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Use of Physiological Modulators in the Management of Anemia due to Menstrual Blood Loss

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Abstract

Background

The use of a combination of physiological modulators has been shown to be effective in restoring hemoglobin (Hb) in women with excessive menstrual bleeding. The improvement of Hb was much more than expected.

Methods

This is a single blind, placebo-controlled clinical trial in 20 anemic women (Hb < 12g/dL) with heavy menstruation. Hemoglobin, iron balance, iron intake, cell count, blood oxygen saturation (SO2), heart rate, blood glutathione (tGSH) and menstrual bleeding were measured after treatment with placebo and a mixture physiological modulators (Agent F) for 28 days.

Results

All parameters improved significantly by the treatment with Agent F, particularly the Hb levels which rose from 10.0 ± 1.41 to 12.8 ± 0.6 mg/dL. The iron related variable (transferrin, ferritin and iron) increased together with the erythrocyte count, SO2, and tGSH, whereas the menstrual blood flow decreased. The improvement of Hb was directly correlated with SO2, platelet count increase and menstrual bleeding decrease.

Conclusions

The treatment with Agent F mobilized the iron storage, increased the platelet count, increased Hb synthesis and reduced the menstrual bleeding. The SO2 and heart rate may be surrogate variables to monitor the improvement of anemia.

Keywords: Iron Deficiency Anemia; Heavy Menstrual Flow; Physiological Modulators; 190 w; 3276 w; 3 Tables

Introduction

Heavy menstrual bleeding is a common problem among women of reproductive age which may affect between 3 % to 19 % of women depending upon ethnicity and race [1-3].

The impact of menstrual blood flow and diet on iron deficiency in women have been previously reported [4-7]. Usually dietary iron intake is considered insufficient to prevent anemia due to menstrual blood loss.

Heavy bleeding during menstruation (> 80 mL) is also a cause of discomfort and poses a consistent annual economic

burden. The treatment of this type of anemia is based on iron supplementation at variable dosages, ranging between 14 mg/day [8] to 60 mg/week or 60 mg/day only during menstruation [9].

In this study the approach to manage anemia was to administer physiological modulators (PM) based on to use natural compounds (physiological) capable of regulating biological functions (modulation). Daily administration of a low quantity of iron, ascorbic acid, and essential amino acids was found to be effective in the recovery of the anemic condition [10]. The aim of this study was to validate previous observations and by adding with some additional variables such as blood oxygen saturation (SO2, heart rate) and blood glutathione (in terms of GSH+, GSSG or tGSH). Agent F was administered daily to women with heavy menstrual bleeding at dosages that allow a projected increase of 5 mL of blood/day.

Material and Methods

Subjects

Twenty women in the age range of 28-43 years were admitted with BMI > 22 to < 25.

Inclusion Criteria

Women with abundant or excessive menstrual flow and Hb < 12 g/dL were included. The enrollment was done in the Irwin Labs (Spoltore-Pescara-Italy) in women addressed to the center by their family doctors. For the admission criteria, the quantity of menstrual blood loss was measured twice based on a score and only women with scores ≥ 4 were admitted with hemoglobin (Hb) <12 g/dL. Two baseline evaluation of menstrual flow score were taken, the first at the moment of the enrollment and the second after the placebo treatment.

Exclusion Criteria

Women were excluded if they had a menstrual blood flow with a score discrepancy of > 1 between the two baseline measures. Subjects under treatment for anemia as well as any type of gastric, neurological, psychiatric disease and any type of cancer were excluded. Concomitant diseases such as hypertension, dyslipidemia and diabetes type II were not considered exclusion criteria provided the corresponding treatment had been stable for at least 2 months and did not change during the trial.

Agent F (Combination of Physiological Modulators)

The dosages of Agent F components were optimized considering an average concentration of Hb of 12 g/dL in women with an average weight of 60 Kg. The blood and plasma volumes were standardized to 4.8 L and and 3.0 L, respectively. The concentration of iron (Fe) in Hb accounts for 0.3464 %, considering the molecular weights of Hb and iron 64.5 KD and 223.4 D, respectively. The complete formula of agent F is reported in the Table 1

Table 1. Composition of the Agent F.

