Food Chemistry **43** (1992) 377–381

Bioavailability in the rat of zinc and iron from the basic salts $Zn_5(OH)_8Cl_2.H_2O$, $Fe(OH)SO_4$ and $Fe_4(OH)_{11}NO_3.2H_2O$

Magdelena Galvez-Morros

Departmento de Patologia II, Universidad Complutense de Madrid, Spain

O. Garcia-Martinez

Instituto de Ciencia de Materiales CSIC, Madrid, Spain

A. J. A. Wright* & Susan Southon

AFRC Institute of Food Research, Norwich Laboratory, Colney Lane, Norwich NR4 7UA, UK

(Received 5 January 1991; accepted 25 February 1991)

The bioavailability of zinc and iron from the basic salts ($Zn_5(OH)_8Cl_2.H_2O$, $Fe(OH)SO_4$ and $Fe_4(OH)_{11}NO_3.2H_2O$) was studied in male Wistar rats. In expt. 1, zinc absorption from $Zn_5(OH)_8Cl_2.H_2O$, extrinsically labelled with ^{65}Zn, was measured and compared to zinc absorbed from either $ZnCl_2$ or $ZnCO_3$. In expt. 2, rats were fed *ad libitum* for 2 weeks on semisynthetic diets containing $ZnCO_3$ or $Zn_5(OH)_8Cl_2.H_2O$ as the zinc source (13 mg Zn/kg diet) and $FeSO_4,7H_2O$, $Fe(OH)SO_4$ or $Fe_4(OH)_{11}NO_3.2H_2O$ as the iron source (35 mg Fe/kg diet). After 2 weeks on the experimental diets, rats were killed, and the zinc and iron status of groups was compared as appropriate. Data obtained from both experiments indicated that the availability of zinc from $Zn_5(OH)_8Cl_2.H_2O$ was similar to that from $ZnCO_3$ and $ZnCl_2$, and hence, the basic zinc salt is of high bioavailability. The availability of iron from the basic iron salts, however, was very poor when compared to ferrous sulphate.

INTRODUCTION

Neutral inorganic salts are generally used as therapeutic aids to correct mineral deficiencies, but in many cases the ideal material for mineral therapy is still being sought. Zinc, for instance, is usually given in the form of zinc sulphate; however, this salt has a very unpleasant taste and in large doses can cause nausea, vomiting and fever (Hurren, 1989), side-effects that are particularly prevalent in children. Zinc given in the form of $Zn_5(OH)_8Cl_2.H_2O$ appears to be better tolerated and this observation is now under further study at Hospital Savero Ochoa, Leganes, Madrid (Vazquez *et al.*, 1990).

Ferrous sulphate, used to correct iron deficiency, also has an unpleasant taste, and is noted for causing constipation, diarrhoea, nausea and epigastric pain (Solvell, 1970). Furthermore, this iron salt cannot normally be used for iron fortification of foods as it is chemically highly reactive, forming off-colours, off-flavours and rancidity in the flour-based products (bread, biscuits, etc.) which are usually used as vehicles for fortification.

Basic salts are compounds similar in composition to neutral salts, but with hydroxyl groups (OH^-) introduced into the molecule, and are generally formed by hydrolysis of neutral salts or by the oxidation of some metals such as zinc, cadmium and lead (though some do occur as natural products). The chemistry of this class of compounds, having intermediate solubility between the highly soluble neutral salts and the relatively insoluble oxides or hydroxides, was initially studied by Feitknecht (1953), but their potential usefulness as mineral supplements or food fortificants has not been investigated.

* To whom correspondence should be addressed.

Food Chemistry 0308-8146/92/$05.00

The aim of the present study was to evaluate the absorption and utilization of zinc from the basic zinc salt $Zn_5(OH)_8Cl_2.H_2O$ and of iron from the basic iron salts $Fe(OH)SO_4$ and $Fe_4(OH)_{11}NO_3.2H_2O$, in comparison to that from the neutral salts $ZnCl_2$, $ZnCO_3$ and $FeSO_4.7H_2O$.

