**Direct Effect of CoCl₂ and NiCl₂ on Citrate Uptake by the Rat Renal Brush Border Membrane**

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Received August 27, 2004 and accepted March 14, 2005

Abstract: Co and Ni are essential but relatively rare elements as to organisms. In the mammalian membrane, these metals are transported by the same carrier proteins. The aim of this study was to investigate the direct effects of CoCl₂ and NiCl₂ on citrate uptake by rat renal brush border membrane vesicles (BBMV). BBMV were prepared by the divalent cation precipitation methods, and citrate uptake was measured by the Millipore rapid membrane filtration technique. The time course of citrate uptake during 120-min of incubation with 1 mM CoCl₂ and NiCl₂ showed a rapid significant inhibition at the early phase and a slight recover at the late phase. Incubation for 1 min of BBMV with 1, 5 and 25 mM CoCl₂ and NiCl₂, respectively, significantly inhibited citrate uptake in a concentration-dependent manner compared with that of 0 mM. We discuss these findings from the point of view that Co and Ni are located in Group VIII of the periodic table.

**Keywords:** Co, Ni, Fe, Citrate, Brush border membrane vesicles (BBMV)

Introduction

The production of hard metal tools started in the 1920s in Europe and Japan. The extremely hard product (90 to 95% of the hardness of diamond) is used for the cutting edges of tools, rock drills, broaches, and dies. Health problems associated with the inhalation of cemented tungsten carbide dust have been described as “hard-metal disease”, and have been reported throughout the world. Two types of respiratory disease have been observed among tungsten carbide workers: bronchial asthma, and disabling and progressive interstitial fibrosis.

Co and Ni are located in Group VIII of the periodic table, both are used as a matrix in hard metals depending on the desired properties of the final product. In bronchial challenge tests, patients with hard metal asthma have been found to be sensitive to Co. Occupational exposure to Ni also causes asthma by allergic processes among hard metal workers. In some hard metal asthma patients, specific IgE against Co-HSA (human serum albumin) or Ni-HSA has been reported.

Increased Co concentration in the urine is a specific indicator of occupational exposure to Co. A significant correlation between Co concentration in the ambient air and in the urine was reported among workers in four studies. β₂-microgloblin (β₂-MG) was also found to be significantly elevated among hard metal tool manufacture workers. Nevertheless, an Italian researcher concluded that the kidney is not a target organ for low-level occupational exposure to Co. Goodpasture’s syndrome in a worker with occupational exposure to hard metal dust was previously reported. This worker caught a life-threatening interstitial lung disease followed by rapidly progressive glomerulonephritis. The authors noted that Co might have played a pathogenetic role through various mechanisms of action. It might interfere with normal immunoregulation.

Renal citrate excretion is important with regard to the prevention of kidney stones, and with respect to acid-base
balance, since the metabolism of citrate generates three HCO₃⁻ ions¹⁶). Citrate is freely filtered in the glomerulus, and its reabsorption in the proximal tubule is the major determinant of the rate of renal excretion. Proximal citrate reabsorption is an active transport and mediated by an apical Na-citrate cotransporter, which carries three Na⁺ with one citrate²⁻, and is thus electrogenic¹⁷) in nature.

In this study, we exposed rat renal brush border membrane vesicles (BBMV) to CoCl₂ and NiCl₂, respectively, and comparatively investigated the direct effect of these exposure on citrate uptake by BBMV to elucidate the renal toxic mechanisms of Co and Ni.

Materials and Methods

Materials

¹⁴C-citrate (1.85 GBq/m mol) was obtained from the New England Nuclear Corp. (Boston, MA). All other chemicals were reagent grade and purchased from commercial sources.

Animals

Experiments were performed using the kidneys of male Wistar rats (CLEA, Japan, Inc) weighing 321–449 g. The animals were maintained in a temperature- and photoperiod-controlled animal house with ad libitum access to a standard diet and tap water.