Ingredients	Quantity	Ingredients	quantity
L-Lysine	57.5 mg	Vitamin C	15 mg
L-Histidine	49.5 mg	Iron	2.5 mg
L-Threonine	45 .0 mg	Vitamin B1	0.35 mg
L-Valine	86.5 mg	Vitamin B2	0.4 mg
L-methionine	10.0 mg	Nicotinamide	4.5 mg
L-Leucine	89.5 mg	Vitamin B6	0.5 mg
L-Phenylalanine	38.5 mg	Folic acid	50.0 mcg
L-tryptophan	9.5 mg	Vitamin B12	0.5 mcg

It was prepared to support the daily production of 5 mL of blood. The amount of essential amino acids to be administered daily was based on the relative quantity of each to produce 0.7 g of Hb (14 % of 5 ml of blood). Agent F was prepared in the form of dry sachet and contained appropriate excipients and flavoring to make the taste acceptable The formula have been described in a previous paper also [10].

Study Design

A placebo identical to Agent F in the form of dry sachet containing arabic gum and flavoring was given starting immediately after the second menstrual flow and continued for 28 days. During the third cycle (even during the menstruation) Agent F was administered in the form of dry sachets to be taken diluted with half a glass of water. The products (placebo and Agent F) were administered in the evening immediately before going to bed and at least two hours after the evening meal. Precise written instructions were given to avoid interference with the food and to take advantage of the peak of gastric acidity during the night which allow a better iron absorption. There was no limitation in the use of tea or coffee provided that the beverages were not taken after the Agent F or the placebo administration. Subjects were given 3 boxes of 10 dry sachets of placebo or Agent F depending on their assigned treatment group. The compliance was assured through the count of the residual sachets at the time of final evaluation. All the subjects were asked to maintain a constant diet and level of activity and to avoid any food supplement or drug for the treatment of anemia.

Menstrual Flow Score

Two variables were measured. The first was the menstrual bleeding which was scored from 1 to 5 based on the number of tampons and/or pads as follows: 1-normal (< 22 tampons/

pads); 2 –abundant (23-29 pads); 3 high- (30-35 tampons/pads); 5-very high (> 35 tampons/pads). The second was the duration of menstruation which was scored from 1 to 3, where 1 means < 2 days, 2 means 3 days and 3 means > 4 days. The total score was the sum of the two scores that could be from 2 to 8.

In case of the calculation of the menstrual flow all the adsorbents used during the menstrual flow were kept by the participants into a plastic bag at 4 ^{O}C. The plastic bag was purposely prepared to minimize the evaporation of the contents. The difference in grams between the weight of tampons/pads when they were dry and after the use was considered a measure of blood loss. This procedure was found to be quite complex and was limited to four medical professional operators (see Table 2) and used only to support the validity of score progression.

Blood Variables

Blood sampling was taken twice (baseline and after treatment) by venipuncture of 16 mL of blood divided into four test tubes (one for serum isolation, two added with citrate, and one with EDTA). Serum was kept at -80 °C for the analysis of tGSH.

The following variables were considered: Hb concentration (mg/dL), hematocrit (Ht) (%), erythrocyte count (106), reticulocytes count (%), platelet count, ferritin (ng/mL), transferrin (mg/dL), mean corpuscular volume (MCV) in fL, mean corpuscular hemoglobin (MCH) in pg, serum iron levels (µg/dL), heart rate (beats/min), and oxygen saturation (SO2). Venous Hb and complete blood count were analyzed using an automated instrument (Cell-Dyn 4000, Abbott Laboratories).

Table 2. List of variables measured at baseline and after 28 days of treatment with placebo (baseline) and after 28 of treatment with Agent F. Average values ± SD.

