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Investigation of Manganese and Iron Absorption by Rat Everted Gut Sac

A.A. Moshtaghie, A.A. Badii and T. Hassanzadeh
Department of Clinical Biochemistry, School of Pharmacy,
Isfahan University of Medical Sciences, Isfahan, Iran

Abstract: The major aim of this investigation was to examine the interactions of manganese and iron absorption by rat Everted Gut Sac (EGS). Iron absorption by rat EGS was completed within 60 min of incubation time, whereas manganese absorption was occurred within 30 min. Absorption of both metals by EGS was dose and time dependent processes. Manganese absorption was reduced by 45% when iron was added to reaction mixture. The absorption of iron was decreased by 15% when manganese was added to the medium. The effects of other factors including glucose and citric acid on intestinal absorption of iron and manganese were also investigated here. Both glucose and citric acid enhanced both metals absorption and the absorption seems to be an energy dependent process.

Key words: Manganese, iron, absorption, EGS

INTRODUCTION

Manganese (Mn) is an essential trace element and is necessary for catalytic processes and acts as a promoter of various enzymes including the brain-specific glutamine synthesis, superoxide dismutase and pyrurate carboxylase (Wedler and Denman, 1984; Keen *et al*., 2000).

Although (Mn) is in an essential trace elements for brain function, but manganese toxicity has been reported to cause brain disorder and also disturbs heme synthesis (Hundell, 1999; Erikson *et al*., 2004). However, there is currently no exact recommended dietary allowance for this element although an estimated safe and adequate daily dietary intake of 2-5 mg per day has been recommended (Hurley and Keen, 1987). Inhalded manganese is also well absorbed through respiratory system (Thompson *et al*., 2006; Rao *et al*., 2003). Manganese shares many similarities with iron from different point of views including structural, biochemical and physiological functions (Reid *et al*., 2006). Previous investigation showed that the amount of manganese absorbed in healthy young woman is strongly associated with iron status which gives greater insight into manganese absorption and retention in humans (Finely, 1999).

Right now there is little known about manganese absorption by human body and the exact mechanism by which manganese absorbed, by intestine cells is still matter of speculation. The major aim of this investigation was to study and compare manganese and iron absorption by rat everted gut sac and the effects of various factors on this process.

MATERIALS AND METHODS

All chemicals used in this project were reagent grade and purchased from Sigma Chemical Company UK. Deionized water was used throughout this investigation. Laboratory glassware was soaked overnight in 10% HNO_3 and washed three times with distilled water. Male wistar rats purchased from Tehran Pasteur Institute (Iran) and were kept in faculty animal house of Isfahan university medical sciences (Isfahan, Iran) at standard conditions and fed on basal diet and water until their weights reached between 200-250 g.

Animals were fasted 24 h prior to the experiments and they were killed by cervical dislocation. Small intestine was removed, cleaned from debris, washed, blotted dried and weighted. The intestine cut into small pieces and proximal duodenum, proximal jejunum and distal ileum were prepared (Moshtaghie *et al*., 1996).

The segments were everted. The everted gut sacs were filled up with Kerebs Ringer Phosphate (KRP) medium, pH 7.4 and suspended in kerebs ringer phosphate medium with or without iron and/or manganese. The incubation mixture was capped and gassed with O_2/CO_2 = 95.5 on water bath shaker at 30°C. At the time of intervals the reaction mixture was removed and the concentration of iron and/or manganese inside the sacs was determined. Iron determination was carried out using spectrophotometry and phenanthrolin reagent was used as chromogen (Brithenham, 1979).

Manganese Concentration was determined using flameless-atomic absorption Zemman (Model 23030).

Corresponding Author: A.A. Moshtaghie, Department of Clinical Biochemistry, School of Pharmacy,
Isfahan University of Medical Sciences, Isfahan, Iran

Preparation of iron and manganese citrate complex: Separate stock standard solutions of $FeCl_3$, $6H_2O$ (3.0 mM) and $MnCl_2$, $4H_2O$ (3.0 mM), were prepared in deionized water and mixed with equal vol of 60.0 mM citric acid. The solutions were adjusted to pH 7.4 with 1 M NaOH and Made up to final concentration of 1.5 mM iron and manganese.

