1987

Influence of Picolinic Acid on the Uptake of Zinc-Amino Acid Complexes by the Everted Rat Gut

D. A. Hill
Roman L. Hruska USMARC

E. R. Peo, Jr.
University of Nebraska-Lincoln

A. J. Lewis
University of Nebraska-Lincoln, alewis2@unl.edu

Published in JOURNAL OF ANIMAL SCIENCE 65 (1987), pp. 173-178.

INFLUENCE OF PICOLINIC ACID ON THE UPTAKE OF ^{65}ZINC-AMINO ACID COMPLEXES BY THE EVERTED RAT GUT[1,2,3]

D. A. Hill[4], E. R. Peo, Jr.[5] and A. J. Lewis

University of Nebraska[6]
Lincoln 68583-0908

ABSTRACT

Three hundred fifty rats were used in three experiments to: 1) validate the everted gut procedure as an in vitro technique for estimating Zn absorption, 2) determine the effect of increasing ratios of picolinic acid (PA) to Zn on Zn absorption and 3) determine the effect of PA on absorption of Zn and amino acid complexes at pH 6, 7 and 8. In the first experiment the time delay between tissue collection with subsequent storage in ice-cold saline and start of tissue incubation was 0, 10, 20 or 30 min. A linear decrease was observed for ^{65}Zn uptake with increasing delay time. Lysine absorption was not affected by delay time. In the second experiment, molar ratios of PA:Zn of 0, .5, 1.0, 1.5, 2.0 and 2.5 with Zn held constant were evaluated. A linear decrease in ^{65}Zn absorption from $^{65}ZnCl_2$ occurred as the molar ratio of PA to Zn increased. In the third experiment, 0 and 5 molar ratios of PA to a constant Zn level were evaluated using $^{65}ZnCl_2$, ^{65}Zn-^{14}C-methionine (ZnMet) and ^{65}Zn-^{3}H-lysine (ZnLys) at pH 6, 7 and 8. The addition of PA decreased Zn absorption regardless of Zn source. The data suggest that the Zn sources used were of similar biological value. The data do not support the theory that PA facilitates Zn absorption.
(Key Words: Rats, Zinc, Methionine, Lysine, Picolinic Acid.)

Introduction

Acrodermatitis enteropathica (AE), a form of Zn deficiency (Moynahan, 1974) and malabsorption in humans, has been treated with diiodohydroxyquin (a chelating agent) since 1953 (Dillaha et al., 1953). Acrodermatitis enteropathica is observed when infants who have been receiving human breast milk are weaned to cow milk or soy-based formulas. This suggests that human milk contains factor(s) that enhance Zn absorption and(or) that cow milk and soy formulas contain factors that depress Zn absorption.

Hahn and Evans (1973) detected a low molecular weight Zn-binding ligand (ZnBl) in rat intestinal mucosal cells and in pancreatric secretions in the dog (Evans et al., 1975). This low molecular weight ZnBL was isolated (Evans and Johnson, 1979) and identified (Evans and Johnson, 1980) as picolinic acid (PA), a metabolite of tryptophan. The authors suggested that the PA in human milk and pancreatic secretions facilitates Zn absorption from the intestine. If PA chelates Zn and facilitates its absorption, then it may do so by: 1) maintaining dietary Zn in a soluble form to allow maximum opportunity for contact with intestinal mucosa, or 2) by being absorbed into the mucosal cell as the Zn-ZnBL complex. If the Zn-PA complex does, in fact, facilitate Zn absorption then presentation of this complex to intestinal tissue would be expected to result in greater and(or) more rapid tissue uptake of Zn. The following experiments were conducted to test this hypothesis.

Materials and Methods

Three hundred fifty male Sprague-Dawley rats were fed a commercial rat diet[7] containing 75.8 ppm Zn (by analysis) for at least 7 d, fasted overnight and then killed by stun-

[1] Published as paper no. 8212, Journal Series, Nebraska Agr. Res. Div.

