Bioavailability of iron in oral ferrous sulfate preparations in healthy volunteers

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The bioavailability of iron in five ferrous sulfate preparations was studied in 10 healthy male volunteers. The preparations were an oral solution, two types of film-coated tablets and two types of enteric-coated tablets. Blood samples were drawn hourly from 8 am to 6 pm on the day before each study day to assess baseline serum iron concentrations and on the study day. Spectrophotometry was used to measure the serum iron concentrations. The area under the curve (AUC), the maximum concentration and the time to achieve the maximum concentration were compared by analysis of variance. The enteric-coated preparations resulted in AUCs less than 30% of the AUC for the oral solution. The two film-coated products produced AUCs essentially equivalent to that of the oral solution. We conclude that the bioavailability of iron in the enteric-coated preparations was low, relative to that of the film-coated products and the oral solution, and that these products should not be considered interchangeable.

On étudie chez 10 volontaires masculins sains la biodisponibilité du fer dans cinq préparations de sulfate ferreux: une solution pour usage buccal, deux sortes de comprimés enrobés et deux sortes de comprimés glutinisés. La veille et le jour même de chaque essai on détermine toutes les heures, de 8 h à 18 h, la sidérémie par spectrophotométrie. On compare par l'analyse de la variance l'aire sous la courbe (ASC), la concentration maximum et le temps qu'il faut pour y arriver. Alors que les comprimés glutinisés donnent une ASC inférieure à 30% de celle qu'on obtient par la solution, celle que donnent les comprimés enrobés est, à toute fin pratique, équivalente à celle-ci. Vu cette différence de biodisponibilité on ne peut considérer ces préparations comme interchangeables.

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The absorption and bioavailability of iron is complex. Although absorption can take place along the entire intestine, it occurs primarily in the duodenum and the jejunum.[1,2] Factors that inhibit absorption, especially in the upper portion of the small intestine, would be expected to affect the bioavailability of iron administered orally. It has been reported that enteric coating of iron products may impair the hematologic response of patients with iron deficiency,[2,3] yet appropriate comparative bioavailability studies have not been performed.

Three cases in which enteric-coated ferrous sulfate tablets failed to alleviate iron deficiency anemia (see pages 565 and 566 of this issue) prompted us to evaluate the relative bioavailability of five ferrous sulfate products.

The impracticality of obtaining radioisotope preparations of each of the five products led us to select a study design that used nonisotope standard plasma iron determinations, as described by Chiou.[4]

Methods

Ten adult male volunteers gave informed written consent to participate in the study, which was approved by the Research Assessment Team and Research Ethics Committee of Sunnybrook Medical Centre, Toronto. All of the subjects were healthy according to their history, findings at physical examination, results of biochemical and hematologic tests and levels of serum ferritin. Nine were nonsmokers, and one was an occasional pipe smoker. Their ages were from 21 to 42 (mean 27.9 [standard deviation 10.5]) years. All were within 15% of their ideal body weight.

← For prescribing information see page 564

The subjects were not allowed caffeinated colas, tea, coffee or chocolate for 24 hours before and during the sampling period. They received 300 mg of each of five ferrous sulfate preparations, at intervals of at least 1 week; the order was determined randomly. The five preparations tested were a ferrous sulfate solution (Mead Johnson Canada, lot AD7506A), two types of film-coated ferrous sulfate tablets (Pharmascience Inc., lot X4578; and Novopharm Limited, lot 11A596), an enteric-coated ferrous sulfate product (Novopharm Limited, lot T123) with a core identical to that of the film-coated Novopharm product and another type of enteric-coated ferrous sulfate product (Apotex Inc., lot 71800).

An indwelling intravenous catheter was inserted into the forearm to permit blood sampling for 2 consecutive days. On the first day samples were drawn hourly from 8 am to 6 pm to assess baseline serum iron concentrations. On the next day a sample was collected at 8 am, the ferrous sulfate preparation was then ingested with 200 ml of water, and over the next 10 hours blood samples were collected hourly. All samples were centrifuged, and the serum was frozen until assayed. Spectrophotometry was used to determine the iron content.[5] In this assay thiourea is added to each tube to chelate copper and eliminate potential interference. This method has within-day and between-days coefficients of variation of less than 6% for all samples.

