Magnesium status and deficiency in cirrhosis of liver due to alcoholism

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Zusammenfassung

Bei 35 Gesunden, 24 Alkoholikern mit einer Lebersteatose und 19 Alkoholikern mit einer Leberzirrhose wurden Plasma, intrazelluläre und membranöse Mg-Konzentrationen bestimmt. Die intrazellulären und membranösen Mg-Konzentrationen wurden in Erythrozyten gemessen. Die Mg-Bestimmungen erfolgten mittels Atomabsorptionsspektroskopie (Video 12, Thermo Electron, Andover, USA).

Die Plasmamagnesiumkonzentrationen in der Kontrollgruppe und den Patienten mit einer Steatose waren nicht statistisch signifikant unterschiedlich (0,98 ± 0,10 mmol/l versus 0,97 ± 0,15 mmol/l).

Die Plasma-Mg-Spiegel wurden in der Gruppe der Leberzirrhotiker mit 0,85 ± 0,12 mmol/l signifikant erniedrigt gemessen im Vergleich zu den anderen beiden Gruppen ($p < 0,05$); jedoch lagen fast alle Plasma-Mg-Spiegel im Normbereich.

Die intrazellulären Mg-Konzentrationen waren bei der Kontrollgruppe und der Steatosegruppe nicht signifikant unterschiedlich (1,88 ± 0,16 mmol/l versus 1,82 ± 0,22 mmol/l). Im Vergleich mit diesen beiden Gruppen fanden sich bei den Leberzirrhotikern signifikant erniedrigte intrazelluläre Mg-Spiegel (1,30 ± 0,25 mmol/l, $p < 0,001$).

Die membranöse Mg-Konzentration war in dieser Gruppe ebenfalls mit 0,16 ± 0,04 mmol/g Membranprotein signifikant erniedrigt (Kontrollgruppe: 0,53 ± 0,05 mmol/g Membranprotein; Steatosegruppe: 0,35 ± 0,04 mmol/g Membranprotein; $p < 0,001$, $< 0,01$).

Ein zellulärer und membranöser Mg-Mangel hängt bei Alkoholismus auch vom Grad der Schwere der Lebererkrankung ab. Zelluläre und membranöse Mg-Konzentrationsbestimmungen eignen sich zur Beurteilung eines Mg-Mangels bei Alkoholismus und Hepatopathien besser als Mg-Plasmaspiegel.

Summary

In 35 healthy subjects, 24 patients with a steatosis and 19 patients with a cirrhosis of the liver due to chronic alcoholism plasma and total intracellular and membrane magnesium concentrations were determined. Cellular and membrane magnesium measurements were performed in red blood cells.

Magnesium was measured by atomic absorption spectroscopy using aVideo 12 apparatus of Thermo Electron Instrumentation, Andover, USA.

In controls and patients with a steatosis there was no significant difference in plasma magnesium concentrations (0.98 ± 0.10 mmol/l versus 0.97 ± 0.15 mol/l). Plasma magnesium concentrations in patients with cirrhosis of liver were measured 0.85 ± 0.12 mmol/l which was significantly decreased as compared to healthy subjects ($p < 0.05$); nearly all plasma values were within the normal range for magnesium. Intracellular magnesium concentrations were not found of significant difference in controls versus patients with a steatosis due to alcoholism (1.88 ± 0.16 mmol/l versus 1.82 ± 0.22 mmol/l). In drinkers with cirrhosis of liver intracellular magnesium levels were significantly lowered as compared to healthy subjects or to the steatosis patients (1.30 ± 0.25 mmol/l, $p < 0.001$).

In addition, membrane Mg concentrations were found significantly decreased in patients with a steatose (0.35 ± 0.04 mmol/g membrane protein*) or a cirrhosis of liver (0.16 ± 0.04 mmol/g m.p.**) in comparison to healthy subjects (0.53 ± 0.05 mmol/g m.p.) (* = $p < 0.01$; ** = $p < 0.001$).

The data presented here show that a cellular and membrane magnesium deficiency may depend on the degree of the liver disease in chronic alcoholism.

Furthermore cellular and membrane magnesium content is a better parameter when determining alterations in magnesium status in alcohol induced diseases of the liver than plasma magnesium concentrations, which can still remain in the normal range even in severe forms of cirrhosis.