Brush border membrane isolation

Each brush border membrane specimen was prepared from the cortex of both kidneys of a single rat. During preparation, all materials were maintained at 4°C. The brush border membrane specimens were isolated by differential centrifugation and magnesium precipitation method¹⁸). Briefly, each rat was anesthetized with 360 mg/kg of chloral hydrate administered intraperitoneally. The abdomen was opened and the renal cortices were removed and placed in isolation buffer consisting of 50 mM mannitol and 2 mM Tris-(hydroxymethyl)-aminomethane (Tris)/HCl at pH7.0. They were then homogenized in a glass Teflon homogenizer (Iuch Co. Ltd., Japan) and a polytron homogenizer. One molar MgCl₂ was added to the resultant homogenate to give a final concentration of 10 mM. The mixture was next stirred for 20 min in a cold room and then centrifuged for 15 min at 3,000 g. The supernatant was collected and centrifuged for 20 min at 43,000 g. The pellet containing the brush border membrane vesicles was resuspended in isolation buffer and centrifuged for 15 min at 3,000 g. The supernatant was centrifuged for 20 min at 43,000 g. The pellet containing the purified brush border membrane vesicles (BBMV) was resuspended in 260 mM mannitol and 20 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES)/Tris at pH7.0 (titrated by 1 M Tris to pH7.0) at a final concentration of about 10 mg protein/ml.

Marker enzyme assays

The purity of the membrane preparations was assessed by evaluating the enrichment of alkaline phosphatase (ALP)¹⁹) and leucine amionopeptidase (LAP)²⁰) in the final preparation as compared with that of the original homogenate.

Protein determination

Protein determination was performed by the method of Lowry et al.²¹) with crystalline bovine serum albumin as a standard.

Citrate uptake

Transport measurements were determined by the Millipore rapid membrane filtration technique²², ²³). For the experiments in which BBMV were incubated with CoCl₂ or NiCl₂, membrane vesicles (10 µl) were incubated at 30°C with 10 µl of incubation solution consisting of 260 mM mannitol, 20 mM HEPES/Tris, pH7.0, with CoCl₂ or NiCl₂. Citrate uptake was initiated by the addition of 40 µl uptake solution consisting of 100 µM ¹⁴C-citrate, 130 mM NaCl and 20 mM HEPES/Tris, pH7.0, at 30°C. After thirty seconds, the uptake was terminated by the addition of 4 ml of ice-cold solution consisting of 130 mM NaCl and 20 mM HEPES/Tris at pH7.0. The solution was then rapidly filtered through a Millipore filter (DAWP02500; pore size: 0.65 µm) and washed twice with 4 ml of ice-cold stop solution under vacuum suction. The background uptake was similarly determined by adding 4 ml of ice-cold solution consisting of 130 mM NaCl and 20 mM HEPES/Tris at pH7.0. The solution was then rapidly filtered through a Millipore filter (DAWP02500; pore size: 0.65 µm) and washed twice with 4 ml of ice-cold stop solution under vacuum suction. The background binding was always <0.5% of the total count for the uptake solution. Radioactivity associated with the filters was measured using a liquid scintillation counter (LSC-3500, Aloka, Japan). All uptake measurements were performed at 30°C in triplicate, and uptake was calculated on the basis of the specific activity measured in each experiments. The background binding value was subtracted from the experimental value, and the vesicular uptake was expressed as picomoles [¹⁴C]-citrate per mg protein.

The values were expressed as the means ± SEM. Groups were compared using one way analysis (ANOVA) followed by Bonferroni test. Statistical significance was accepted at P<0.05.
Results

Purity of the BBMV preparation

The purity of the BBMV preparations was assayed by examining BBM-specific marker enzymes. The specific activities of the enzymes, ALP and LAP, in the BBM fraction were enriched by $12.4 \pm 4.3$ and $10.3 \pm 6.8$ (mean ± S.D.) over those in the original homogenate.

Effect of Co concentration on citrate uptake by BBMV

The effect of incubation of BBMV with Co is illustrated in Fig. 1. Incubation with 1, 5 and 25 mM Co significantly inhibited the citrate uptake compared with that incubated with 0 mM. There is a significant difference between citrate uptake incubated with 0.2 mM and 1 mM, between 1 mM and 5 mM. These findings indicate that incubation with Co resulted in a concentration-dependent inhibition of citrate uptake.

