INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
12–775/2013/15–2–372–376
http://www.fspublishers.org

Full Length Article

Comparison of Absorption Characteristics of Iron Glycine Chelate and Ferrous Sulfate in Caco-2 Cells

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Abstract

The study was conducted to compare the absorption characteristics of iron glycine chelate (Fe-Gly) and ferrous sulfate ($FeSO_4$) in Caco-2 cells. Effects of several factors, including concentration, time and temperature on iron (Fe) transport were investigated. In Caco-2 cell model, Fe transport amount in Fe-Gly treatment was higher than that in $FeSO_4$ from the apical side (AP) to the basolateral (BL) side. However, there was no significant difference between the $FeSO_4$ and Fe-Gly treatments in the direction of BL to AP. Similar results were also found on Fe transport rate of Fe-Gly and $FeSO_4$ across Caco-2 monolayers. The apparent permeability coefficient of Fe-Gly was significantly higher than that of $FeSO_4$ in the direction of AP to BL. The ratio of the apparent permeability coefficient between AP to BL and BL to AP was greater than 1.0 for both forms of Fe resources. The Fe transport amount in Fe-Gly treatment was higher than that in $FeSO_4$ treatment, no matter the incubation temperature was 37°C or 4°C. However, it significantly decreased, when the incubation temperature dropped from 37°C to 4°C. The results indicate that Fe of Fe-Gly can be easily absorbed than $FeSO_4$ in Caco2 cells. © 2013 Friends Science Publishers

Keywords: Fe-Gly; $FeSO_4$; Caco-2 Cell; Concentration; Time; Temperature

Introduction

Iron (Fe) plays an important role in physiological functions, for instance, carries oxygen, forms a part of the oxygen-carrying proteins (hemoglobin and myoglobin) and serves as a cofactor in enzymes that involving in oxidation-reduction reactions (Glahn *et al*., 2002; Kloots *et al*., 2004). In terms of oxygen transport and cellular respiration, Fe is an indispensable element for fish (Javed and Saeed, 2010). In order to meet the growth requirement, an addition of 38 to 80 mg of Fe was given to the diet of swine or poultry (Kratzer *et al*., 1994; 1998). However, studies have shown that a relatively low absorption of nonheme Fe in maize, soybeans and wheat, ranging from 2 to 20% (Hallberg *et al*., 1997; Tapiero *et al*., 2001). Therefore, many Fe additives were used in the animal diets to provide enough Fe source, such as Fe sulfate, Fe carbonate, Fe proteinate, and Fe chelate.

The bioavailability values vary greatly among different Fe sources. Compared with ferrous sulfate ($FeSO_4$), chelated or proteinated source of Fe had higher bioavailability which was 125 to 185% at the same level of $FeSO_4$ (Henry *et al*., 1995). Langini *et al*. (1988) reported that the Fe absorptions of weanling rats which given infant formula labeled with [^{59}Fe] glycine and [^{59}Fe] sulfate were 30.9 and 15.8%, respectively. Layrisse *et al.* (2000) found that the absorption rate of Fe in iron glycine chelate (Fe-Gly) was almost two times of that in $FeSO_4$. We also reported a better bioavailability in ferrous glycine than ferrous sulfate in piglet (Feng *et al*., 2007; 2009; Ma *et al*., 2012). However, the data of absorption characteristics between Fe-Gly and $FeSO_4$ is still limited.

Caco-2 line that derived from the human colon adenocarcinoma is able to differentiate spontaneously (Sanchez *et al*., 1996). It can present many absorption characteristics analogous to intestinal cells during culture, such as formatting a monolayer of the cells and expressing several morphological and functional characteristics of the mature enterocyte (Rousset, 1986; Artursson, 1990; Gan *et al*., 1997). Caco-2 cell model has been used extensively in numerous of biological, biochemical and toxicological studies, as well as in the intake or transport study of trace elements, such as iron, zinc, copper, chromium, and so on (Zödla *et al*., 2005; He *et al*., 2008; Villarroel *et al*., 2011). Thus, the purpose of the present study was to compare the absorption characteristics between Fe-Gly and $FeSO_4$ using the Caco-2 cell lines.