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Introduction

Severe chronic alcohol ingestion is known to cause hypomagnesemia [4, 6, 9, 10, 26]. Many attempts have been made to clarify the nature of the underlying defects. Typical reasons may be a magnesium deficient nutrition, malabsorption, increased intestinale or sweat excretion, hyperaldosteronism or disorder in catecholamine metabolism [2]. Alcohol ingestion can also cause the increased urinary excretion of magnesium and zinc in humans and animals [2, 5, 7, 17] and may lead to tissue depletion of magnesium [21, 23].

Furthermore it is known that moderation of alcohol intake reduces blood pressure and leads to an increase in lowered cellular magnesium stores [6, 18].

To test the hypothesis that depending on the degree of the liver insufficiency the most severe forms of hypomagnesemia can be observed, in the present study the magnesium status in controls and drinkers with a steatosis and cirrhosis of liver was investigated.

As plasma magnesium concentrations in alcoholism have often been described, but constitute only less than 3 % of total body magnesium content [25], intracellular and membrane magnesium concentrations were of interest.

Patients and methods

35 patients – non drinkers – served as controls (C) and 24 drinkers with a steatosis (S) and 19 drinkers with a cirrhosis of liver (CL) were studied; all patients were treated in the Marienhospital Clinic of Bottrop, Germany.

The diagnosis of liver disease was obtained from routine bioptic puncture.
The mean alcohol consumption was 15.4 ± 2.9 g/day in the steatosis patients and 22.5 ± 3.6 g/day in the patients with cirrhosis of liver ($p < 0.05$). The clinical data are shown in Tab. 1.

Tab. 1: Clinical data of 35 healthy subjects (C), 24 patients with a steatosis (S) and 19 patients with cirrhosis of liver (CL) due to chronic alcoholism (means ± SD).

	C	S	CL
BP (mmHg, systolic/ diastolic)	116.2 ± 9.9/ 83.0 ± 7.8	127.6 ± 10.4/ 91.0 ± 6.0	149.7 ± 6.8/ 94.7 ± 8.5*
Serum creatinine (mg %)	1.04± 0.11	1.01± 0.12	1.09± 0.10
Age (yr)	47.7 ± 13.4	45.9 ± 11.3	50.2 ± 10.1
Sex (m/f)	20/15	12/12	10/9
Renal discase	–	–	–
Hyperlipidemia	–	14*	12*
Smoker	–	5	3

* As compared with C, $p < 0.05$

Plasma and total and membrane erythrocytic magnesium concentrations were measured as previously described by atomic absorption spectroscopy [8, 9, 11].
For magnesium measurements a Video 12 apparatus of Thermo Electron Inc., Andover, USA was used.
10–20 ml of heparinized blood were drawn from each patient and thereafter centrifuged at 2,500 g. Then plasma for magnesium determinations was removed.
The erythrocytes were washed with an isotonoc lithium acetate solution adjusted to pH 7.4. Magnesium was measured at room temperature after the erythrocyte pellet had been frozen and rethawed in magnesium-free tubes. The magnesium concentrations were measured in the hemolysate and were not corrected for trapped extracellular volume.
The determination of membrane Mg content was performed in erythrocyte membranes.
Erythrocytes were washed 3 times by sedimenting in a swinging-bucket rotor (2300 G for 10 min) and resuspending the pellet in 5 volumes of phosphate-buffered saline (150 mmol/L sodium chloride and 5 mmol/L sodium phosphate, pH 8.0). The buffy coat was carefully aspirated each time from the surface of the pellet. Hemolysis was initiated by rapidly and thoroughly mixing 1 mL packed cells with approximately 40 mL of 5 mmol/L sodium phosphate at pH 8.0. The membrane ghosts were pelleted by centifugation at 22,000 g for 10 min in an angle head rotor. The supernatant was removed with a top aspirator. The tube was tipped and rotated on its axis so that the loose ghosts slid from a small hard button, rich in contaminating proteases, which could then be aspirated. After two more identical wash cycles the ghost membranes were creamy white and morphologically intact [3, 15].
The membrane pellet was then dissolved in 1 mL doubly distilled water. The magnesium content in this solution was measured by atomic absorption spectroscopy (Video 12). Thereafter measurements of the membrane protein content were performed using Coomassie Blue (Serva) according to Bradford's method [1].
Magnesium content was then referred to the amount of membrane proteins.
Calibration curves were established using solutions with known magnesium concentrations in the lower, upper, and intermediate range (Seronorm charge no. 176, Pathonorm H charge no. 21, Pathonorm L charge no. 20, Merck, Darmstadt, Germany). For each sample a mean value was calculated from 5 measurements.
The intraassay variation coefficient was 4.8 % in 10 consecutive measurements and the interassay variation coefficient was between 6 and 9 %.
Statistical analysis was performed using ANOVA. Data are mean ± SD, p values below 0.05 were considered significant. The reported p values are 2-tailed.