Two experiments were performed. In the first experiment, ^{65}Zn absorption and re-excretion from extrinsically radiolabelled meals containing $Zn_5(OH)_8Cl_2.H_2O$, $ZnCO_3$ or $ZnCl_2$ were compared. In the second experiment rats were fed a semisynthetic (SS) diet, containing either $ZnCO_3$ or $Zn_5(OH)_8Cl_2.H_2O$ as the zinc source and either $FeSO_4.7H_2O$, $Fe(OH)SO_4$ or $Fe_4(OH)_{11}NO_3.2H_2O$ as the iron source, for 2 weeks. At the end of this period, rats were killed, and the zinc and iron status of groups was compared as appropriate.

MATERIALS AND METHODS

Synthesis of basic salts

The $Zn_5(OH)_8Cl_2.H_2O$ was prepared by hydrolysis of $ZnCl_2$ with NaOH at 60° for 24 h (Nowacky & Silvermand, 1957), $Fe(OH)SO_4$ by hydrolysis of $Fe_2(SO_4)_3$ at 120° for 15 days (Parada-Cortina *et al.*, 1983) and $Fe_4(OH)_{11}NO_3.2H_2O$ by hydrolysis of $Fe(NO_3)_3.9H_2O$ with urea at 60° under nitrogen (Lopez-Delgado *et al.*, 1988). All the precipitates were filtered, washed with distilled water, ethanol and acetone, dried in a vacuum and kept over phosphorus pentoxide. The prepared salts were characterised by elemental analysis, IR spectroscopy, electron microscopy and X-ray diffraction.

Experiment 1

Fifty male Wistar rats (150–170 g) were randomly allocated to one of three groups and housed singly in polypropylene cages with stainless steel gridded tops and bottoms in a room at 21° having a light/dark cycle of 12 h. All rats were given a SS control diet *ad libitum* for 7 days. The SS diet was the same as previously described (Wright *et al.*, 1989), except that $FeSO_4.7H_2O$ was added at 174 mg/kg diet equivalent to Fe added at 35 mg/kg. After an overnight fast each rat was weighed and given a 5 g meal of cooked starch–sucrose–water (1:1:6) paste containing 130 μg Zn either as $Zn_5(OH)_8Cl_2.H_2O$ (20 rats), $ZnCl_2$ (15 rats) or $ZnCO_3$ (15 rats) extrinsically labelled with 37 kBq ^{65}Zn ($ZnCl_2$, 3·7–92·5 MBq/mg Zn; Amersham International, Aylesbury, UK). Rats were allowed a maximum of 1 h in which to consume the test meal and were whole-body counted immediately after consuming the meal (designated day 0) and then again each day until the end of the experiment (day 14). Whole-body radioactivity was measured using an NE 8112 small-animal whole-body gamma counter (NE Technology, Beenham, Berkshire, UK) as described by Fairweather-Tait & Wright (1984). The counts obtained each day, after correction for counting efficiency, background and decay, were expressed as a percentage of the counts obtained from the same animal on day 0. It was assumed that unabsorbed ^{65}Zn would have been excreted within 5 days of dosing and that absorbed ^{65}Zn should have entered equilibrium with body zinc pools.

The logarithm ($\log_{10}$) of the percentage whole-body retention, between days 5 and 14, was plotted (y-axis) against time (x-axis) for each animal and, from linear regression analysis, an estimate of true ^{65}Zn absorption from the test meal on day 0 was obtained (antilog of intercept on y-axis), together with an estimate of the fractional daily rate of loss of body ^{65}Zn (1 − antilog slope of regression), an indication of whole-body zinc turnover. This technique for estimating true absorption makes allowance for endogenous losses of ^{65}Zn over the experimental period through zinc re-excretion into the small intestine. Any animal that consumed less than two-thirds of the test meal was excluded from the final results because of the possibility of a dose-related response.