To cite this paper: Ma, W.Q., J. Wu, Z. Zhuo, H. Sun, M. Yue and J. Fe, 2013. Comparison of absorption characteristics of iron glycine chelate and ferrous sulfate in Caco-2 cells. *Int. J. Agric. Biol*., 15: 372–376

Materials and Methods

Cells

Caco-2 cells during 20-40 serial passages (Shanghai Institute of Biochemistry and Cell Biology, SIBS, CAS) were cultured according to the method described by Mazariegos *et al.* (2004). Briefly, cells were cultured in 25 cm^3 flasks with Dulbecco's minimum essential medium (DMEM, Gibco) which supplemented with 10% fetal bovine serum (FBS), 4.5 g/L glucose, 2% L-glutamine, 1% non-essential amino acids and 100 U/L penicillin/streptomycin. The incubation condition was 37°C, 5% CO_2 and 90% relative humidity. Suspended the Caco-2 cells, which were in the logarithmic growth phase and reseeded them on the polycarbonate membrane of 6-Transwell plug-in Petri dish at a density of $1x10^4$ cells /cm^2.

Assessment of Caco-2 Model

The cells were evaluated for study use on the 20-22th days. Caco-2 cells were similar to the epithelial cells morphologically (observed by inverted phase contrast microscope, Olympus CKX41, Japan), transmembrane resistance value (466.75 Ω·cm^2) and mannitol permeation rate (0.85%) could meet the requirements of tightness, integrity and permeability. Besides, cells differentiated well with polarity, which tested by alkaline phosphatase activity. Therefore, Caco-2 model could be used for intestinal absorption *in vitro*.

Fe Solutions

Fe-Gly and $FeSO_4$ were made into 1 moL/L stock solution with double-stilled water. Filtrated the stock solution with aseptic Millipore filter and diluted into different multiple with D'Hanks buffer. Atomic absorption spectrometry (Shimadzu AA-6501; Paleologos *et al.,* 2002) was used for the measurement of Fe concentration.

Transport Assays in Caco-2 Cells

We conducted the bidirectional transport of $FeSO_4$ and Fe-Gly using caco-2 monolayers prepared as above. Transports from the apical side (AP) to the basolateral side (BL): Sample solutions (1.5 mL) were placed on the apical side of Caco-2 cells, served as the donor chamber, while the basolateral side was supplemented with D'Hanks (2.6 mL) buffer as a receiver. Whereas, transports from BL to AP: BL with 2.6 mL sample served as the donor chamber and AP with 1.5 mL D'Hanks buffer as the receiver. Triplicate wells were used for each treatment.

Transport Study on Concentration

Sample solutions (1.5 mL) containing different Fe concentration (0.5, 5, 10 and 20 μmol/L) were placed on the side of the donor chamber, while the side of the receiver chamber was supplemented with 2.6 mL D'Hanks buffer. The experiment was performed in the oscillation sink with 37°C constant temperature and at a speed of 50 rpm. Transport of Fe across the cell monolayer was tested by withdrawing 200 μL of sample from the receiver chamber at different time points, and an equivalent amount of pre-warmed D'Hanks buffer was immediately given to replace this volume. The experiment was ended after 120 min.

Transport Studies on Time and Temperature

As for the study of transports across Caco-2 monolayers on time and temperature, we collected 200 μL of sample from the receiver chamber at the specific time points (30, 60, 90 and 120 min) and just changed the temperature condition from 37°C to 4°C, respectively. The other details were same as the different Fe concentration study.