Results

The results are shown in Tab. 2.
In controls and patients with a steatosis there was no significant difference in plasma magnesium concentrations, but in patients with cirrhosis of liver due to chronic alcoholism plasma magnesium was significantly lowered as compared to the control group ($p < 0.05$). Nearly all plasma magnesium values were found in the normal range (0.8–1.2 mmol/l) (Fig. 1).
Intracellular magnesium concentrations were not found of significant difference in controls versus patients with

Tab. 2: Plasma, intracellular and membrane magnesium concentrations in 35 controls (C), 24 patients with a steatosis (S) and 19 patients with cirrhosis of liver (CL) due to alcoholism (means ± SD).

	plasma Mg (mmol/l)	intracellular Mg (mmol/l)	membrane Mg (mmol/g m. p.)
C	0.98 ± 0.10	1.88 ± 0.16	0.53 ± 0.05
S	0.97 ± 0.15	1.82 ± 0.22	0.35 ± 0.04**
CL	0.85 ± 0.12*	1.30 ± 0.25***	0.16 ± 0.04***

* As compared with healthy subjects, $p < 0.05$.
** As compared with healthy subjects, $p < 0.01$.
*** As compared with healthy subjects, $p < 0.001$.

steatosis due to alcoholism. In drinkers with cirrhosis of liver intracellular magnesium levels were significantly lowered as compared to controls or steatosis patients; all magnesium values in this group were found below the normal range for red blood cell magnesium content (1.6–2.2 mmol/l) ($p < 0.001$) (Fig. 2).

In addition, membrane Mg concentrations were found significantly decreased in the steatosis patients and in the patients with a cirrhosis of liver as compared to healthy subjects ($p < 0.01$; $p < 0.001$) (Fig. 3).

There was no correlation between plasma or intracellular or membrane Mg concentrations or blood pressure values in the studied groups.

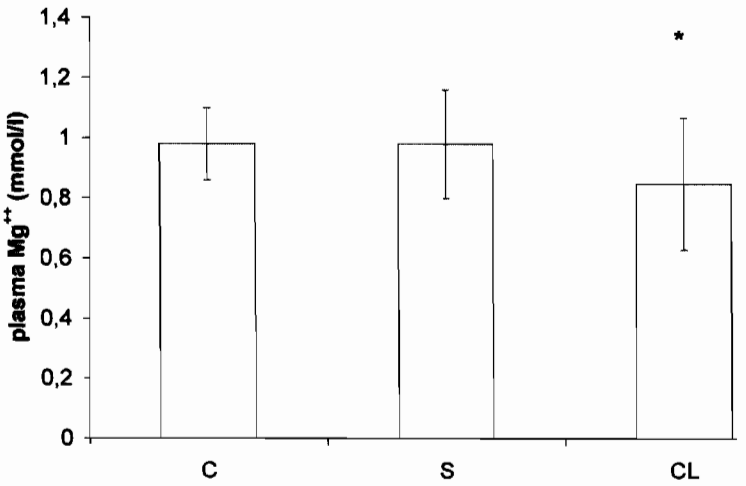

Fig. 1: Plasma Mg concentrations in 35 healthy subjects (C), 24 drinkers with a steatosis (S) and 19 drinkers with a cirrhosis of liver (CL) (means ± SD; * = $p < 0.05$).