Variable	Measure	Baseline (placebo)	28 days (F)	Dunnet
Age	Years	34.7 ± 4.6		
Body weight	Kg	53.5 ± 3.01		
Hb	g/dL	10.0 ± 1.41	12.8 ± 0.62	p <0.01
Ht	%	36.2 ± 3.45	40.7 ± 4.53	p <0.01
Fe	mcg/dL	51 ± 10.3	65 ± 9.3	p <0.01
Transferrin	mg/dL	308 ± 30.7	339 ± 34.4	p <0.01
Ferritin	ng/mL	97 ± 22.6	118 ± 30.4	p <0.01
RBC	10^6	3.95 ± 0.221	4.17 ± 0.141	p <0.01
Reticulocytes	%	1.7 ± 0.73	2.0 ± 0.60	p <0.01
Platelets	10^3	119.4 ± 12.63	145.3 ± 13.25	p <0.01
MCV	fL	91.9 ± 9.08	97.7 ± 10.26	p <0.01
MCH	pg	25.2 ± 3.09	30.8 ± 1.97	p <0.01
tGSH[a]	meq/L	1.41 ± 0.349	1.54 ± 0.392	P< 0.01
Heart rate	beats/min	78 ± 2.6	72 ± 2.27	p <0.01
SO2	%	93.9 ± 1.05	96.3 ± 0.82	p <0.01
Menstrual flow	score	5.3 ± 1.69	3.3 ± 1.24	p <0.01

[a]The tGSH was measured in 10 cases only.

Serum iron was determined with a chemistry analyzer (LX 20, Beckman-Coulter) as were ferritin and transferrin (Immunolite 2500, Diagnostic Product Corporation). SO2 and heart rate were measured with a SIM fingertip pulse oximeter (SFPO-01). In a set of 10 subjects it was also possible to measure the whole blood tGSH according to the method described by Richie, et al [11].

Iron Intake

The iron content in foods was calculated twice, the first during the second week of placebo treatment and the second during the second week of Agent F treatment. A questionnaire [12] was used where the weekly intake of different portions of cereal, meat, fish, fruit, vegetables, dairy products, grains, alcohol (wine, beer, and other alcoholic drinks), other beverages (coffee, tea, soft drinks) was reported. The iron content of foods was according to the INRAN tables [13].

Statistical Analysis

Hb was considered as a primary variable. Twenty was determined to be and adequate number of cases to obtain a power 90 % with an α 0.05 and 1-β 0f 90 comparing two sets of interdependent data (Dunnet). Average values and SD were calculated for all the data. For all the variables the coefficient of correlation "r" between the difference before and after the treatment with Agent F (placebo vs Agent F) was calculated taking α 0.01 as limit of significance. No correlation was possible with tGSH due to the limited number tested (10 cases only).

Results

Nineteen subjects completed the study, the compliance was > 95 % and no side effects were reported. One patient was excluded after the treatment with placebo due to a difference > 1 in the bleeding baseline score. Data are reported in Table 2.

Table 3. Bleeding score and menstrual blood flow.

First measure		Second measure		Third measure*	
Bleeding score	Blood loss [g]	Bleeding score	Blood loss [g]	Bleeding score	Blood loss [g]
4	65	4	67	2	35
8	85	8	88	6	71
6	71	6	73	4	51
7	87	7	85	5	59
Correlation coefficient "r" = 0.9472 p<0.01					

*After the treatment with Agent F.

The tGSH was measured in 10 cases only due to technical reason (poor condition of the samples treated with EDTA). All the variables increased, whereas of the heart rate decreased in some subjects. The difference between the two sets of data (before and after 28 days of treatment) were statistically significant.

The correlation between the bleeding score and the menstrual blood loss was found to be statistically significant and the relative data corresponding to the four women are listed (see Table 3). As previously described, the first and the second measure were taken respectively before and after placebo as a variable for the admission criteria, the third was taken under the treatment with Agent F.

The average daily intake of iron during the placebo treatment was 9.9 ± 0.59 mg/day (range 8.9-11.1 mg/day). A similar quantity was calculated during the treatment with Agent F, on average 9.7 ± 0.56 mg/day (range 8.9-10.8 mg/day). The total intake of iron in the two 28-day periods was almost identical and a calculated average for the placebo was 277 mg, whereas during the Agent F treatment the amount was 342 mg considering that 70 mg that were contained in the Agent F.

Table 4. Correlation matrix among the difference between placebo values and after F administration baseline: value of "r".