The SS control diet was again provided *ad libitum* 6 h after the test meals had been consumed, a period of time sufficient to allow clearance of the test meals from the stomach, until the end of the experiment 2 weeks later. All faeces were collected from day 0 to day 4, pooled for each rat, and counted for ^{65}Zn. The pooled faecal ^{65}Zn for each rat was then added to the day 4 whole-body ^{65}Zn retention, any discrepancy between the total accountable ^{65}Zn and the original dose (day 0) being presumed to have been lost via urinary excretion.

Experiment 2

Eighty immature male Wistar rats (75–80 g) were allocated to one of four groups of 20 and housed as in experiment 1. Rats were fed for 5 days *ad libitum* on the same SS control diet used in experiment 1. Rats then received one of the following experimental SS diets for a further 14 days, with zinc added at 13 mg/kg and iron added at 35 mg/kg diet: group 1 (control group) received control SS diet, which contained zinc carbonate (25 mg/kg) and ferrous sulphate (174 mg/kg); group 2 (basic zinc group) received SS diet containing $Zn_5(OH)_8Cl_2.H_2O$ (100 mg/kg diet) and ferrous sulphate; group 3 (basic iron sulphate group) received SS diet containing zinc carbonate and $Fe(OH)SO_4$ (106 mg/kg diet); and group 4 (basic iron nitrate group) received SS diet containing zinc carbonate and $Fe_4(OH)_{11}NO_3.2H_2O$ (80 mg/kg diet). Food was provided in restricted amounts (20 g/day; *c.* 80 to 100% of *ad libitum* intake) in order to equalise food intake, and hence zinc and iron intake, between groups. Body

weights and food intakes were recorded regularly. At the start of the experimental feeding period, rats in the control and basic zinc group were injected subcutaneously with 37 kBq ^{65}Zn (in 0·2 ml saline) into the scruff of the neck and whole-body counted immediately and then again every day until the end of the experiment. Five days were allowed for equilibration of subcutaneously injected ^{65}Zn with body zinc pools, and then whole-body retention from days 5 to 14 was plotted in $\log_{10}$ format against time. From regression analysis an estimate of the daily loss of body ^{65}Zn was made as an indication of zinc turnover. The iron status of rats in the control and basic iron groups was assessed via measurement of liver iron content, red blood cell count (RBC), packed cell volume (PCV), mean cell volume (MCV) and haemoglobin (Hb) concentration. The zinc status of rats in the control and basic zinc group was assessed via plasma and femur zinc concentration.

Rats were killed by an intraperitoneal injection of sodium pentabarbital (1 ml, 160 mg/ml, Euthatal; Rhone Poulenc, Dagenham, Essex, UK) and exsanguinated by cardiac puncture. Livers were removed from the control and basic iron groups, together with femurs of the right hind limb from the control and basic zinc group. RBC, PCV, MCV and Hb concentration were determined promptly on samples of heparinised whole blood from the control and basic iron groups, using a semiautomated Coulter counter (model CBC-5, Coulter Electronics, Luton, UK). Livers were rinsed in isotonic saline, blotted dry, freeze-dried, weighed and ground to an homogeneous powder. Femurs were oven-dried overnight at 80° and then weighed. Plasma (control and basic zinc group) was separated from whole blood and deproteinised with 3·5% (w/v) perchloric acid. Freeze-dried liver powder and oven-dried femurs were ashed in a muffle furnace for 48 h at 480°C, the ash was dissolved in a minimum volume of hot concentrated HCl (11·7M) and the solution was further diluted with distilled water. The iron (liver) and zinc (femur) contents were determined by atomic absorption spectroscopy, using a PU 9000 (Pye Unicam, Cambridge, UK). Deproteinated plasma was analysed directly for zinc.

Statistical analysis

All data were checked for variance homogeneity. Where the variance was not homogeneous, data were transformed ($\log_{10}$ format) prior to one-way analysis of variance. Where the variance ratio (F) showed a treatment effect ($P < 0·05$), t-tests between any two means (x_1 and x_2) having n replicates (n_1 and n_2) were performed using the standard error of the difference of the means (SED) calculated from the residual mean square (RMS) as follows:

$$SED = \sqrt{(RMS(1/n_1 + 1/n_2))}; \qquad t = (x_1 - x_2)/SED$$

The residual degrees of freedom were used to estimate the level of significance of t.

