ALUMINIUM INTERFERENCE WITH IRON ABSORPTION BY EVERTED GUT SAC

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SUMMARY : Iron absorption by everted gut sac (EGS) and the effect of aluminium in this process has been investigated. Incubation of freshly prepared rat EGS in Earle's medium pH=7.4 containing either Fe (II) and or Fe (III) showed that maximum absorption occurred within 30 min of incubation time. The absorption of Fe (II) or Fe (III) by EGS was dose dependent. Maximum absorption was carried out in the presence of 8 mM of iron followed by a gradual reduction thereafter. Aluminium (400 ug/1) reduced iron uptake by 24% . Apo-transferrin when saturated with aluminium (30-90%) and inserted inside the sac caused reduction in iron uptake. The higher the percentage of saturation the lower the iron uptake by EGS. Suggesting the probable role of transferrin in iron uptake. The mechanism of iron absorption by EGS and the effect of aluminium in this process has been discussed.

Key Words : Aluminium, iron, transferrin.

INTRODUCTION

Aluminium is the most abundant metal in earth's crust. According to the recent literature, this element enters blood circulation via either dialysis fluid and/or aluminium phosphate binders which are used to reduce serum phosphate concentration in chronic renal failure who are undergoing hemodialysis (1,2).

There are a number of stages by which iron metabolism being achieved. The first stage is the uptake of iron from Lumen by intestinal mucosal cells. In mucosal cells the exact mechanism by which iron transportation occurs is still a matter of speculation. Iron enters to the ferritin molecule or binds to a carrier protein (3). This carrier protein has recently been isolated and characterized by our laboratory (4). It is a β-glycoprotein and is responsible for the transportation of iron inside mucosal cells (5).

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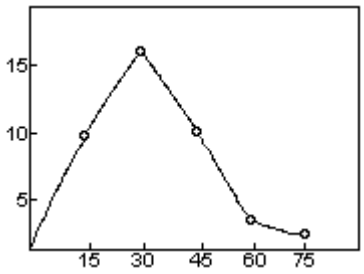
In blood iron binds to serum apo-transferrin and via transferrin receptors enters cells for heme synthesis (6,7) or other biochemical pathways (8).

Due to chemical similarities between aluminium and iron, aluminium has been reported to bind to serum transferrin (9).

The binding of aluminium to serum transferrin has been investigated using a number of biochemical techniques including spectrophotometric titration (10,11), affinity chromatography (12), gel filtration chromatography (13) and equilibrium dialysis (14). Aluminium transferrin complex binds to the same receptor at placental membrane (15) by which it inters the cells and interferes with heme synthesis (16,17). In chronic renal failure patients with aluminium overload a series of pathophysiological disorders appear such as dialysis osteomalacia (18), dialysis dementia (19) and hypo-chromic microcytic anemia (20).

The present project has been undertaken to study the

Figure 1: Effect of incubation time on iron absorption by E.G.S.



influence of aluminium on iron absorption in connection to the appearance of hypo-chromic microcytic anemia in patients with aluminium everload. Rat everted gut sac was used as a model study in this investigation.

MATERIALS AND METHODS

All chemicals used in this project were reagent grade and obtained from sigma chemical Company U.K. Deionized water was used throughout this study. Laboratory glassware was soaked overnight in 10% HNO_3 and washed three times with bi-distilled water with aluminium concentration less than 1 ug/l. Plastic ware was washed with 1 mM EDTA and rinsed with bi-distilled water three times. Aluminium concentration in bi-distilled water was determined using flameless-atomic absorption spectrophotometry (7). Male Wistar rats were kept in faculty animal house at standard conditions and fed on basal diet and water until their weights reached between 200-250 grams. Animals were fasted 24 h prior to the experiment and they were killed by cervical dislocation. Small intestine was removed, cleaned from debries, washed, blotted driy and weighted. The intestine cut into small pieces and proximal duodenum, jejunum segments were prepared.

