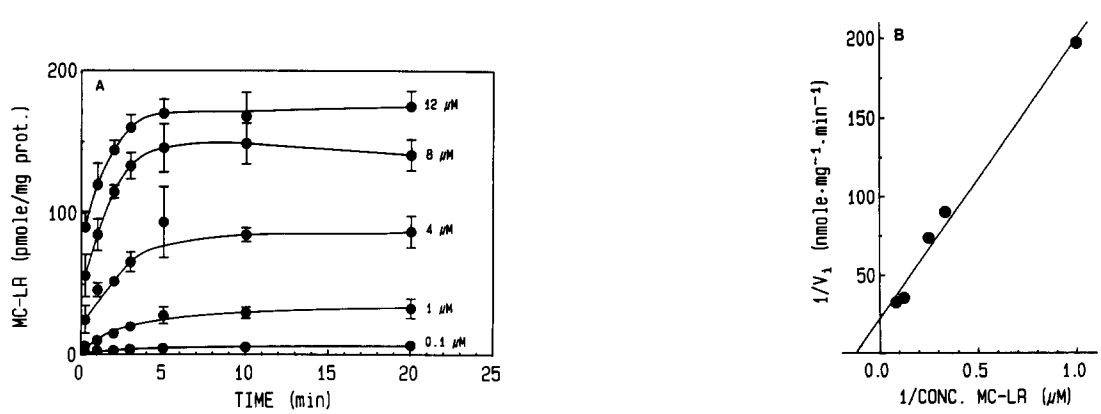

Fig. 3. Calculated total uptake of MC-LR in freshly isolated rat hepatocytes (A). Hepatocytes were exposed to 0.5 μM of ^{3}H-DMC-LR diluted with unlabelled MC-LR to give concentrations from 1 μM to 12 μM (0.1 μM was undiluted ^{3}H-DMC-LR). The positive intercepts with the Y axis, if curves are extrapolated to zero time, indicate a rapid 'adsorption' of MC-LR by the hepatocytes. Values are means ± S.D. ($N = 3$). The initial uptake rates V_i shown in the Lineweaver-Burk plot (B) were determined from the linear part of the uptake curves ($t < 4$ min).

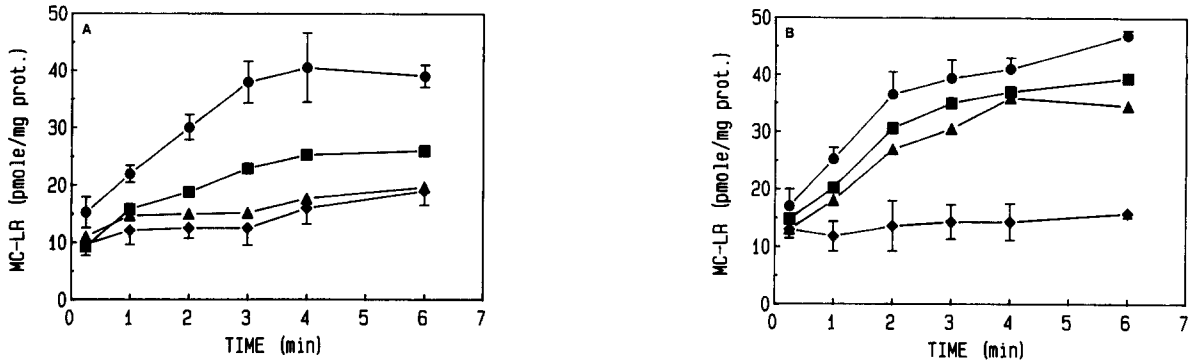

Fig. 4. Effect of bile salts on the uptake of ^{3}H-DMC-LR/MC-LR in freshly isolated rat hepatocytes. Cells were pre-incubated for 30 s with various concentrations of (A) cholate (CHA) and (B) taurocholate (TCHA) after which a mixture with 0.5 μM ^{3}H-DMC-LR and 2.5 μM MC-LR was added. Numbers represent mean values ($N = 3$) of the calculated total uptake of MC-LR. S.D. is shown only for control cells and cells exposed to the highest bile salt concentration. S.D. was less than 5.0 at each mean value in the two other curves (10 and 20 μM of CHA and TCHA, respectively). ●, control; ■, 10; ▲, 20; and ◆, 100 μM of respective bile salt.