Data reduction and statistical analysis

After the iron concentration was determined for each sample the area under the concentration–time curve for both the baseline day and the study day was calculated from 0 to 10 hours with use of the trapezoidal rule. To establish relative bioavailability, areas determined on each baseline day were subtracted from those determined on the study day. The mean area under the curve (AUC) for each treatment was compared with the mean AUC for the oral solution to determine relative bioavailability.

Because of the apparent effect of blood sampling on serum iron concentrations a second estimate of relative bioavailability was obtained by subtracting the study day concentration at 8 am from all other concentrations determined that day. The concentration–time profiles were then calculated for each subject during each treatment. The mean AUC was calculated as previously described. A mean concentration–time profile was estimated. The highest concentration (C_{max}) apparent after subtraction in each of the profiles and the time to achieve this concentration (T_{max}) were also calculated.

The AUC, C_{max} and T_{max} for each treatment were compared statistically by analysis of variance (ANOVA). When data were subtracted, simple two-way ANOVA was used; when subtraction was not done or baseline data were considered a 2×5 factorial design was used. Fisher's protected least significant difference test and the Newman–Keuls multiple range test were used to find significant differences between the effects of the preparations. In addition, least squares linear regression was used to test the correlation between the 8 am baseline concentrations and baseline AUCs and the 8 am study day concentrations. A paired *t*-test was used to assess differences between the iron concentrations at 8 am on the baseline and study days. The *a priori* level of significance was less than 0.05.

A good estimate of the intrasubject coefficient of variation for the iron AUC was unknown before the start of the study. However, as a pharmacokinetic variable the AUC has had a between-days coefficient of variation of 10% on average in bioavailability studies.[6-9] If the coefficient for iron were 10% and a difference in AUC (bioavailability) of 10% were important, then 10 subjects would be required; this would allow for acceptable error rates ($\alpha = 0.05$; $\beta = 0.2$), as predicted by an *a priori* power calculation.[10]

Results

None of the subjects experienced vomiting or any significant untoward effects from the drugs. The only side effect attributed to iron was nausea (in one subject) on the day the solution was given.

When analysed by week of therapy the mean serum iron concentrations at 8 am differed significantly between weeks (Fig. 1). Furthermore, the baseline concentrations at 8 am were significantly higher than the study day concentrations at 8 am ($t = 2.75$, 49 degrees of freedom, $p = 0.008$); this trend was observed in all the subjects. Apparently negative serum iron concentrations occurred when the study day concentrations at 8 am were subtracted from other study day concentrations and when the baseline AUC was subtracted from the study day AUCs. Clearly, a negative concentration and bioavailability cannot occur; therefore, nega-

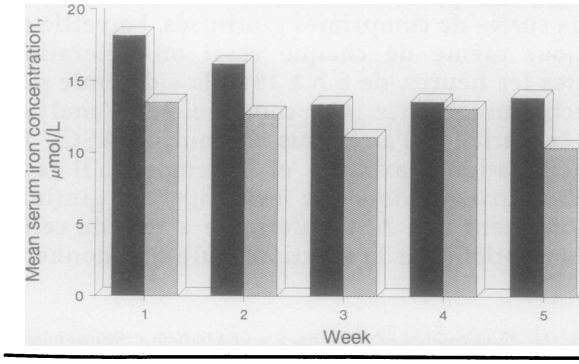

Fig. 1 — Mean baseline serum iron concentrations (black bars) and predose study day concentrations (striped bars) in samples collected at 8 am by week of therapy.

tive values reflected both the variability in concentrations and the trend for the concentrations to decrease with continued blood sampling.

The AUCs from 0 to 10 hours for each subject during each treatment were compared (Table I). Both types of enteric-coated tablets produced an AUC not significantly different from the baseline AUC. The two types of film-coated tablets and the oral solution resulted in concentrations significantly higher than the baseline values; however, the differences in AUC between the solution and either type of film-coated tablet were not significant. Subtraction of the baseline AUC from the study day AUC resulted in an AUC representative of the iron systemically available from each preparation (Table I). Less than 30% of the iron in the enteric-coated products was bioavailable, relative to the oral solution, whereas 100% of the iron in the film-coated products was bioavailable.