Calculations

Apparent permeability coefficient ($P_{app,}$ cm/s)

P_{app} was calculated by the following formula:

$$P_{app} = \frac{dQ}{dt \times 60 \times A \times C_0}$$

Where, dQ/dt (μmoL/min) is the flux rate of mass transport across the monolayers, A is the surface of insert membrane(4.7 cm^2), C_0 is the initial concentration (μmoL/mL) of sample in the donor chamber.

The following equation was applied to revise the values for minimizing sampling errors.

$$TR_{cum} = A_n + \frac{V_{sn}}{V_R} \sum_{i=0}^{n-1} A_i$$

Where, TR_{cum} is the revised mass transport across the monolayers, A_n is the transport amount, which is actual measured, V_{Sn} is the volume of collected sample, V_R is the volume of the donor chamber.

The transport rate is calculated using the following equation:

$$\text{Transport rate}(\%) = \frac{\text{Fe transport amounts in the receiver chamber}}{\text{Fe transport amounts in the donor compartment}} \times 100\%$$

Statistical Analysis

Values presented in the study are given as the mean ± SEM. One-way analysis of variance (ANOVA) and paired comparison of LSD were used in analyzing the effects between different treatments in SPSS 11.5 software. P value of < 0.05 was considered significant, while < 0.01 were regarded extremely significant.

Results

Effect of Concentration on Fe Transport

The bidirectional transports of Fe-Gly and $FeSO_4$ are concentration-dependent at the temperature of 37°C (Fig. 1).

Table 1: Apparent permeability coefficient (P_{app}) of Fe-Gly and $FeSO_4$ at different concentrations across Caco-2 cell monolayers

Concentration (μmol/L)	Apparent permeability coefficient (P_{app} , $\times 10^{-6}$cm/s)					
	$FeSO_4$			Fe-Gly		
	AP→BL	BL→AP	R*	AP→BL	BL→AP	R*
0.5	4.70±0.67^{b}	0.72±0.05$^{\beta}$	6.50	10.40±0.62^{a}	0.64±0.08$^{\alpha}$	16.14
5	3.37±0.14^{b}	0.34±0.02	9.99	6.74±0.21^{a}	0.33±0.04	20.71
10	2.39±0.12^{b}	0.27±0.0	8.96	5.48±0.25^{a}	0.21±0.03	25.43
20	1.60±0.11^{b}	0.18±0.03	8.77	3.55±0.39^{a}	0.15±0.04	23.57

Note: Experiments were conducted 120 min at 37°C. The ab or αβ represents the differences between Fe-Gly and $FeSO_4$ treatments under the same concentration and direction, without common letters are considered significantly (P<0.05). R represents the apparent permeability coefficient ratio

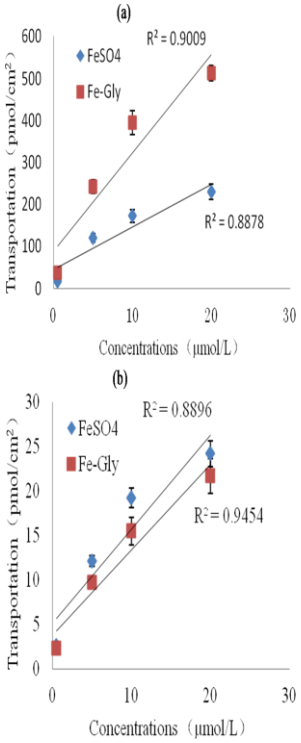

Fig. 1: Effects of concentration (0.5–20 μmol/L) on Fe bidirectional transport across Caco-2 monolayers (37°C, 120 min). (a) shows the transport of Fe-Gly and $FeSO_4$ from apical to basolateral side. (b) is the results in the inverse direction. The regression lines indicate the positive correlation between transport amount and concentration of Fe

Fe transport amount in Fe-Gly treatment is higher than that in $FeSO_4$ from AP to BL side, but there is no obvious difference in the direction of BL to AP. The same results were also found on Fe transport rate of Fe-Gly and $FeSO_4$ across Caco-2 monolayers (Fig. 2).