[2] Radio-labeled zinc-amino acid complexes donated by ZINPRO Corp., Chaska, MN 55318.

[3] The technical assistance of R. M. Hill, Dept. of Agr. Biochem. and Joy Kovar, Dept. of Anim. Sci. is gratefully acknowledged; appreciation is expressed to Diana J. Smith for manuscript preparation.

[4] Current address: Roman L. Hruska USMARC, Clay Center, NE 68933.

[5] To whom reprint requests should be addressed.

[6] Dept. of Anim. Sci.

[7] Wayne LAB-BLOX, Div. of Allied Mills, Chicago, IL 60606.

Received December 22, 1986.
Accepted March 11, 1987.

TABLE 1. EXPERIMENTAL SOLUTIONS

Item	Concentration
Isotonic saline solution[a]	
NaCl	8.5322 g/liter
KCl	.2982 g/liter
to 1 litter with distilled deionized water	
Saline – buffer solution[abc]	
NaCl	8.1816 g/liter
TRIS	.9692 g/liter
$CaCl_2:2H_2O$	.0147 g/liter
$MgCl_2:6H_2O$	.2170 g/liter
D(+) mannose	7.2062 g/liter

[a]To 1 liter with distilled deionized water.

[b]pH adjusted with 1 N HCl.

[c]Pre-oxygenated for 60 min before use by constant bubbling of O_2 into solution.

ning and exsanguination. A duodenal sac was prepared for the everted gut by previously defined procedures (Manis and Schachter, 1962). Everted gut sections were held in an ice-cold isotonic saline solution (table 1) until just prior to the start of incubation. One-half milliliter of pre-oxygenated isotonic saline-buffer solution (table 1) was put into the gut sac and the sac was tied. The gut sac was placed into a test tube containing 10 ml of the same saline-buffer solution used inside the gut sac. ^{65}Zn was then added to the buffer in the test tubes. The incubation period was 60 min at 37 C in a shaking water bath (90 oscillations/min). Picolinic acid, when used, was added to the outer buffer containing the ^{65}Zn. After 60 min of incubation, the gut sacs were removed from the isotopic-labeled buffer solution, carefully opened, and the inside of the sac (serosal side) was gently rinsed with 3 ml distilled water. The concentration of isotope was then determined in contents inside the sac with the rinse, the gut tissue itself, and the labeled buffer solution. Activity of ^{65}Zn was determined using a deep-well gamma counter. The tissue was then solubilized for analysis of ^{14}C-methionine or ^{3}H-lysine (TS-1 tissue solubilizer)[8] in liquid scintillation cocktail (Biocount scintillation cocktail)[8]. Nutrient absorption was considered to be the activity (cpm for ^{65}Zn and dpm for ^{14}C and ^{3}H) inside of the gut sac plus the activity in the gut wall itself divided by the sum of the activity found in all three fractions.

Zinc analysis of the diet sample was accomplished by dry-ashing duplicate 5-g samples at 550 C for 8 h. The ash was then extracted twice with 10 ml hot 6 N HCl and twice with hot distilled deionized water. The extract was filtered and analyzed by atomic absorption spectrophotometry[9] as described by Perkin-Elmer (1971).

In the first experiment the tissue was stored in ice-cold isotonic saline solution for 0, 10, 20 or 30 min to evaluate the effect of delay time between tissue collection and start of tissue incubation on Zn absorption. Forty-eight rats weighing 266 ± 22 g were used in the experiment. A ^{65}Zn-^{3}H-lysine complex (.8 μM Zn and .8 μM lysine/intestinal segment) was used at pH 7.4 to evaluate Zn and lysine absorption uptake from everted gut loops at normal body pH.

In the second experiment the effect of molar ratios of PA to Zn (.8 μM) from $^{65}ZnCl_2$[10] of 0, .5, 1.0, 1.5, 2.0 or 2.5 on Zn absorption at pH 8, a pH level similar to pancreatic secretions was determined. Seventy-two rats with an average weight of 198 ± 34 g were used.