Table II shows the AUCs after the second estimate of bioavailability was done. Fig. 2 shows the mean concentration–time profiles for each treatment. There were significant differences (of 70 μmol·h/L or greater) between the enteric-coated preparations and the three other treatments. No significant differences were apparent between either of the film-coated preparations and the oral solution, and there was no difference between the two types of enteric-coated tablets. However, less than 20% of the iron in the enteric-coated products was bioavailable, relative to the oral solution, and 100% of the iron in the two film-coated preparations was bioavailable.

The observed mean C_{max} did not differ significantly between the solution and the film-coated preparations (Table II). The two enteric-coated preparations had a significantly lower C_{max} than the other products, although the differences between the enteric-coated preparations were not significant. A difference of at least 10 μmol/L between treatments would have been significant.

The mean T_{max} for each treatment is also given in Table II. It took less time to achieve the C_{max} with the oral solution and the film-coated products than with the enteric-coated products. The differences in T_{max} between the enteric-coated products and between the oral solution and the film-coated preparations were not significant; a difference of 2.0 hours would have been significant.

Discussion

Baseline values were thought to be necessary to evaluate the serum iron concentrations before treatment. Because of the study design various methods can be used to calculate bioavailability. All require subtraction and include the methods

Table I — Area under the curve (AUC) for serum iron concentrations (μmol · h/L) on the baseline and study days

Subject no.	Oral solution		Film-coated products				Enteric-coated products			
			Pharmascience		Novopharm		Novopharm		Apotex	
	Baseline	Study	Baseline	Study	Baseline	Study	Baseline	Study	Baseline	Study
1	284.96	296.19	142.48	347.27	157.22	377.42	150.02	171.96	192.87	307.71
2	82.43	248.14	60.35	183.24	74.58	321.31	126.94	150.11	102.06	164.52
3	127.24	219.02	219.50	367.07	87.13	233.05	135.62	157.18	92.24	113.90
4	164.48	211.88	112.88	122.45	69.18	149.14	143.74	86.48	150.83	156.70
5	149.12	160.82	114.89	196.39	119.57	310.09	141.92	103.63	170.24	143.23
6	107.58	225.82	136.61	218.42	58.99	99.66	183.43	161.51	124.42	150.74
7	80.45	227.06	174.29	445.26	68.14	269.20	148.42	93.85	99.08	88.68
8	109.18	382.04	156.96	235.56	187.70	195.59	174.83	147.65	161.21	191.28
9	75.53	292.54	75.34	309.13	135.98	83.50	87.89	91.00	95.94	70.50
10	249.37	257.60	205.65	121.09	198.92	216.48	195.54	116.53	156.86	222.96
Mean	143.03	252.11	139.90	254.59	115.74	225.54	148.84	127.99	134.58	161.02
Standard deviation (SD)	72.07	60.43	51.64	108.52	52.08	96.72	30.73	32.94	36.28	68.61
95% confidence limits										
Upper	193.81	294.69	176.28	331.05	152.42	293.69	170.48	151.20	160.14	209.36
Lower	92.22	209.53	103.51	178.12	79.04	157.40	127.19	104.78	109.01	112.68
Mean AUC*		109.08		114.69		109.80		−20.85		26.44
SD		92.20		106.51		104.34		37.02		44.78
95% confidence limits for mean AUC*										
Upper		174.04		189.74		183.32		5.24		58.00
Lower		44.12		39.65		36.29		−46.93		−5.11
Bioavailability, %†		100		105.15		100.67		−19.11		24.25

*After subtraction of baseline AUC from study day AUC.
†Relative to oral solution.

used in this study. However, regardless of the methods used the results are similar, as demonstrated by the two estimates of bioavailability reported here.