The segments were everted. The everted gut sacs were filled up with Earl's medium pH=7.4 and suspended in Earle's medium with or without iron. The incubation mixture was capped and gassed with O_2 / CO_2 = 95/5 in water bath shaker at 37°C. At predetermined time intervals, the reaction mixtures were removed and the concentration of iron inside the sacs was determined. Iron determination was carried out using spectrophotometry and phenanthrolin reagent as chromogen (21). Protein concentration was determined by Lowry method (22).

Preparation of aluminium and/or iron citrate complex

Separate standard solutions of aluminium chloride were prepared in bi-distilled water and mixed with equal volumes of citric acid 1:20. The solutions was adjusted to pH=7.4 with 1 M NaOH and made up to final concentration. The solution was always prepared freshly on the day of the experiment.

The final concentration of Al, Fe (II) and Fe (III) in each solutions was determined by above mentioned method. Aluminium and/or iron transferrin complex were prepared as reported elsewhere (14).

RESULTS

Establishment of optimum conditions for iron absorption by EGS

Preliminary experiments were carried out to establish optimum conditions necessary for iron uptake study by EGS. The effect of incubation time on this process was investigated first. To follow this EGS was prepared and incubated in a series of volumetric flasks in Earle's medium containing 2 mM/l iron as Fe (III) in complex with citric acid 1:20. At 15 min intervals time EGS was removed from medium and washed with saline. The iron containing solution inside the sac was determined and the results are presented in Figure 1. It shows that maximum Fe (III) uptake occurs within 30 min of incubation time (Figure 1). The level of iron uptake was then off, suggesting that the mucosal cells gradually lose their ability to take up iron. Fe (II) and Fe (III) uptake by EGS was studied and compared.

Figure 2: Comparative study of Fe (II) (o–o), and Fe (III) (•–•) absorption by E.G.S.

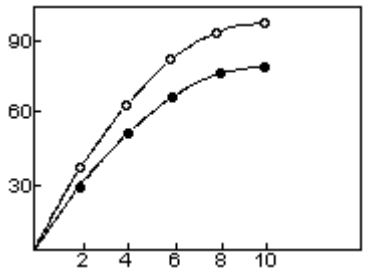
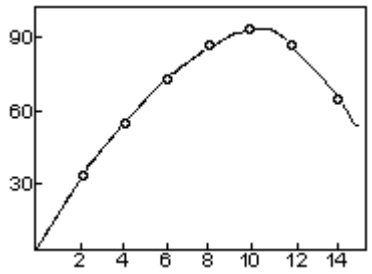


Figure 3: Fe (II) absorption by E.G.S.



It was found that iron as Fe (II) complex with citric acid 1:20 being absorbed much more efficiently than Fe (III) (Figure 2). The Fe (II) absorption was 20 percent higher than Fe (III). In order to find out whether iron uptake by EGS was dependent on the concentration of Fe (II), to a series of volumetric flasks varying concentrations of iron (0-12 mMol/l) as Fe (II) in complex with citric acid was added. The reaction mixtures were incubated for 30 min at the same conditions mentioned in methods. At the end of incubation time, EGS from each volumetric flask was removed and iron containing inside the sacs was measured. The data presented in Figure 3 showing that there was a gradual increase in iron uptake by EGS up to 8 mM/l and the iron absorption then decreased.

Effect of aluminium on iron absorption by EGS

In order to investigate the effect of aluminium on Fe (II) uptake by EGS, to a series of volumetric flasks containing EGS and 8 mM/l Fe (II) as a complex with citric acid 400 ug/l of aluminium without and/ or with citric acid 1:20 was added. The reaction mixture were incubated for 30 min. At the end of incubation time the iron concentration inside the sacs were measured and compared with control in which no aluminium was added. It was found that addition of 400 ug/1 of aluminium to reaction mixture decreased iron uptake by approximately 20 percent (Figure 4).