In the third experiment a 3 × 2 × 3 factorial arrangement of treatments was used with three Zn sources, $^{65}ZnCl_2$, ^{65}Zn-^{14}C-methionine (ZnMet) and ^{65}Zn-^{3}H-lysine (ZnLys), 0 or 5 molar ratio of PA to .8 μM Zn, and pH levels of 6, 7 or 8. The pH levels were considered to be similar to those found

[8]Research Products International, Mt. Prospect, IL 60056.

[9]Perkin-Elmer Model 303, Norwalk, CT 06856.

[10]New England Nuclear, Boston, MA 02118.

TABLE 2. EFFECT OF DELAY TIME BETWEEN TISSUE COLLECTION AND START OF INCUBATION ON ^{65}Zn ABSORPTION (PERCENT OF ZN DOSE FROM ^{65}Zn-^{3}H-LYSINE COMPLEX)

	Absorption			
Delay, min	Serosal[a]	Gut[b]	Total[c]	N[d]
0	5.34	24.38	29.72	10
10	5.27	21.05	26.32	13
20	5.50	16.07	21.57	13
30	4.90	20.19	25.09	12
SE	.48	1.22	1.33	

[a]No differences (P>.58).

[b]Linear and quadratic effects (P<.01).

[c]Values are the sum of the serosal and gut analysis; Linear effect (P<.01), quadratic effect (P<.02).

[d]N = number of observations per treatment.

in the small intestine and pancreatic secretions. Absorption of ^{65}Zn and amino acids was determined as described previously. Average weight of the 230 rats used in this experiment was 271 ± 101 g.

The response criteria were analyzed by orthogonal contrasts as described by Steel and Torrie (1980) and calculated after SAS (1979). Specific statistical components tested were: Exp. 1, the regression of time; Exp. 2, the regression of the effect of added PA on Zn absorption; and Exp. 3, the regression components of levels of pH, the main effect of PA, inorganic vs organic Zn, ZnMet vs ZnLys and all possible interactions.

TABLE 3. EFFECT OF DELAY TIME BETWEEN TISSUE COLLECTION AND START OF INCUBATION ON LYSINE UPTAKE (PERCENT OF ^{3}H-LYSINE DOSE)

	Absorption[a]			
Delay, min	Serosal	Gut	Total[b]	N[c]
0	12.76	7.48	20.24	10
10	14.44	7.45	21.89	13
20	11.57	7.41	18.98	13
30	11.34	8.10	19.44	12
SE	2.86	1.74	4.45	

[a]No differences (P>.52).

[b]Sum of serosal and gut dose.

[c]N = number of observations per treatment.

Results and Discussion

Results of Exp. 1 are presented in table 2 as percentage of dose for ^{65}Zn uptake. A linear (P<.01) and quadratic (P<.02) decrease in Zn absorption was observed for total ^{65}Zn uptake into gut tissue as delay time increased. The percentage of ^{65}Zn recovered on the serosal side of the sac was not affected by delay time. Lysine uptake was not affected (table 3) either to the serosal side of the gut sac or into the gut tissue. Lysine uptake was considerably more variable across treatments than Zn uptake. Lysine absorption to the serosal side averaged 12.5%, while total lysine absorption averaged 20.1%. Zinc absorption to the serosal side of the gut sac averaged 5.3% of the test dose, while total Zn absorption ranged from 21.6 to 29.7%. The Zn absorption data indicate that time between sacrifice and start of incubation should be minimized.