RESULTS

Experiment 1

Values for ^{65}Zn absorption from a starch:sucrose radiolabelled test meal and fractional rates of whole body ^{65}Zn turnover were similar for the control ($ZnCl_2/ZnCO_3$) and basic zinc ($Zn_5(OH)_8Cl_2.H_2O$) groups (Table 1).

Table 1. Comparison of food intake, body weight gain, ^{65}Zn absorption, rate of whole body ^{65}Zn turnover and zinc status in rats fed $ZnCO_3$, $ZnCl_2$ or $Zn_5(OH)_8Cl_2.H_2O$

Dietary treatment		Control groups		Basic zinc ($Zn_5(OH)_8Cl_2.H_2O$) (mean (SE))
		$ZnCO_3$ mean (SE)	$ZnCl_2$ mean (SE)	
Expt. 1[a]				
Number of replicates	(n)	13	13	19
Body weight	(g)	251·7 (2·6)	250·1 (3·1)	247·4 (2·5)
^{65}Zn absorption	(%)	48·4 (3·0)	45·1 (2·1)	40·0 (2·4)
Fractional rate of ^{65}Zn loss/day		0·0169 (0·0005)	0·0171 (0·0006)	0·0158 (0·0005)
Expt. 2[b]				
Number of replicates	(n)	20		20
Food intake	(g)	277·0 (3·1)		278·0 (1·8)
Body weight gain	(g)	103·0 (1·9)		99·0 (2·0)
Plasma zinc	(μg/ml)	1·86 (0·034)		1·76 (0·040)
Femur zinc	(μg/g dry wt)	145·8 (3·1)		148·2 (3·8)
Fractional rate of ^{65}Zn loss/day		0·0098 (0·0008)		0·0101 (0·0008)

[a] ^{65}Zn absorption from a single test meal using adult rats: there were no significant differences between groups.
[b] Longer-term feeding experiment using immature, fast growing rats: there were no significant differences between groups.

Table 2. Expt. 2: Comparison of food intake, body weight gain, RBC, MCV, PCV, Hb concentration, liver dry weight, liver iron concentration and total liver iron in rats fed for 2 weeks on SS diets containing either $FeSO_4.7H_2O$ (control) or the basic iron salts $Fe(OH)SO_4$ or $Fe_4(OH)_{11}NO_3.2H_2O$ at 35 mg Fe/kg diet

Dietary treatment		Control	Basic Fe salts		Significance[a] of variance ratio (F)	SED[b] (57 df)
			Sulphate	Nitrate		
Food intake	(g)	277·7	266·7	276·2	$P < 0.05$	4·17
Weight gain	(g)	102·9	106·2	103·0	n.s.	2·80
RBC	($\times 10^{12}$/l)	7·00	3·98	5·40	$P < 0.001$	0·267
MCV (GM)[c]	(fl)	62·47	61·48	57·32		
$\log_{10}$ MCV		1·7957	1·7887	1·7583	$P < 0.001$	0·00951
PCV	(l/l)	0·439	0·243	0·31	$P < 0.001$	0·0149
Hb	(g/l)	151·0	97·5	108·4	$P < 0.001$	3·46
Liver:						
dry weight	(g)	3·112	2·797	2·580	$P < 0.001$	0·0637
Fe (GM)[c]	(μg/g dry wt)	223·3	115·5	131·2		
$\log_{10}$ Fe concentration		2·3489	2·0624	2·1179	$P < 0.001$	0·02442
Total Fe (GM)[c]	(mg)	694·5	321·8	337·8		
$\log_{10}$ total iron		2·8417	2·5076	2·5287	$P < 0.001$	0·02395

[a] Statistical comparisons performed, using one-way-analysis of variance (AOV).
[b] Standard error of the difference between means with 57 degrees of freedom (df).
[c] Geometric mean: mean values with unequal variance were $\log_{10}$ transformed prior to AOV.