The effects of bile acids on the ^{3}H-DMC-LR uptake

In the presence of bile acids the total uptake of MC-LR was reduced. Both cholate and taurocholate showed a similar pattern of concentration-dependent inhibition (Fig. 4A, B). At 100 μM both bile acids completely inhibited the uptake of ^{3}H-DMC-LR/MC-LR.

In a reversed experimental setting, we tried if MC-LR would inhibit the uptake of bile acids. The uptake of ^{14}C-cholate and ^{14}C-taurocholate could also be reduced by adding excess concentrations of MC-LR (Fig. 5A, B). However, the uptake was inhibited to a lesser extent than the uptake of ^{3}H-DMC-LR by cholate and taurocholate.

Discussion

Theoretically, the amphiphatic properties of microcystins could enhance their penetrating capacity into cell membranes. Some amphipathic cyclic peptides such as, e.g., cyclosporin [28], valinomycin [29] and gramicidin [29] are capable of penetrating the cell membrane, the two latter actually acting as ionophores. However, in the present study the membrane penetrating capacity or surface activity of both MC-LR and 7-dm-MC-RR was low. At a surface pressure of about 15 mN/m, 1 μM of MC-LR and 7-dm-MC-RR caused a 3 and 6% increase in the membrane area, respectively. The corresponding change in the surface pressure, when the surface area was held constant, was of the magnitude 0.5 mN/m or less. This is a small change when compared to, e.g., phospholipase A2 which readily penetrates into a non-substrate phospholipid monolayer and increases the surface pressure with about 3–4 mN/m [30]. The surface pressure of human erythrocytes is 31–35 mN/m [31] and it is reasonable to assume that other eukaryotic cells have a surface pressure in their plasma membranes close to these values. Since the observed membrane penetrating capacity of MC-LR and 7-dm-MC-RR was negligible at or above 30 mN/m, there is

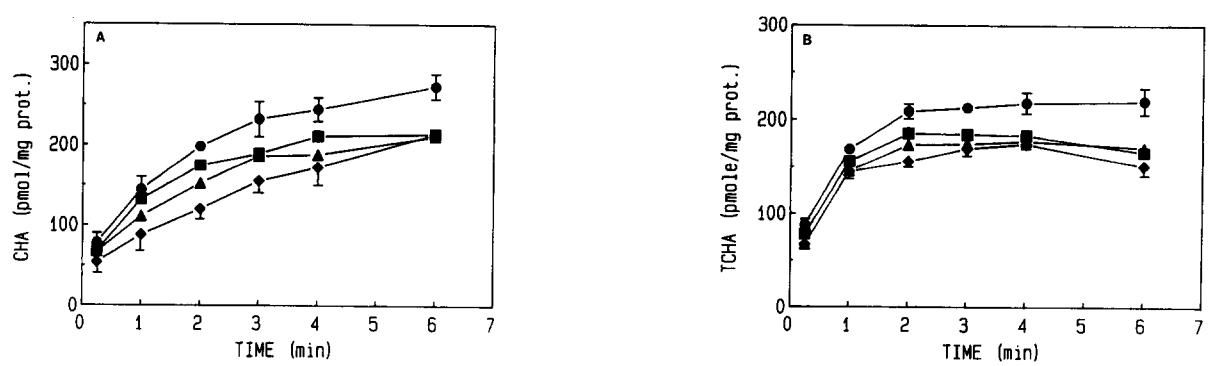

Fig. 5. Effect of MC-LR on the uptake of bile salts in freshly isolated rat hepatocytes. Cells were preincubated for 30 s with various concentrations of MC-LR after which (A) 1.8 μM [^{14}C]cholic acid (CHA) or (B) 1.0 μM [^{14}C]taurocholic acid (TCHA) were added. Numbers represent mean values ($N = 3$). S.D. is shown only for control cells and the cells exposed to the highest MC-LR concentration. S.D. was less than 25.0 at each mean value in the two other curves (10 and 20 μM). ●, Control; ■, 10 μM; ▲, 20 μM; and ◆, 50 μM of MC-LR.

TABLE II

Inhibition of the hepatocellular ^{3}H-DMC-LR/MC-LR uptake in the presence of bile acid transport inhibitors

Hepatocytes were pre-incubated for 30 s with the different concentrations of the inhibitor substances, after which a mixture with 0.5 μM ^{3}H-DMC-LR and 3.5 μM MC-LR was added. Numbers represent mean values ± S.D. ($N = 3$) of the calculated total uptake of MC-LR. The inhibitor substances are: antamanide (AA), rifampicin (RA) and sulfobromophthalein (SBP). Cholic acid (CHA) is included for comparison.