Table 1 shows the P_{app} of Fe-Gly and $FeSO_4$ at different concentrations across Caco-2 cell monolayers. The P_{app} of Fe-Gly is significantly higher than that of $FeSO_4$ in the direction of AP to BL. Besides, the ratio of the p_{app} value between AP to BL and BL to AP is greater than 1.0 for the both forms of iron resources.

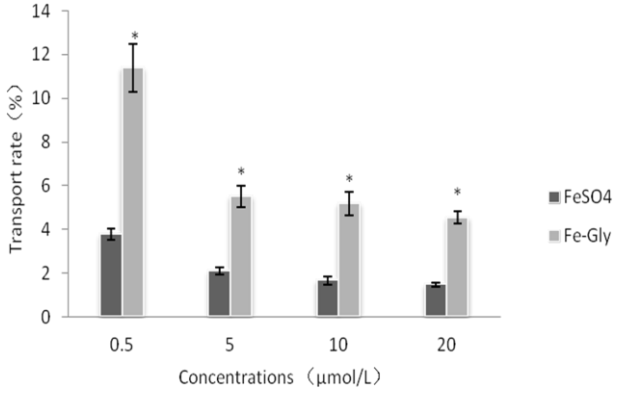

Fig. 2: Transport rate of Fe-Gly and $FeSO_4$ across Caco-2 monolayers (AP→BL). Bars within an Fe concentration with asterisk are significantly (P<0. 05) different

Effect of Time and Temperature on Fe Transport

Fe transport amount of Fe-Gly and $FeSO_4$ across Caco-2 monolayers are linear increased with the time prolonged (Fig. 3). The bidirectional Fe transport of Fe-Gly and $FeSO_4$ are time-dependent at the temperature of 37°C. It also showed similar pattern as the concentration on Fe transportation, Fe transport amount in Fe-Gly is higher than in $FeSO_4$ from AP to BL side.

Effect of temperature on Fe transport was shown in Table 2. Compared with $FeSO_4$, Fe transport amount in Fe-Gly treatments were both higher when incubated in 37°C or 4°C. However, it decreased (P < 0.05) when the incubation temperature dropped from 37°C to 4°C.

Discussion

Iron amino acid chelate has been proven to be one of the original models for animals to absorb Fe compound supported by studies on Fe absorption mechanism (Saltman, 1965). Many studies have showed that Fe-Gly has a high bioavailability in the bodies of rats, human beings and other animals, compared with $FeSO_4$, (Ashmead, 2001; Allen, 2002). Langini *et al*. (1988) feed weaning rats with infant formula food which added the same level of Fe-Gly or $FeSO_4$ marked by isotope [^{59} Fe]. The study showed that the iron absorption rate of $FeSO_4$ was 15.8%，while the rate of Fe-Gly was 30.9%. Layrisse *et al*. (2000) reported that the

Table 2: Effect of temperature on transport of Fe-Gly and $FeSO_4$ across Caco-2 monolayers (10 μmol/L, 37°C, 120 min)

Transport amount (pmol/cm^2)	AP→BL	
	37°C	4°C
$FeSO_4$	172.34±5.62^a	120.41±2.79^b
Fe-Gly	394.55±12.31^a	278.55±10.40^b

Note: Measurements without common letters in the same row are considered significantly different (P<0.05)