Experiment 2

Mean values for food intake, body-weight gain, plasma and femur zinc concentration, and fractional rate of ^{65}Zn turnover were similar for the control and basic zinc groups (Table 1).

Significant differences were observed, however, when the control and basic iron groups were compared. Food intake was significantly lower when rats were fed diets containing the basic iron sulphate salt, compared to the control and basic iron nitrate groups, although body weight gain of rats in each of these groups was similar (Table 2). RBC, PCV, Hb concentration, liver dry weight and total liver iron content were all significantly lower ($P < 0.001$) for the basic iron groups compared to the controls, with the basic iron sulphate group consistently having the lowest values (Table 2).

DISCUSSION

Neutral inorganic salts are commonly used in dietary supplements and in therapeutic aids and as food fortificants. However, there may be several problems associated with the use of these salts. The occurrence of the unpleasant side-effects of inorganic zinc sulphate is greatly enhanced when large quantities are used, as in oral zinc tolerance tests and in diagnostic tests for zinc malabsorption (Sullivan *et al.*, 1979), where the increase in plasma zinc from the fasting level is measured. The side-effects of such tests are particularly prevalent in children and in many cases tests cannot be accomplished because of vomiting of the oral zinc sulphate dose. Studies with the basic zinc salt $Zn_5(OH)_8Cl_2.H_2O$ at the Hospital Savero Ochoa, Leganes, Madrid, indicates that this salt is much better tolerated (Vazquez *et al.*, 1990).

Ferrous sulphate, used to correct iron deficiency, also has a number of unpleasant side effects, such as constipation and epigastric pain, which may reduce compliance to therapy (Solvell, 1970). Ferrous sulphate is well absorbed and utilised by the body. However, it is highly chemically reactive, which often precludes its use as a food fortificant because of the formation of off-colours and off-flavours.

Basic iron salts have an intermediate reactivity between the highly reactive neutral salts and the relatively unreactive oxides or hydroxides and, if reasonably well absorbed, may prove useful as food fortificants.

The present study undertook to investigate the bioavailability of zinc and iron from basic salts, using the extrinsic radiolabel-tag method (one meal) or longer-term feeding (2 weeks) of fast-growing, immature, rats. Results show that the absorption and utilization of zinc from the basic zinc salt ($Zn_5(OH)_8Cl_2.H_2O$) was similar to that from $ZnCl_2$ and $ZnCO_3$. In the first experiment (using adult 250 g rats) the percentage absorption of ^{65}Zn and subsequent whole-body ^{65}Zn turnover from a single test meal containing $Zn_5(OH)_8Cl_2.H_2O$, $ZnCl_2$ and $ZnCO_3$ were not significantly different.

There is evidence that some mineral complexes, for example Fe(III)–EDTA, are absorbed intact and then a portion is rapidly excreted from the body via the urine (Helbock & Saltman, 1967) rather than being totally utilized. To check that there were no differences in the urinary excretion of zinc between groups in the present study, total faecal output was collected for each rat for the first 4 days after ingestion of the radiolabelled test meals. Carcass and faecal radioactivity over this period

accounted for the total original dose in all groups, indicating that there was little, if any, urinary excretion of the isotope and hence no significant loss of any zinc absorbed from the three zinc compounds via this route. In the second longer-term feeding experiment, using young rapidly growing rats, no significant differences in whole-body ^{65}Zn turnover or zinc status, as judged by plasma and femur zinc concentration, was observed between the basic zinc ($Zn_5(OH)_8Cl_2.H_2O$) and control ($ZnCO_3$) groups. Body weight gain and food intake were not adversely affected by inclusion of basic zinc rather than zinc carbonate in the SS diet.