The results of increasing the molar ratio between PA and Zn (Exp. 2) are presented in table 4. The addition of PA decreased the absorption of ^{65}Zn from $^{65}ZnCl_2$ at pH 8. There was a linear decrease (P<.01) in Zn absorption as the PA molar ratio was increased. The ability of PA to chelate Zn is clear (Evans and Johnson, 1980), but a physiological role for PA is not evident from this experiment because graded levels of PA depressed Zn absorption. A quadratic effect (P<.01) of PA on total Zn absorption and

TABLE 4. EFFECT OF INCREASING MOLAR RATIO OF PICOLINIC ACID ON ABSORPTION OF ^{65}Zn AT pH 8.0 (PERCENT OF $^{65}ZnCl_2$ DOSE)

	Absorption			
PA molar ratio	Serosal[a]	Gut[b]	Total[c]	N[d]
0	3.18	28.68	31.86	12
.5	2.53	24.61	27.14	12
1.0	2.65	20.70	23.35	12
1.5	2.50	19.86	22.36	12
2.0	2.40	17.27	19.67	12
2.5	2.26	16.66	18.92	11
SE	.21	.82	.92	

[a]Linear effect (P<.01).

[b]Linear and quadratic effects (P<.01).

[c]Linear and quadratic effects (P<.01); sum of serosal and gut dose.

[d]N = number of observations per treatment.

Zn absorption into the gut tissue was detected and is believed to be an indication that the first increments of PA addition resulted in the greatest percentage decrease in Zn absorption.

The effects of Zn source, pH and addition of PA on ^{65}Zn absorption are presented in table 5. A depression in ^{65}Zn absorption with the addition of PA was evident for total ^{65}Zn absorption and ^{65}Zn absorption to the serosa. The addition of a 5 M PA ratio to Zn reduced total ^{65}Zn absorption to 53% and serosal absorption to 60% of absorption levels without PA. However, absorption of ^{65}Zn from the three sources responded differently to the addition of PA at the three pHs, which resulted in pH × PA interactions ($P<.02$) for ^{65}Zn absorption in the gut tissue and for total absorption. Zinc from $^{65}ZnCl_2$ was absorbed into the gut tissue more readily than were the organic Zn sources ($P<.04$). There was a similar trend ($P<.09$) for total Zn uptake.

Absorption of the radiolabeled amino acids are presented in table 6. Absorption of amino acids into the gut tissue resulted in a pH × PA interaction caused by the increased lysine absorption in the presence of PA at pH 7. The same response also resulted in a Zn source × pH interaction for amino acid uptake to the serosal side of the gut sac and total amino acid absorption. Across both PA treatments, there was more lysine (14.2% of dose) found on the serosal side of the gut sac than methionine (5.1% of dose).

Although the amino acid uptake is characterized by much higher variability than Zn uptake, the Zn-amino acid complexes do not appear to be absorbed intact. If the Zn-amino acid complexes were absorbed intact, similar ranges between percentage of dose of Zn and complexed amino acid would be expected.

Methionine is absorbed at a faster rate than lysine in vivo (Kidder and Manners, 1978). The mechanisms of absorption of neutral amino acids (including methionine) and basic amino acids (including lysine) are energy-dependent (Davenport, 1982). The buffers were oxygenated prior to, but not

TABLE 5. ABSORPTION OF ^{65}Zn (PERCENT OF DOSE) AS AFFECTED BY SOURCE, pH AND ADDED PICOLINIC ACID (PA)

Location	No PA			5 M PA		
	$ZnCl_2$	ZnMet	ZnLys	$ZnCl_2$	ZnMet	ZnLys
	pH 6					
Serosal[a]	4.00	4.41	4.56	3.04	3.16	2.93
Gut[b]	25.09	22.64	21.67	11.95	9.45	9.54
Total[cd]	29.09	27.05	26.23	14.99	12.61	12.47
N[e]	8	11	14	10	14	14
	pH 7					
Serosal[a]	5.34	6.23	6.94	4.61	4.04	3.22
Gut[b]	25.96	21.06	21.25	13.86	14.24	15.44
Total[cd]	31.30	27.29	28.19	18.47	18.28	18.66
N[e]	12	12	10	12	12	12
	pH 8					
Serosal[a]	4.19	4.03	5.39	1.77	2.04	2.50
Gut[b]	26.49	25.39	24.08	11.75	12.10	12.62
Total[cd]	30.68	29.42	29.47	13.52	14.14	15.12
N[e]	12	11	17	12	12	17

[a]Serosal side: no PA vs PA, pH quadratic, pH quadratic × PA interaction ($P<.01$); pH linear ($P<.03$).