This study further demonstrates not only the need for more critical evaluation of which ferrous sulfate preparations are interchangeable but also the problems inherent in the design of such a study. Although some method of subtraction must be used to calculate bioavailability the need for an entire day of blood sampling is questionable, since we observed similar results with the two methods. Furthermore, these two methods displayed variability greater than that observed in most bioavailability trials. In most pharmacokinetic studies the AUC generally has a coefficient of variation of between 10% and 15%.[6-10] However, in our study the baseline AUCs from week to week had an intrasubject coefficient of variation of 29% on average, as compared with 36% for the predose study day AUCs. This unexpectedly large variability means that differences of 10% cannot be confidently detected. However, the differences observed between the enteric-coated products and the oral solution or either of the film-coated preparations can still be reported with confidence since they were so large. In fact, there is less than a 1% chance that the bioavailability of iron in either of the enteric-coated products is greater than 50%, relative to the oral solution.

Conclusions

The film-coated preparations and the oral solution achieved peak concentrations significantly sooner and had higher maximum concentrations than the enteric-coated products. We conclude that enteric-coated ferrous sulfate preparations have extremely low bioavailability, relative to film-coated products and oral solutions, and that the enteric coating is solely responsible for this effect, since the only difference between the Novopharm enteric-coated and film-coated products was the coating.

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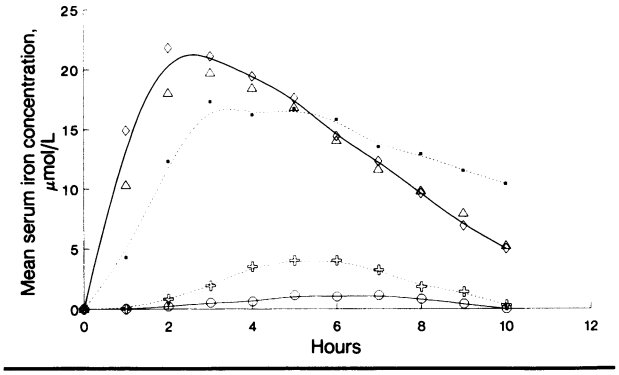

Fig. 2 — Mean serum iron concentration–time profile for each ferrous sulfate treatment, as determined by subtracting study day concentration at 8 am from all other study day concentrations. Diamonds represent oral solution, triangles Pharmascience film-coated product, dots Novopharm film-coated product, crosses Apotex enteric-coated product and circles Novopharm enteric-coated product.

Table II — AUC after subtraction of study day serum iron concentration at 8 am from all other study day concentrations

Subject no.	Oral solution	Film-coated products		Enteric-coated products	
		Pharmascience	Novopharm	Novopharm	Apotex
1	134.52	218.32	277.85	25.53	101.61
2	158.15	128.01	221.31	40.12	6.98
3	130.90	246.07	162.53	12.04	6.28
4	101.15	44.32	69.55	7.93	38.83
5	47.74	56.37	180.08	0	15.20
6	115.83	25.47	24.28	1.92	1.99
7	167.26	245.00	156.20	13.85	20.54
8	188.72	78.20	66.04	9.23	41.39
9	232.54	219.12	33.50	1.00	0.50
10	148.04	48.04	86.47	46.50	19.17
Mean	142.49	130.89	127.78	15.81	25.25
SD	50.26	91.61	84.68	16.37	30.33
95% confidence limits					
Upper	177.89	195.43	187.44	27.35	46.62
Lower	107.08	66.35	68.12	4.27	3.87
Bioavailability, %	100.00	91.86	89.68	11.10	17.72
Maximum concentration, µmol/L	22.10	20.40	19.20	3.40	4.90
Time to achieve maximum concentration, h	2.32	3.11	3.74	5.69	5.60

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Upcoming Meetings

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Le 12–14 oct 1989: L'Association des médecins de langue française du Canada 61e Congrès-exposition international: Un coeur en santé pour mieux vivre
Centre de conférences du complexe Guy Favreau et la Place du Complexe Desjardins
Suzanne de Montigny ou Diane Bircher, Association des médecins de langue française du Canada, 510–1440 ouest, rue Sainte-Catherine, Montréal, PQ H3G 2P9; (514) 866-2053, FAX (514) 866-0506