RESULTS

Earlier reports have shown that iron absorption occurs through duodenum. In order to study the possible major site of manganese absorption various parts of small intestine were chosen: the proximal 8 cm of duodenum, the proximal 11 cm of jejunum and the distal 14 cm of ileum. Variations in the absorptive surface of three segments compensated by differences in their length. To follow this, EGS was prepared and incubated in kerebs ringer phosphate medium including 100 mg L^{-1} Mn, for 1 h. It shows that the absorption of Mn by duodenum is approximately 12-14% higher than jejunum and/or lleum respectively (Fig. 1).

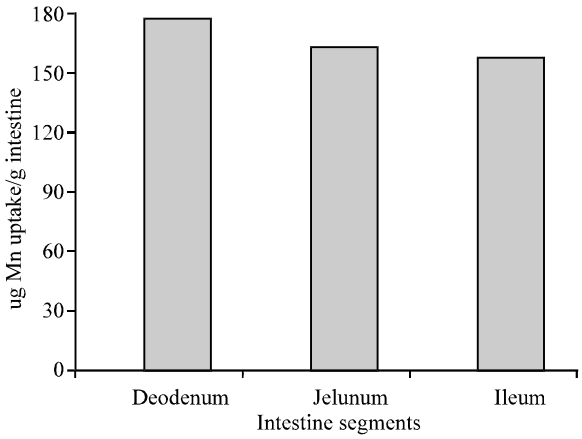

Fig. 1: Mn absorption by the small intestinal segments

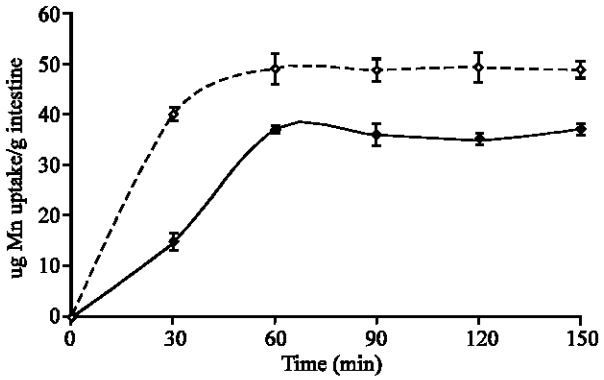

Fig. 2: The effect of incubation time on Fe (◇-) and Mn (-◆-) absorption by rat EGS

Next experiments were carried out to study and compare the effects of other factors which may necessary for iron and manganese uptake by EGS. The effects of incubation time on this process was studied first. To follow this, EGS was prepared and incubated into two series of volumetric flasks in kerebs ringer phosphate medium containing 100 mg L^{-1} Fe and/or Mn as complex with citric acid (1:20).

At intervals time EGS was removed from medium and washed with saline. The iron and manganese containing solution inside the sac were determined and the results show that maximum Fe and Mn uptake occurs within 30-60 min of incubation time, respectively (Fig. 2). Significant reduction by 30% in iron absorption was seen when Mn was added to the medium. The level of iron uptake was then decreased, suggesting that the mucosal cells gradually lose their abilities to take up iron (Fig. 2).

Fe II and Fe III uptake by EGS was studied and compared. It was found that iron as Fe II complex with citric acid 1:20 being absorbed more than Fe III (Fig. 3). The Fe II absorption was 25% Fe II higher than Fe III.