[b]Gut: no PA vs PA ($P<.01$); inorganic Zn vs organic Zn ($P<.04$); pH linear ($P<.03$); pH quadratic × PA interaction ($P<.01$).

[c]Total: Sum of serosal and gut dose; no PA vs PA ($P<.01$); pH quadratic ($P<.01$).

[d]SE: serosal side, 1.52; gut absorption, .49; total absorption, 1.57.

[e]N = number of observations per treatment.

TABLE 6. ABSORPTION OF RADIOLABELED AMINO ACIDS (PERCENT OF DOSE) AS AFFECTED BY Zn SOURCE, pH AND ADDED PICOLINIC ACID (PA)

Location	No PA		5 M PA	
	ZnMET	ZnLys	ZnMET	ZnLys
	pH 6			
Serosal[a]	6.19	19.58	4.90	21.55
Gut[b]	10.27	15.64	8.15	10.06
Total[cd]	16.46	35.22	13.05	31.61
N[e]	13	14	13	13
	pH 7			
Serosal[a]	8.17	8.62	5.71	18.01
Gut[b]	10.93	8.38	8.55	8.43
Total[cd]	19.10	17.00	14.26	26.44
N[e]	10	9	10	11
	pH 8			
Serosal[a]	3.25	9.47	2.24	7.97
Gut[b]	5.72	7.03	4.55	6.47
Total[cd]	8.97	16.50	6.79	14.44
N[e]	9	11	9	16

[a]Serosal side: Met vs Lys, pH linear ($P<.01$); (Met vs Lys) X pH linear PA interaction ($P<.01$).

[b]Gut: pH linear ($P<.01$); no PA vs PA ($P<.05$).

[c]Total: Sum of serosal and gut dose; Met vs Lys, pH linear ($P<.01$); (Met vs Lys) X pH linear interaction ($P<.04$).

[d]SE: serosal side, 2.43; gut absorption, 1.62; total absorption, 3.62.

[e]N = number of observations per treatment.

during the 60-min incubation. The neutral amino acid absorption mechanisms may be more prone to oxygen limitation than basic amino acid absorption systems.

Currently there is general agreement among researchers regarding the existence of (a) low molecular weight Zn-binding ligand(s) in human milk, but its(their) identity(ies) remain controversial (Song and Adham, 1978; Cousins and Smith, 1980; Evans, 1980; Hurley and Lonnerdal, 1982; Robello et al., 1982). If the ligand found in milk facilitates Zn absorption, then it is reasonable to expect that presentation of the ligand to intestinal tissue would enhance Zn absorption as well. Oestreicher and Cousins (1982) found that picolinate did not enhance Zn absorption in isolated, vascularly perfused rat intestines. Menard and Cousins (1983) used rat intestine brush border membrane vesicles and found Zn uptake to be depressed by picolinate. However, Seal and Heaton (1983), used everted rat duodenal sacs with $ZnCl_2$ as a control, and found a fourfold increase in Zn uptake when picolinate was added to a system using a 30-fold higher concentration of zinc than was used in this study and a 50:1 ratio of PA:Zn. Others have shown that Zn absorption is not improved by the addition of picolinic acid in cattle (Flagstad, 1981), sheep (Ivan and Lamand, 1981) or pigs (Hill et al., 1986).

The data presented indicate that the Zn sources used in these experiments are of similar biological value, and do not support the theory that picolinic acid enhances Zn absorption.

Literature Cited

Cousins, R. J. and K. T. Smith. 1980. Zinc-binding properties of bovine and human milk in vitro: Influence of changes in zinc content. Amer. J. Clin. Nutr. 33:1083.

