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Chronic exposure to cyanobacterial lyophilisate reveals stronger effects than exposure to purified microcystins – a MRI study

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Introduction

Microcystins are potent hepatotoxins, tumor promoters, and carcinogens. Although they are present in surface water bodies worldwide little has been done to assess the effects of chronic exposure to these substances in human population. This may partly be due to the fact that acute intoxication by these substances is rare in man. Recent experience from Brazil where a number of dialysis patients died due to the presence of microcystins in the water used for dialysis is a serious warning. Chronic exposure to microcystins is less dramatic, but more widespread. Therefore the aim of our study was to test magnetic resonance imaging (MRI) as a noninvasive and harmless method for the early detection of changes in liver tissue after chronic exposure to microcystins. It should be emphasized that living organism are in most cases chronically exposed to toxic cyanobacteria and/or to their dissolved contents, which consist of a broad range of biologically active substances and not only microcystins.[1-4] Therefore the second aim of our study was to assess the possible synergistic interactions between these substances in chronic intoxication by using either purified microcystins or cyanobacterial lyophilisate (CL).

Methods

Male albino rats (Wistar) weighing 165-190 g were used in all experiments. The animals were kept at standard room condition (room temperature 24 °C, daily-night interval at 12 hours, exposed to 60 lux artificial light). The number of animals was kept at minimum. The experiments were performed with the guidelines of IST. Either cyanobacterial lyophilisate or purified microcystins were injected i.p. in the intervals of 3 days for 2 months, the total cumulative dose of microcystins (either in the lyophilisate or purified substance) was 2 LD_{50}.

Magnetic resonance imaging of the rat's liver was performed on a Bruker Biospec system with a 2.35 T horizontal bore magnet on animals anesthetized with i.p. injection of Xylazin (15 mg/kg), Ketanest (100 mg/kg) and atropine (0.3 mg/kg). In this study, standard T1 weighted spin-echo magnetic resonance imaging method with echo time 18 ms and repetition rate 400 ms was used. Signal averaging of 10 signal acquisitions was used to improve the signal to noise ratio. In order to extract the liver volume, rats were imaged in seven consecutive slices in two experiments, first in transverse and then in coronal slice orientation. Imaging filed of view and slice thickness was at each experiment adjusted so that imaging volume covered the whole liver region. Thus, field of view was in the range 8-10 cm and slice thickens 3-4 mm. T1 weig-

hted MR images had good contrast between liver and surrounding tissues that allowed precise liver region selection and its area calculation for each slice. Liver volume was later calculated as a sum of liver areas in all slices multiplied by the slice thickness.

Results and discussion

In acute experiments MRI revealed enlargement of liver in both groups of experimental animals, and there was no difference whether they received only purified microcystins or the same amount of microcystins present in the CL. Significant differences have been observed between the group that received CL or purified microcystins only in the chronic experiments. Liver from the animals injected with the microcystin- LR showed only minor changes on the signal intensity on MRI images, and patho -morphological examination of abdominal organs on sacrificed animals showed degenerative changes in one animal only. The MR images of the liver from all animals injected with CL showed irregular changes in the signal intensity and nodular formations have been observed. Patho -morphological examination of sacrificed animals showed granular structure of liver edges, and enlargement of kidneys. However, degenerative changes characterised as periportal fibrosis and fatty infiltrations were observed in both groups of chronically treated animals. Changes were more pronounced in the case of the animals treated with CL. No tumours were detected in any of the treated animals, which is in agreement with the data from other authors[5] that showed that longer exposures and higher dosages are necessary to produce carcinogenic effects.

Extracts from toxic cyanobacteria induce DNA damage in vitro as well as in vivo.[7] This implies that microcystins can no longer be treated merely as tumour promoters. The enlargement of kidney shown in our experiments suggests that exposure to toxic cyanobacteria also affects other organs, which has already been proposed.[8] Microcystins could be responsible for these effects, but the combined action of the constituents of cyanobacteria is evidently important as kidney enlargement has been observed only in the group of animals treated with CL. The data show that not only microcystins but also other components of the cyanobacterial bloom can affect the health of population.[6] Therefore the health risk of hepatotoxic cyanobacteria estimated from the microcystin content is underestimated.

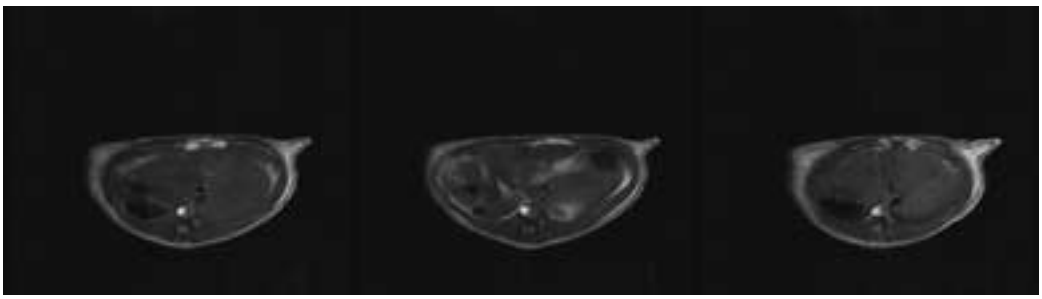

Figure 1. MR images of the liver exposed to cyanobacterial lyophilisate or to purified microcystins alone. A. Control liver is of a dark gray and homogeneous colour. B: Chronic exposure to cyanobacterial lyophilisate (CL) results in liver degeneration shown as non-homogeneous signal from the liver seen as white patches. C) Chronic exposure to microcystins gives rise to only slight changes in the liver structure as seen by MRI.

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