In order to determine whether iron and manganese uptake by EGS was dependent on the concentration of

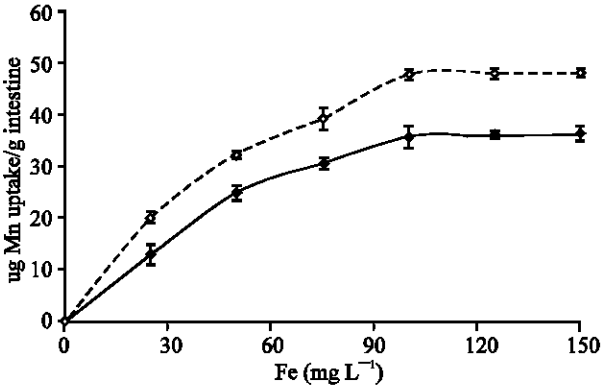

Fig. 3: Fe (II) (◇-) and Fe (III) (-◆-) absorption by rat EGS

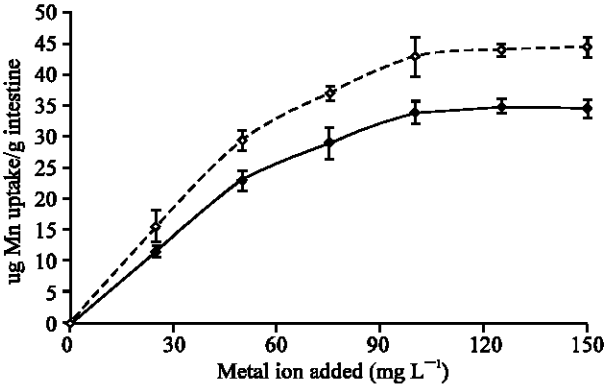

Fig. 4: Manganese Mn (-◆-) and iron Fe (◇-) uptake by rat EGS. Effect of concentration

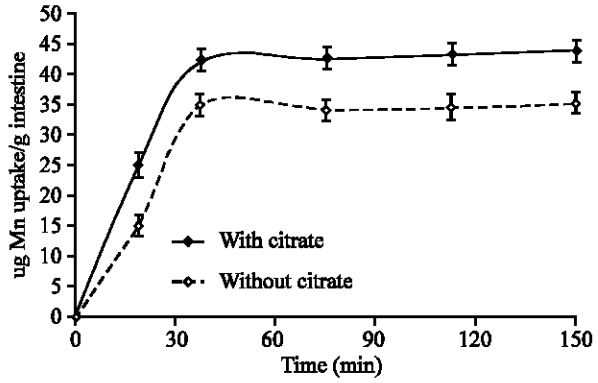

Fig. 5: Effect of citric acid on manganese uptake by rat EGS

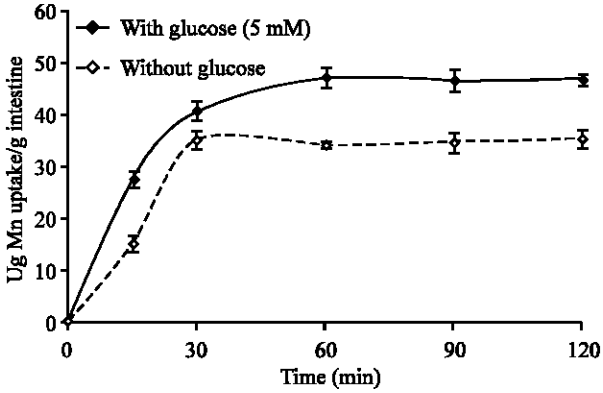

Fig. 6: Effect of glucose on manganese uptake by rat EGS

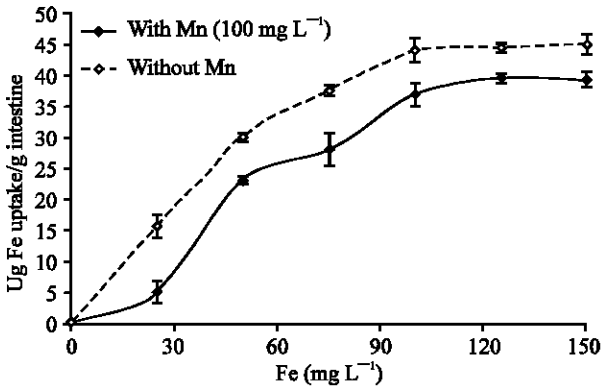

Fig. 7: Effect of on manganese on iron absorption by rat EGS

these elements, two series of volumetric flasks containing varying concentrations of metals were selected and next experiment was performed. Addition of varying concentration of Fe and/or Mn (0-200 mg L^{-1}) to the reaction mixtures showed that there was a gradual increases in iron and/or Mn uptake by everted gut sacs which is up to 100 mg L^{-1} (Fig. 4).