In contrast to the good solubility of the basic zinc compounds, the solubility of the basic iron compounds was poor. Since the complete exchangeability of an extrinsic ^{59}Fe radiolabel could not be guaranteed, it was felt that the results from any radiotracer experiment may be invalid and that examination of the bioavailability of these compounds was limited to longer-term feeding experiments rather than to a single test meal. While growth was not impaired, results from experiment 2 clearly demonstrate that both basic iron salts had a poor bioavailability in comparison to ferrous sulphate. The control group fed $FeSO_4.7H_2O$ as the iron source had twice the level of liver iron stores and significantly higher RBC, PCV and Hb concentrations compared to rats fed either of the basic iron salts. Values for Hb concentrations in the basic iron groups were borderline for iron-deficiency anaemia. Although mean values for MCV were lower for both the basic iron groups compared to controls, this was significant only for the basic iron nitrate group. There was some evidence that rats found the SS diet containing the basic iron sulphate salt slightly less palatable since food intake was marginally lower in this group.

In conclusion, this study indicates that the basic iron salts studied, because of their very poor bioavailability, appear to be of little use in the field of iron supplementation or fortification, though they may find use in the agrochemical industry as slow-release iron compounds. However, zinc from the basic zinc compound $Zn_5(OH)_8Cl_2.H_2O$ is as well absorbed and utilized as zinc from sources that are regarded as having a relatively high zinc bioavailability. Since it appears that orally administered zinc from this source may be better tolerated, especially in children, the possibility that $Zn_5(OH)_8Cl_2.H_2O$ could be used to replace $ZnSO_4$ as a dietary supplement or as a therapeutic agent to correct zinc deficiency should be considered.

REFERENCES

Fairweather-Tait, S. J. & Wright, A. J. A. (1984). The influence of previous iron intake on the estimation of bioavailability of Fe from a test meal given to rats. *Br. J. Nutr.*, **51**, 185–91.

Feitknecht, W. (1953). The solid hydroxy salts of bivalent metals. *Fortschr. Chem. Forsch.*, **2**, 670–757.

Helbock, H. J. & Saltman, P. (1967). The transport of iron by rat intestine. *Biochem. Biophys. Acta*, **135**, 979–90.

Hurren, C. (1989). Zinc. *WHICH? Way to Health*, October 1989, 6–7.

Lopez-Delgado, A., Garcia-Martinez, O. & Galvez-Morros, M. (1988). Synthesis, characterization and thermal behaviour of $Fe_4(OH)_{11}NO_3.2H_2O$. *J. Therm. Anal.*, **34**, 843–52.

Nowacky, W. S. & Silvermand, S. (1957). The crystal structure of zinc hydroxychloride II. *Acta Crystallographica*, **10**, 787–9.

Parada-Cortina, C., Andres, S. L. & Garcia-Martinez, O. (1983). Nuevas aportaciones al estudio de hidroxisulfatos de Fe III $Fe(OH)SO_4$ y $Fe_3(SO_4)_2.H_2O$. *An. Quim.*, **79B**, 567–71.

Solvell, L. (1970). Oral iron therapy-side effects. In *Iron Deficiency: Pathogenisis, Clinical Aspects, Therapy*, ed. L. Hallberg, H.-G. Harwerth & A. Vannotti. Academic Press, London, pp. 573–83.

Sullivan, J. F., Letton, M. & Burch, E. (1979). A zinc tolerance test. *Lab. Clin. Med.*, **93**, 485–91.

Vazquez, C., Galvez-Morros, M., Millon, P. & Garcia-Martinez, O. (1990). Hidroxicloruro de zinc versus sulfato en la sobrecarga oral de zinc. *Nutr. Clin.*, **10**, 33–7.

Wright, A. J. A., Southon, S. & Fairweather-Tait, S. J. (1989). Measurement of non-haem iron absorption in non-anaemic rats using ^{59}Fe: can the Fe content of duodenal mucosal cells cause lumen or mucosal radioisotope dilution, or both, thus resulting in the underestimation of Fe absorption? *Br. J. Nutr.*, **62**, 719–27.