Effect of level of saturation of transferrin with aluminium on iron uptake by EGS

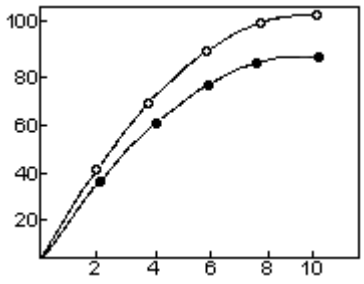
Since transferrin may play an important role in iron uptake from intestinal mucosal cells, the role of this b-glycoprotein on iron uptake was investigated. Apo-transferrin was saturated with aluminium at different degree of saturation. To a series of volumetric flask same amount of transferrin (2 mg/1) with different amount of aluminium was added. The concentration of iron in all reaction mixture was 8 mM/1. They were incubated for 30 minutes. At the end of incubation period, the EGS from each flask was removed and treated as mentioned earlier, the iron concentration inside the sacs was measured and the data are presented in Figure 5. It shows that the higher the level of saturation of transferrin with aluminium, the lower the amount of iron uptake.

DISCUSSION

The consumption of aluminium phosphate binders in chronic renal failure patients causes reduction in phosphate absorption from diet with subsequent reduction in serum phosphate level in these patients. On the other hand aluminium by itself may interfere with iron uptake by intestinal mucosal cell and disturb iron metabolism in these patients (23). As hypo-chromic microcytic anemia is one of the most prevalent disorder in renal failure with aluminium overload. The data which has been presented in this manuscript shows that iron absorption by EGS depends on many factors including form of iron and incubation time. The results presented in Figure 1 suggest that maximum absorption occurs within 30 min of incubation time which might be due to the death of mucosal cells which in turn might be correlated to the consumption of energy.

The transportation of many cations including sodium, potassium and calcium across the membranes require energy which is provided by ATPase system (24). The uptake of iron by EGS cell has also been reported to be energy dependent (13) and the reduction in iron absorption after 30 min of incubation time might be due to reduction of this system.

Figure 4: Effect of aluminium on iron absorption by E.G.S (o–o). No aluminium, (•–• with aluminium).



We have found that the iron uptake depends on the form of Fe, in which the degree of ferrous ion absorption is much higher than ferric (25). These findings are in agreement with our previous observations concerning aluminium transfer across mucosal cell (23). We had concluded that citric acid accelerates the absorption process (26). The data which has been presented in this paper shows that aluminium interferes with iron absorption and reduces iron uptake by mucosal cell (Figure 4). Review of the literature suggests that 10% of aluminium might be absorbed from the environment and may enter blood circulation. The absorption of aluminium from intestinal cell might be mediated by transferrin.

Using affinity chromatography technique and intestinal cell homogenate, we have been able to Purify and later identify this protein (4). The role of this protein in aluminium transport in blood circulation has already been reported (9). In this study we found that the level of saturation of transferrin with aluminium has also been influenced by iron uptake of the mucosal cells. It is obvious that when all iron binding sites of the protein being occupied by aluminium this may lead to a reduction in iron uptake, which it can confirm the important role of transferrin in iron uptake by intestinal mucosal cells. However more investigations needs to be carried out to elucidate the exact mechanism by which aluminium interferes with iron uptake by the mucosal cells.

REFERENCES

1. Berlyne GM, Pest D, Ben-Ari J, Weinberger J, Stern M, Gilmore GR and Levine R : Hyperaluminemia from aluminium resins in renal failure. Lancet, 11:494-496, 1970.

2. Alfrey AC : Aluminium Toxicity, Advances in clinical chemistry, 23:69-71, 1983.

3. Kristin W and Hamish NM : Induction of ferritin synthesis by iron is regulated at both the transcriptional and translational levels. J Biol Chem, 263:8938-8942, 1988.

4. Moshtaghie AA and Sabet-Jahromi M : Identification of transferrin in cytosol isolated from intestinal Mucosal cells. Biochem Society Transaction, 21:715, 1992.

5. MacGillivray RTA, Mendz E, Shewale JG, Sinha SK, Lineback SJ and Brew K : The Primary structure of human Serum transferrin. J Biol Chem, 258:3545-3553, 1983.

6. Karin M and Minty B : Receptor mediated endocytosis of transferrin in developmentally totipotent mouse tetra carcinomas stem cell. J Biol Chem, 256:3245, 1981.