Citric acid has been previously reported to enhance iron absorption through small intestine. In this project when the effect of citric acid on Mn absorption was

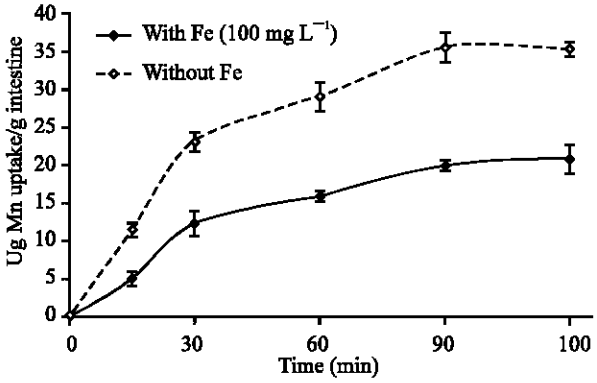

Fig. 8: Effect of iron on manganese absorption by rat EGS

investigated, there was a 25% elevation in Mn absorption (Fig. 5). Addition of 5 mM glucose to the reaction mixtures also enhanced Mn uptake by 20% (Fig. 6). Finally the intestinal interactions between Mn and Fe were studied (Fig. 7). It shows the effect of Mn (100 mg L^{-1}) on intestinal absorption of iron and Fig. 8 shows the effect of identical concentration of Fe (100 mg L^{-1}) on Mn uptake by everted gut sac. Addition of Mn reduced Fe absorption by approximately 48%, respectively.

DISCUSSION

A previous study by this laboratory found that manganese interferes with iron uptake by transferrin. Since transferrin also has been reported to play an important role in iron and cadmium uptake by intestine (Moshtaghie *et al*., 1996) it is obvious that there should be possibility of the interaction between Mn and Fe absorption.

Initial examination in this project showed that the uptake of manganese in duodenum section of intestine is much higher than ileum and jejunum Fig. 1. Secondly, it has been reported that high dietary iron depress manganese absorption (Finely, 1999) and addition of iron to an intestinal perfusate decreased manganese absorption (Thompson *et al*., 2006). These findings are in good agreement with the present data in this manuscript that uptake of either iron and/or manganese by rat everted gut sac is a time and dose dependent processes (Fig. 2 and 3) and addition of iron to the incubation medium lead to the reduction of manganese uptake by intestinal cells. Heilig *et al*. (2005) reported that intestinal manganese uptake is upregulated by iron deficiency and is thought to be mediated by Divalent Metal Transporter 1 (DMT1), an iron regulated factor known to play a role in dietary absorption. Interestingly, the reduction in manganese uptakes by gut in the presence of iron is much higher than the reduction of iron uptake in the presence of manganese. It seems that the existence of iron could

be a major regulator in manganese uptake by rat everted gut sac.

These findings consistent with the recent observations by Thompson *et al.* (2006) that individuals chronically exposed to manganese are at high risk for neurotoxin effects of this metal absorption from the lung to the blood and it be modified by iron status. High dietary iron levels inversely affect intestinal uptake of manganese.

On the other hand it has been reported that manganese inhibited iron absorption both in solutions and in a diet. Fractional iron absorption is strongly dose dependent. Suggesting a direct competitive inhabitation of manganese and iron absorption (Rossander-Hulton *et al.*, 1991). These observations are in agreement with our findings that the absorption of both metals by EGS is not only a time dependent process but also depends on the concentration of either Mn or Fe in the solutions. Also in confirmation to all these findings is the report of Thompson *et al.* (2006) that high dietary iron levels inversely affect intestinal uptake of Mn.