Effect of Co incubation time on citrate uptake by BBMV

Figure 2 shows the time course of citrate uptake after incubation with 1 mM Co. The uptake at 1 sec, 1 min, 10 min, 60 min and 120 min are significantly inhibited compared with that at 0. Although the difference is not statistical significant, the uptake after 1 sec indicate a slight recover. These findings suggest that the time course of citrate uptake with 1 mM CoCl₂ shows a rapid inhibition at the early phase and a slight recover at the late phase.

Effect of Ni concentration on citrate uptake by BBMV

The effect of preincubation of BBMV with Ni is illustrated in Fig. 3. Preincubation with 1, 5 and 25 mM Ni significantly inhibited the citrate uptake compared with that incubated with 0 mM. There is a significant difference between citrate uptake incubated with 0.2 mM and 1 mM, between 5 mM and 25 mM. These findings indicate that incubation with Ni resulted in a concentration-dependent inhibition of citrate uptake.

Effect of Ni incubation time on citrate uptake by BBMV

Figure 4 shows the time course of citrate uptake after incubation with 1 mM NiCl₂ for 1 min. The values are the means ± SEM.
incubation with 1 mM Ni. The uptake at 1 s, 1 min, 10 min, 60 min and 120 min are significantly inhibited compared with that at 0. Although the difference are not statistical significant, the uptake after 1 s indicate a slight recover. These findings suggest that the time course of citrate uptake with 1 mM NiCl₂ shows a rapid inhibition at the early phase and a slight recover at the late phase.

Discussion

There are no other reports, to our knowledge, which describe the effect of Co on the transport system in the renal brush border membrane. Other metals, such as, Cd inhibit Na-D-glucose transporter (24) in the rat small intestine brush border membrane, the L-alanine (25) and L-glutamate (26) co-transporter system in the rat renal brush border membrane, and mercury also inhibits the Na⁺-Pi-cotransporter (27). In our previous studies, cadmium (28), inorganic mercury (29), platinum (30,31) and vanadium (25) were found to inhibit the citrate uptake in a concentration- and time-dependent manner. This inhibition might be due to the direct interaction of these metals with the Na-citrate cotransporter. In our present study, preincubation of BBMV with 1, 5 and 25 mM CoCl₂ and NiCl₂ for 1 min also significantly inhibited the citrate uptake compared with that of BBMV for 0 mM (Figs. 1, 3). The time course of citrate uptake during 120-min incubation with 1 mM CoCl₂ and NiCl₂ shows a rapid inhibition at the early phase and a slight recover at late phase (Figs. 2, 4). These findings also might have been due to a direct interaction of these metals with the Na-citrate cotransporter, and at higher concentrations, this binding might be irreversible.

High occupational exposure to Ni was found to induce renal tubular dysfunction in a chemical plant that produces several Ni compounds (33). The time-weighted average exposure to Ni in the plant greatly exceeded the TLV of 0.05 mg/m³, was 0.75 mg/m³ on average with a range of 0.04–2.86. Among Ni workers, significant elevations of lysozyme concentration, β₂-MG and NAG activity have been reported (33). Although the concentration of Co and Ni in this
study (0–25 mM) might be too high, these exposure could be accidentally occurred.

Co and Ni are located in Group VIII of the periodic table as with Fe. Although these metals are essential for mammals, the distribution of Co and Ni within organisms is minute amount compared with that of Fe. In the mammalian membrane, Co, Ni, and Fe are also transported by the same carrier.

Analysis of the time course of citrate uptake during 120-min preincubation of BBMV with 1 mM CoCl$_2$ or NiCl$_2$ shows a rapid significant inhibition at the early phase and a slight recover at late phase. We believe that an explanation for the resulting recover is as follows. Even at low concentrations, Co or Ni might be bound to the cotransporter. This binding may cause a decrease in citrate uptake, although it is functional and reversible. Evidence exists that transferrin binds to V$^3+$. Co and Ni are located in Group VIII of periodic table as with Fe. In a late phase, these metals might then be removed from the BBMV. As a result, citrate uptake indicates a slight recover.

To further clarify the mechanism responsible for renal disturbance induced by Co, Ni and Fe exposure, in vivo experiments using Co- Ni- and Fe-intoxicated rats are needed.

References

23) Jenkins AD, Dousa TP, Smith LH (1985) Transport of...