7. Konopka K and Romslo I : Studies on the mechanism of Pyrophosphate mediated uptake of iron from transferrin by isolated rat liver mitochondria. Eur J Biochem, 111:239-244, 1981.

8. Pock OP : Role of iron in the synthesis of collagen in connective tissue. Fed Proc, 30:984-990, 1971.

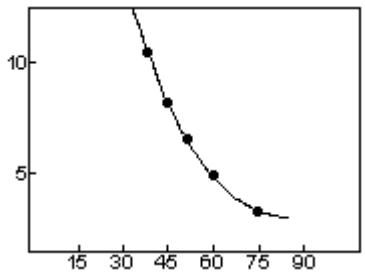
9. Moshtaghie AA and Skilen AW : Binding of Aluminium to transferrin and lactoferrin. Biochem Society Transactions 19(S):916-917, 1986.

10. Moshtaghie AA, Ani M and Bazrafshan : Comparative binding study of aluminium and chromium to human transferrin. Effect of iron. Biological Trace Element Research, 32:39-46, 1992.

11. Moshtaghie AA and Skillen AW : Comparative bindings studies of aluminium and iron to Serum transferrin. J Islam Acad Sci, 3:280-283, 1990.

12. Moshtaghie AA and Skillen AW : Desferrioxamine treatment in aluminium overload patients : An aluminium binding study.

Figure 5: Level of transferrin saturation with aluminium on iron absorption by E.G.S.



Indian Journal of Pharmacology, 23:75-80, 1991.

13. Leung FY, Hoodsman AB, Muirhead N and Henderson R : Ultra-filtration studies In vitro of serum aluminium in dialysis patients after DFO chelation therapy. Clin Chem, 31:20-23, 1985.

14. Skillen AW and Moshtaghie AA : The binding of aluminium by human transferrin and the effect of desferrioxamine. An equilibrium dialysis study in : Aluminium and other trace elements in renal disease (AW Taylor) 1st. Edition Bailliere Tindall, London, pp 81-85, 1986.

15. Skillen AW and Moshtaghie AA : The effect of aluminium on the interaction between transferrin and it's receptor on human placental Plasma membrane. In "Aluminium and other trace elements in Medicine, Ed by AW Taylor, Bailliere Tindals, London, pp 86-90, 1986.

16. Moshtaghie AA and Skillen AW : Study of the relationship between aluminium toxicity and heme synthesis. Journal of Medical Sciences, 15:46-52, 1990.

17. Mosthaghie AA and Skillen AW : Aluminium uptake by rat isolated hepatocytes and it's effect on Mitochondria oxygen uptake. J Isl Acad Sci, 3:11-14, 1990.

18. Ellis HA and Peart RM : Azotaemic renal osteodystrophy. A quantitative study on iliac bone. J Clin Pathol, 26:83-101, 1973.

19. Arieff AI : Aluminium and pathogenesis of dialysis encephalopathy. Am J Kidney Dis, 6(S):317-321, 1985.

20. Kaiser L and Schwartz KA : Aluminium induced anemia. Am J Kidney Dis, 6:384-352, 1985.

21. Brithenham G : Spectrophotometric Plasma iron determination from finger puncture specimens. Clin Chemica Acta, 91:203-211, 1979.

22. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ : Protein Measurnet with foline reagent. J Biol Chem, 193:265-275, 1951.

23. Moshtaghie AA, Ani M and Taher M : Aluminium interference with iron uptake by rat everted gut sackes. Clinical Chemistry, 36:970 (Abs), 1990.

24. Slon JC : Enzymatic basis for active transport of Na+ and K+ across cell membrane. Physiol Rev, 45:571, 1965.

25. Underwood EJ : Iron in "Trace elements in human and animal Nutrition, Academic Press, New York, 13:16, 1987.

26. Slanina P, Fech W, Loof LG, Slorach S and Cederger A : Dietary citric acid enhances absorption of aluminium in antacids. Clin Chem, 32:539-541, 1986.

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