Davenport, H. W. 1982. Intestinal digestion and absorption of protein. In: Physiology of the Digestive Tract – An Introductory Text (5th Ed.). pp 205–210. Year Book Medical Publishers, Inc., Chicago, IL.

Dillaha, C. J., A. L. Lorincz and O. R. Aavik. 1953. Acrodermatitis enteropathica – A review of the literature and report of a case successfully treated with diodoquin. J. Amer. Med. Assoc. 152:509.

Evans, G. W. 1980. Normal and abnormal zinc absorption in man and animals: The tryptophan connection. Nutr. Rev. 38:137.

Evans, G. W., C. I. Grace and H. J. Votava. 1975. A proposed mechanism for zinc absorption in the rat. Amer. J. Physiol. 228:501.

Evans, G. W. and P. E. Johnson. 1979. Purification and characterization of a zinc-binding ligand in human milk. Fed. Proc. 38 (3 part 1):703 (Abstr.).

Evans, G. W. and P. E. Johnson. 1980. Characterization and quantitation of zinc-binding ligand in human milk. Pediatr. Res. 14:876.

Flagstad, T. 1981. Zinc absorption in cattle with a dietary picolinic acid supplement. J. Nutr. 111:1996.

Hahn, C. J. and G. W. Evans. 1973. Identification of a low molecular weight ^{65}Zn complex in rat intestine. Proc. Soc. Exp. Biol. Med. 144: 793.

Hill, D. A., E. R. Peo, Jr., A. J. Lewis and J. D. Crenshaw. 1986. Zinc-amino acid complexes for swine. J. Anim. Sci. 63:121.

Hurley, L. S. and B. Lonnerdal. 1982. Zinc binding in human milk: Citrate versus picolinate. Nutr. Rev. 40:65.

Ivan, M. and M. Lamand. 1981. The influence of intraruminal and intraduodenal infusion of picolinic acid on metabolism of ^{65}Zn in sheep. Ann. Rech. Vet. 12:337.

Kidder, D. E. and M. J. Manners. 1978. Digestion of protein. In: Digestion in the Pig. pp 150–

171. Scientechnica, Bristol.

Manis, J. G. and D. Schachter. 1962. Active transport of iron by intestine: Features of the two-step mechanism. Amer. J. Physiol. 203:73.

Menard, M. P. and R. J. Cousins. 1983. Effect of citrate, glutathione and picolinate on zinc transport by brush border membrane vesicles from rat intestine. J. Nutr. 113:1653.

Moynahan, E. J. 1974. Acrodermatitis enteropathica: A lethal inherited human zinc-deficiency disorder. Lancet 2:399.

Oestreicher, P. and R. J. Cousins. 1982. Influence of intraluminal constitutents by zinc absorption by isolated, vascularly perfused rat intestine. J. Nutr. 112:1978.

Perkin-Elmer. 1971. Analytical methods for atomic absorption spectrophotometry. Perkin-Elmer, Norwalk, CT.

Rebello, T., B. Lonnerdal and L. S. Hurley. 1982. Picolinic acid in milk, pancreatic juice and intestine: Inadequate for role in zinc absorption. Amer. J. Clin. Nutr. 35:1.

SAS. 1979. SAS User's Guide. Statistical Analysis System Institute, Inc., Cary, NC.

Seal, C. J. and F. W. Heaton. 1983. Chemical factors affecting the intestinal absorption of zinc in vitro and in vivo. Brit. J. Nutr. 40:317.

Song, M. K. and N. F. Adham. 1978. Role of prostaglandin E_2 in zinc absorption in the rat. Amer. J. Physiol. 234:E99.

Steel, R.G.D. and J. H. Torrie. 1980. Principles and Procedures of Statistics – A Biometrical Approach (2nd Ed.). McGraw-Hill Book Co., New York, NY.