Time (min)	Calculated total uptake of MC-LR (pmol/mg cell protein)				
	Control	AA (5 μM)	RA (30 μM)	SBP (50 μM)	CHA (100 μM)
0.25	15.2±2.7	9.2±1.0	7.5±3.1	7.2±2.1	9.7±2.0
1	21.9±1.5	8.5±2.5	8.1±1.9	8.1±3.2	12.0±2.5
2	30.0±2.0	9.3±1.3	7.2±2.4	7.9±1.1	12.5±1.8
3	37.9±3.7	9.1±1.1	9.0±2.5	8.9±2.2	12.5±3.0
4	40.5±6.1	8.5±2.1	9.2±2.9	10.0±1.5	16.0±2.9
6	39.0±2.1	8.8±2.1	8.5±1.0	8.2±3.0	19.0±2.6

no support for the assumption that these toxins could penetrate cell membranes by simple diffusion. 7-dm-MC-RR, which showed a somewhat higher membrane penetrating capacity, is in fact less toxic both in vivo [11] and in vitro [32].

The cell studies show that the cell specificity of microcystins is due to a requirement of a liver specific, active transport system. The non-hepatic cells and the Hep G2 cells did not take up ^{3}H-DMC-LR to any significant extent. Thus, the intracellular concentration of microcystins can reach effective levels only in hepatocytes which possess a transport mechanism for microcystins.

It is known that the hepatocyte membrane contains carriers responsible for the active uptake of bile acids [17]. These carriers are often referred to as the bile acid transport system. The real number of different carriers is still controversial but it is known that they can act as a multispecific system, showing high affinity for many organic substances other than bile acids [17,28,33,34]. We observed a complete inhibition of the ^{3}H-DMC-LR uptake in hepatocytes when they were incubated in presence of the bile acid transport inhibitors antamanide, rifampicin and sulfobromophthalein. The strongest evidence, however, for a shared uptake mechanism for ^{3}H-DMC-LR and bile acids is the fact that the uptake of ^{3}H-DMC-LR could be inhibited by bile acids in a concentration-dependent manner. The inhibition of bile acid uptake by MC-LR could also be demonstrated, although the uptake of both ^{14}C-taurocholate and ^{14}C-cholate was only slightly reduced by MC-LR. This low uptake inhibition could be explained by toxic and/or morphological effects induced by MC-LR, influencing the structural properties of the hepatocyte membrane. The fact that the Hep G2 cells did not take up MC-LR is in agreement with the hypothesis of the bile acid transport system as a carrier for microcystins. Previous studies have shown that other types of hepatoma derived cell lines do not possess this transport system [35–37]. The uptake of MC-LR reached a plateau relatively fast (after 5–6 min). This may be due to some of the cellular effects of MC-LR, inhibiting further uptake.

In our experiments with hepatocyte suspensions we used mixtures of ^{3}H-DMC-LR and MC-LR. The pharmacokinetics, toxicological properties, tissue affinities and even the chromatographic behavior of these two variants of the same peptide, are closely related (Refs. 24, 25, 38, and Meriluoto, J.A.O., Nygård, S.E., Dahlem, A.M. and Eriksson, J.E., unpublished results). The possible small differences are not important for the main goal of this study, i.e., to investigate the cellular specificity of MC-LR uptake and the involvement of the bile acid transport system in the uptake process.

Interestingly, the K_m value obtained for ^{3}H-DMC-LR/MC-LR in this study (8 μM) is close to the K_m value of the bicyclic peptide phalloidin (12 μM [39]). Phalloidin, a mushroom toxin from *Amanita phalloides*, is taken up by the cholate transporter [33,40] and shows in a similar fashion as microcystins very high organ specificity for the liver [23]. Although the microcystin- and phallodin-induced lesions are similar, the molecular mechanisms underlying these effects seem to be different [41,42].

The uptake of DMC-LR and other microcystins by the multispecific bile acid transport system provides a plausible explanation for the cell specificity and organotropism of these toxins. However, more detailed information is required on the inhibition kinetics of DMC-LR uptake and on the driving forces of the uptake. Studies elucidating these questions are in progress.

Acknowledgements

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