Some dietary factors including carbohydrate have been reported to influence manganese absorption (Lee and Johnson, 1988). Data of present investigation showed that addition of glucose have increasing effects on either iron and/or manganese absorption by intestine. It could be assumed that either glucose or carbohydrate containing glucose could be involved in the production of chemical energy (ATP). This might be needed for active transport process. Previous findings by Slon (1965) showed that some cations including potassium and calcium transportation across intestine membrane required energy which is provided by ATPase located in the membrane following releasing of energy from ATP molecules (White and Munro, 1988). It seems that manganese absorption may however follow this mechanism.

We think at this point more investigations should be done to elucidate deeply the exact mechanism by which manganese absorption performs by intestine.

REFERENCES

Brithenham, G., 1979. Spectrophotometirc plasma iron determination from finger puncture specimens. Clin. Chemica. Acta, 91: 203-211.

Erikson, K.M., A.W. Dobson, D.C. Dorman and M. Aschner, 2004. Manganese exposure and induced oxidative stress in rat brain. Sci. Total Environ., pp: 334-335: 409-416.

Finely, J.W., 1999. Manganese absorption and retention by young women is associated with serum ferritin concentration. Am. J. Clin. Nutr., 70: 37-43.

Heilig, E., R. Molina, T. Donaghey, J.D. Brain and M. Wessling-Resnick, 2005. Pharma c kinetics of pulmonary manganese absorption. Evidence for increased susceptibility of manganese loading in iron-deficlents rats. Am. J. Physiol Lung Cell Mol. Physiol., 288: 887-893.

Hundell, H.K., 1999. Effects from Environmental Mn exposure; A review of the evidence from non-occupational exposure studies. Nourol. Toxicol., 20: 379-397.

Hurley, L. and C. Keen, 1987. Manganese in Mertz W. Ed., Trace Elements in Human and Animial Nutrition. 5th Edn. San Diego, Academic Press, pp: 85-223.

Keen, C.L., J.L. Ensunsa and M.S. Celgg, 2000. Manganase Metabolism in Animals and Human Including the Toxicity of Manganese In: Sigel, H. (Ed.), Manganase and its Role in Biological Process. Marcel Dekker, ine., New York, pp: 89-121.

Lee, D.Y. and P.E. Johnson, 1988. Factors affecting absorption and excretion of 54 Mn in rats. J. Nutr., 118: 1509-1516.

Moshtaghie, A.A., M. Taghikhani and M. Sandughchin, 1996. Role of intestinal mucosal cell transferrin in cadmium interference with iron absorption by everted gut sac. Clin. Chem. Enzyme. Comms., 7: 177-185.

Rao, D.B., B.A. Wang, B.E. McManus, A.M. McElveen, A.R. James and D.C. Dsman, 2003. Inhaled iron unlike manganese is not transported to the rat brain via the alfactory pathway. Toxicol. Applied Pharmaol., 193: 116-126.

Reid, S.D., A.J. Blake, C. Wilson and J.B. Love, 2006. Syntheses and strutres of dinuclear double strand helicates of divalent manganese iron, cobalt and zinc. Inorg. Chem., 45: 636-643.

Rossander-Hulton, L., M. Brune, B. Sandstrom, B. Lonnerdal and L. Hallbery, 1991. Competitive inhibition of iron absorption by manganese and zinc in human Am. J. Clin. Nutr., 54: 152-156.

Slon, J.C., 1965. Enzymatic bassis for active transport of Na^+ and K^+ across cell membrane. Physiol. Rev., 45: 571-578.

Thompson, K., R. Molaina, T. Donaghey, J.D. Brain and M. Wessling-Resmck, 2006. The influence of high iron diet on rat lung manganese absorption. Toxicol. Applied Pharmacol., 210: 17-23.

Wedler, F.C. and R.B. Denman, 1984. Glutamine synthetase: The major Mn (II) enzyme in mammalian brain. Curr. Top. Cell. Reg., 24: 153-169.

White, K. and H.N. Munro, 1988. Induction of ferritin subunit synthesis by iron is regulated at both the transcriptional and translational levels. J. Biol. Chem., 263: 8938-8942.