Is Lead Uptake Through the Immature Intestine Related to the Number of Free Pb^{2+} Cations in Acidic Solution?

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INTRODUCTION

Children absorb a greater proportion of consumed lead than adults do and this difference may be based on physiological development. The mechanism for this variation has not been adequately described, but it is possible an enhanced uptake of metal via poorly developed intercellular junctions in the immature intestinal tract and changes in the density of transporters in the enterocyte may play a significant role (Pacha 2000).
In solution Pb forms complexes with organic and inorganic ligands. The inorganic forms of Pb found in soil (Pb II oxide, Pb II sulfide, Pb II chloride), all have the same biological toxic endpoint but with very different bioavailability (Ballatori 2002).
Lead species are transported into the cell through DMT1 or an H^+ active process in the free Pb^{2+} metal state (Ballatori 2002). Geochem computational calculations indicate that the addition of NTA to 10µM $Pb(NO_3)_2$ in NO_3^- transport medium (pH 5.5), will complex Pb reduce the percentage of free Pb^{2+} cations in solution. The hypothesis is that Pb is transported through the cell monolayer exclusively in the free Pb^{2+} state. The complexing of Pb^{2+} with NTA will further reduce bioavailability.

METHODS

A confluent cell culture monolayer was used as a model for the intestinal absorption of lead. The CACO-2 cell line displays a polarised morphology when grown on supporting matrices and expresses a range of functional transport processes similar to those used in the intact human intestine.

Cell Culture

Cells were cultured at 37 ^{0}C in DMEM supplemented with: Glucose 4500mg/L, 2mM L-glutamine, 10% FBS, 1% Pen/strep, 1% NEAA and 5% CO_2/95% air atmosphere until reaching 70% confluence. Cells were then harvested using 0.25% trypsin / 0.02% EDTA solution. Harvested cells were seeded onto 0.9cm^2 Falcon collagen coated inserts and fed every second day until day 21.

Transepithelial transport study

The transepithelial transport experiments were performed on day 21 at 37 °C, in freshly prepared 10 µM $Pb(NO_3)_2$ complexed with NTA at concentrations from 1 µM to 7.4 µM. The Pb/NTA complex was delivered onto the apical compartment of the monolayer at pH 5.5. The basolateral

compartment contained modified krebs medium at pH 7.4. The procedure was terminated after 60 minutes by washing the monolayer with ice cold phosphate buffer solution. Cell lysate, apical and basolateral washings were analysed for Pb content using OES and MS Gas Chromatography.

RESULTS AND DISCUSSION

The results of this study found (fig 1)

1. A decreasing trend in lead uptake retrieved from the cell lysate with increasing Pb/NTA complexes.
2. The transport of Pb into the basolateral compartment with increasing Pb/NTA complexes was not significantly different.
3. A decrease in the percentage of free Pb^{2+} cations in solution with an increase in Pb/NTA complexes was observed.
4. The presence of one or more complex Pb species appears to inhibit or impede Pb uptake into the cell and slightly impede transport into the basolateral compartment

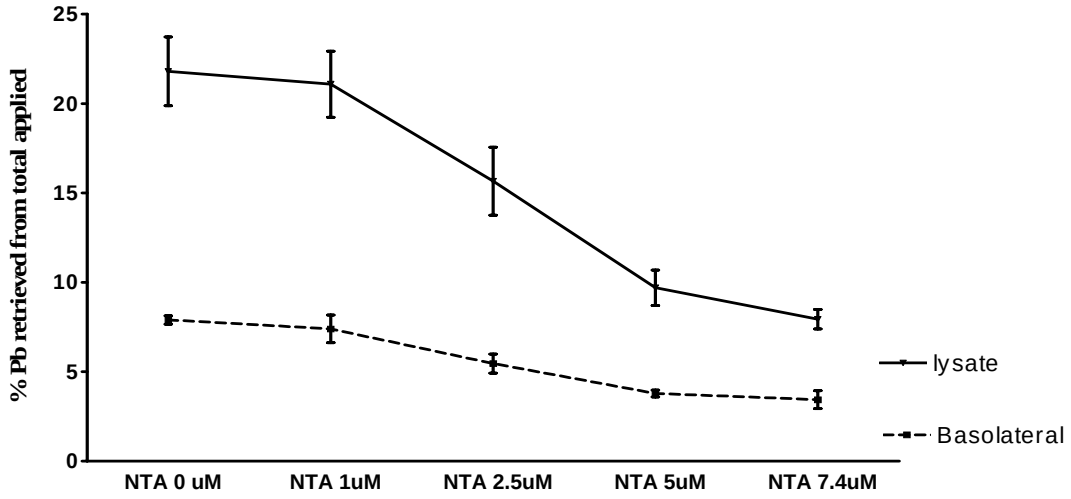

Fig. 1. Percentage of Pb retrieved from 10 µM $Pb(N0_3)_2$ when complexed with NTA

CONCLUSION

A decrease in the specific active uptake of free Pb^{2+} cations through the CACO-2 cell monolayer was assumed in this study but not proven.. Lead may continue to enter the cell not only as free Pb^{2+} cations but also as a complex form slowing down the rate of uptake. In regards to bio availability of Pb species further studies are required to confirm exclusivity for free Pb^{2+} cation transport.

REFERENCES

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