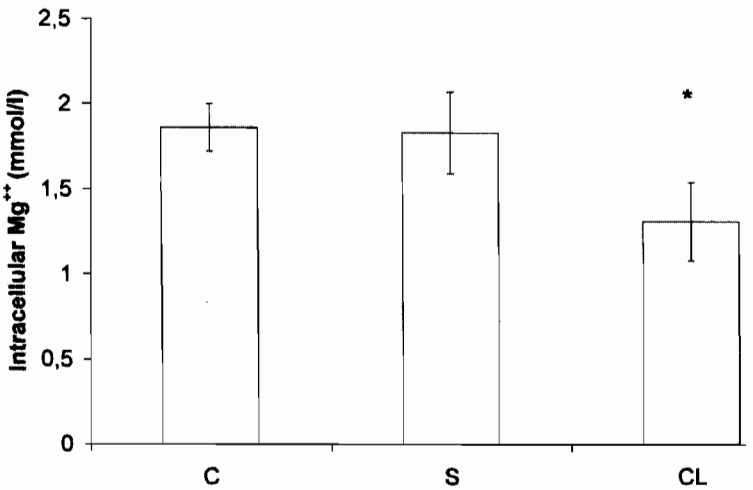

Fig. 2: Intracellular Mg concentrations in 35 controls (C), 24 drinkers with a steatosis (S) and 19 drinkers with a liver cirrhosis (CL) (means ± SD; * = $p < 0.001$).

Discussion

Severe chronic alcohol consumption is known to cause hypomagnesemia [4, 6, 26] and is often associated with arterial hypertension [16, 19, 20, 22, 24]. Many attempts have been made to clarify the nature of the underlying defects.Typical reasons may be a magnesium deficient nutrition, malabsorption, an increased intestinale or sweat excretion, hyperaldosteronism or disorder in catecholamine metabolism [2]. Alcohol ingestion can also cause the increased urinary excretion of magnesium and zinc in humans and animals [2, 5, 7, 17] and may lead to a tissue depletion of magnesium [21].

In a recent study *Martell* et al. investigated the alcohol intake in 1024 normotensives and essential hypertensives and its relation with plasma lipids [18]. They concluded in the population studied both the alcohol intake and the proportion of heavy drinkers were significantly higher in the hypertensive versus the normotensive group. Total cholesterol and triglycerides were also higher in the hypertensive versus the normotensive group.

It is known that the moderation of alcohol intake reduces blood pressure, although the exact mechanism has not been yet established. *Hsieh* et al. stu-

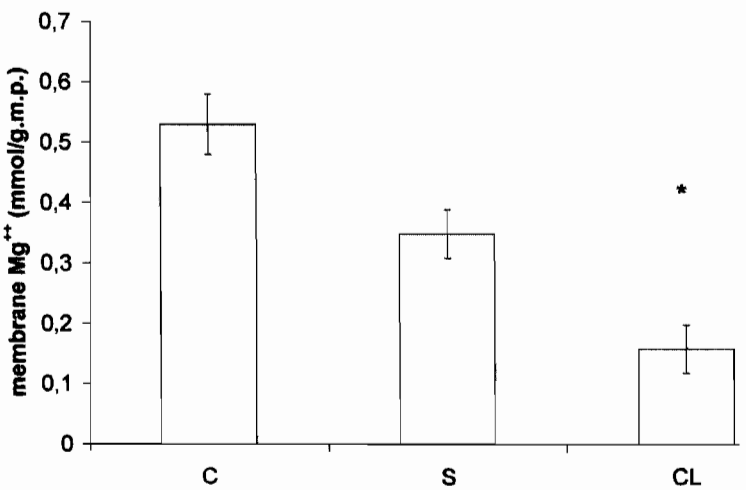

Fig. 3: Membrane Mg concentrations in 35 healthy subjects (C), 24 drinkers with a steatosis (S) and 19 drinkers with a cirrhosis of liver (CL) (means ± SD; * = $p < 0.001$).

died the effects of alcohol moderation on blood pressure and intracellular cations in mild essential hypertension and documented a fall in mean blood pressure correlated positively with the reduction in weekly alcohol consumption [6]. They described also an increase in intraerythrocyte Mg concentration after a reduction in alcohol consumption.

In the presented study here, we found plasma Mg concentrations in the normal range in steatosis and liver cirrhosis patients due to a chronic alcohol consumption. Intracellular and membrane Mg concentrations were found significantly decreased in the drinkers with a cirrhosis of liver as compared to controls, the normal range or patients with a steatosis ($p < 0.001$).

For assessing total body magnesium status intracellular or membrane magnesium levels are a better parameter than plasma magnesium levels [12–15, 25].

In conclusion the data presented here show that an intracellular or membrane magnesium deficiency depends on the degree of the liver disease in chronic alcoholism. Furthermore intracellular or membrane magnesium concentrations are better parameters when determining alterations in magnesium status in alcohol induced diseases of the liver than plasma magnesium concentrations, which can still remain in the normal range even in severe forms of liver cirrhosis.

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