Oct. 12–14, 1989: Société québécoise de biochimie clinique 10th Annual Meeting
Hôtel Plaza de la Chaudière, Hull, PQ
Dr. Jean Alain, Department of Biochemistry, Centre hospitalier de Gatineau, PO Box 2000, Gatineau, PQ J8P 7H2; (819) 561-8349

Oct. 12–14, 1989: Urologic Oncology, Today and Tomorrow (in cooperation with the University of Toronto, Toronto Hospital and the US National Institutes of Health)
Metro Toronto Convention Centre
Credits: 19 hours, category 1, American Urological Association
Jeanie McGoldrick, Stratagem Communication, 604–2 Sheppard Ave. E, Willowdale, Ont. M2N 5Y7; (416) 229-2331, FAX (416) 229-6443

Oct. 13–14, 1989: Arthritis Health Professions Association Annual Conference: War Games Versus Teamwork — a Positive Approach to Problem Solving
Glenway Country Club, Newmarket, Ont.
Dr. J. Carter Thorne or Ieva Fraser, York County Hospital, 596 Davis Dr., Newmarket, Ont. L3Y 2P9; (416) 895-4521, ext. 2299

Oct. 14, 1989: "Hands-On" ENT for the Family Physician
Le Meridien Hotel, Vancouver
Continuing Medical Education, 105–2194 Health Sciences Mall, University of British Columbia, Vancouver, BC V6T 1W5; (604) 228-2626

Oct. 16–17, 1989: Cardiopulmonary Monitoring of the Critically Ill Patient
Royal York Hotel, Toronto
Conference and Seminar Services, Humber College Professional Services, 205 Humber College Blvd., Etobicoke, Ont. M9W 5L7; (416) 675-5077, FAX (416) 675-0135

Oct. 16–17, 1989: Focus on Patient Assessment
Hotel Beausejour, Moncton, NB
Maggie Swithenbank, program manager, Conference and Seminar Services, Humber College Professional Services, 205 Humber College Blvd., Etobicoke, Ont. M9W 5L7, (416) 675-5077, FAX (416) 675-0135; or Gwen Villamere, chairperson, Continuing Education in Nursing, (416) 249-8301

Oct. 18–20, 1989: Quality Assurance in Health Care
World Trade and Convention Centre, Halifax
Ingrid Norrish, director, Conference and Seminar Services, Humber College Professional Services, 205 Humber College Blvd., Etobicoke, Ont. M9W 5L7; (416) 675-5077, FAX (416) 675-0135

Oct. 19–20, 1989: Psychiatric Occupational Therapy: Preparing for the '90s
Clarke Institute of Psychiatry, Toronto
Patti Pettit or Marge Murphy, Education Office, Clarke Institute of Psychiatry, 250 College St., Toronto, Ont. M5T 1R8; (416) 979-6845

Oct. 19–21, 1989: Learning Disabilities Association of Canada 7th National Conference: Lighting the Way
Hotel Newfoundland, St. John's
Judy Davis, conference coordinator, 12 Colville St., St. John's, Nfld. A1E 3J8; (709) 739-0611 or (709) 579-7273

Oct. 19–21, 1989: Practical Orthopaedics for the Primary Care Physician
Sheraton Cavalier and Theatre in the Mall, University Hospital, Saskatoon
Continuing Medical Education Office, University Hospital, Saskatoon, Sask. S7N 0X0

Oct. 20, 1989: La Leche League Manitoba Conference: Controversies in Breastfeeding
St. Boniface Hospital Research Centre, Winnipeg
Credits: 5 hours, CFPC
Leslie Sanders, 321 Marlton Cr., Winnipeg, Man. R3R 1A6; (204) 832-4180

Oct. 20, 1989: University of Western Ontario's Research Day IV in Family Medicine
Sheraton Armouries Hotel, London, Ont.
Mrs. Toula Gerace or Dr. John Sangster, Byron Family Medical Centre, 1228 Commissioners Rd. W, London, Ont. N6K 1C7; (519) 472-9670

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