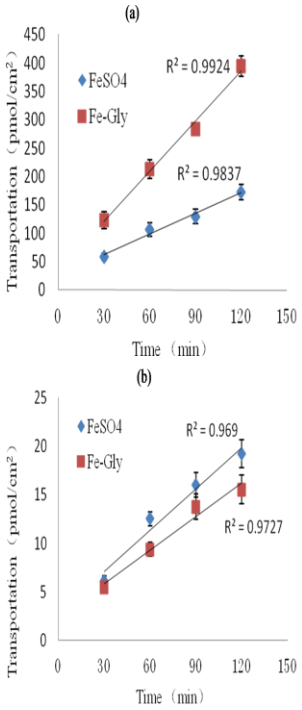

Fig. 3: Effect of time (0-120 min) on Fe transport across Caco-2 cell monolayers (37°C, 10μmol/L, 120 min). (a) shows the transport of Fe-Gly and $FeSO_4$ from apical to basolateral side. (b) is the results in the inverse direction. The regression lines indicate the positive correlation between transport amount and concentration of Fe

Fe absorption rate of Fe-Gly was higher than that of $FeSO_4$ by nearly 2-fold in human being body. Ashmead (2001) supplied Fe-Gly and $FeSO_4$ as Fe fortification for anemia, at the dose of 5 mg/kg weight. After 28 days experiment, the apparent biological utilization rate of Fe-Gly was 90.9%, but the rate of $FeSO_4$ was only 26.75% with plasma ferrtin were monitored as biomarker. Nielsen *et al*. (2005) conducted an experiment treating anemia with Fe-Gly offered as Fe fortificants for nutrition. After 6-week therapy, they found that the mean heme value of patients in the Fe-Gly treatment group was significantly higher than the value of inorganic iron group (12.1±1.8:10.7±1.7 g/dl). In America, Fe-Gly as one of new iron fortificants for nutrition has been applied to milks of infant and food (Fox *et al*., 1998; Giorgini *et al*., 2001).

This study was designed to compare the bidirectional transport of Fe-Gly and $FeSO_4$ using Caco-2 cells. It proved that Fe of Fe-Gly can be easily absorbed than that of $FeSO_4$ in Caco-2 cells. We found that the transepithelial transport of Fe-Gly and $FeSO_4$ were concentration- and time-dependent from both directions. And the transport amounts were much greater in the direction of AP to BL than those in BL to AP transports. The P_{app} of Fe-Gly and $FeSO_4$ decreased as the concentration increased and the P_{app} of transport from AP to BL divide by that in inverse direction is greater than 1.0 for the both two forms of iron sources. The culture temperature has significant effect on transport amount. These results suggest that the absorption of $FeSO_4$ and Fe-Gly in Caco-2 cells is mainly through active transport. It was also found that the transport amount of Fe-Gly was notably higher than that of $FeSO_4$ at the same concentration or temperature. All the results taken into account, we speculate that Fe-Gly may have specific or non-specific intestinal active transit system, except for some ionic iron dissociating from Fe-Gly before uptake and being transported through the apcial membrane follow the same pattern as $FeSO_4$ absorption.

The absorption mechanism of Fe-Gly is still unknown exactly. Zhu *et al*. (2006) proposed at least some iron dissociates from EDTA and is reduced just as simple inorganic iron at the brush border membrane of the enterocyte when study iron uptake by Caco-2 cells from NaFe-EDTA and $FeSO_4$. Pizarro *et al*. (2002) found that Fe-Gly competes for the absorption pathway of nonheme Fe. They enter the common nonheme Fe pool and activate the same transporter on the cells for transportion. They concluded that one reason for better absorption of Fe-Gly is glycine can chelate and protect Fe from inhibitors.

In conclusion, our work confirmed that part of Fe-Gly absorbed by the same pattern as $FeSO_4$ absorption mechanism, but it is likely that Fe-Gly is also using specific or non-specific intestinal active transit system. For further study, transport kinetics of Fe-Gly and relevant proteins of Fe transporter should be tested for revealing the mechanism.

Acknowledgements

This work was supported by the Project supported by the National Natural Science foundation (No.31272398), New-Century Training Program Foundation for Talents from the Ministry of Education of China (No. NCET-10-0727), and the Natural Science Foundation for Distinguished Young Scholars of Zhejiang province, China (No. R3110085).

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(Received 31 August 2012; Accepted 17 October 2012)