	Hb	Ht	Fe	Trans	Ferr	RBC	Ret	Plat	HR	SBO2	score
Hb	1										
Ht	-0.310	1									
Fe	0.176	-.094	1								
Trans	-0.188	-.460	0.293	1							
Ferr	-.657*	0.353	0.122	0.105	1						
RBC	0.149	0.051	0.202	-.002	0.425	1					
Ret	-0.372	-.031	-.334	-.048	0.189	-.186	1				
Plat	-0.279	0.386	-.232	-.217	0.002	-.179	-0.219	1			
HR	-0.218	-.174	0.143	0.331	0.283	0.202	0.431	-0.129	1		
SBO2	0.707*	-.162	0.175	0.112	-.661*	-.053	-0.110	-0.236	-0.092	1	
Score	-0.207	0.265	-.337	-.212	-0.158	-.274	-0.084	0.885*	-0.049	-.124	1

Hb-hemoglobin; Ht-hematocrit; Fe-plasma iron; Trans-transferrin; Ferr-ferritin; RBC- erythrocytes count; Ret- reticulocytes %; Plat-platelets count; HR-heart rate; SBO2-oxygen saturation; score-menstrual bleeding

*p <0.01

The correlation among the differences between placebo values and after the Agent F treatment are reported in Table 4.

The tSGH values that were measured in 10 cases could not be correlated with any of the other variables due to the limited number of data points.

A significant negative correlation was found between ferritin vs Hb and vs SO2. A significant positive correlation was seen between SO2 vs Hb and between platelet count vs bleeding score.

No significant correlation was found among all the other variables.

Discussion

The results of the current study confirm what was shown in a previous report (10) despite the fact that the two studies had the same limitations of a short period of treatment.

The anemic condition in general is improved using high quantity of iron, between 30 to 80 mg of elemental iron given for 3 to 6 months,that may cause gastric discomfort (nausea, flatulence,abdominal pain, constipation and diarrhea). Gastrointestinal symptoms occur up to 30 % of the people taking oral iron that may end up with a reduced adherence to the supplementation and in some case to the therapy withdrawal also[14]. Modified release iron preparation have been shown in a meta-analysis study to be no better tolerated than standard formulations [15] Usually 20 mg/day of iron is considered as "low effective dose". [16]. Despite the side effects of ferrous sulfates were not shown to be dose dependent, the meta-analysis of the clinical trials reported in the literature was related to dosages between 20 and 80 mg/day only without considering lower dosages [16,17]. The injection of iron directly into the blood or intramuscular is not free of side effects and cannot be considered a valid alternative approach due to the complexity of the treatment that should be used in case of severe anemia only [14].

In the literature there are no study reporting the reduction of blood loss during menstruation, a part of the use of oral contraceptives.
Another important point is the cost related to the anemic condition monitoring that usually is undertaken measuring many variables such as ferritin and transferrin which represent a substantial part of the cost.

In the current study we showed that following the concepts of Physiological Modulation the menstrual blood flow can be reduced and the anemia can be reversed. Furthermore the monitoring of the anemic condition can be easily carried out using simple variables such as heart rate and SO2

In this study the increase of iron bioavailability cannot be explained on the basis of the iron intake (about 10 mg/day with food + 2.5 mg/day with Agent F) since the quantity of iron contained in the Hb after the treatment was much higher than the amount that was administered even considering a complete absorption.

The blood loss was measured using a score and showed a reduction of 2 points which correspond to 15-30 mL of blood. This quantity can be important for the daily discomfort but is not consistent with the modification of the total Hb level improvement since 30 mL of blood- with 12.8 g/dL of Hb- corresponds to a total amount not exceeding 3.8 g of Hb

Blood loss reduction can be due to the increase of platelet count ("r"0.885; $p<0.01$) that has a favorable impact on coagulation. However, this high correlation has to be taken with caution since the two variables belong to different scales (parametric for platelets and non-parametric for bleeding) and, the bleeding score has the bias of a ceiling effect.

The improvement of erythrocytes in terms of count and Hb content (as MCV and MCH) can be a factor that may mechanically limit the blood loss through the micro-vessels and vascular lacunaes of the uterus. This effect may belong to a more efficient pentose phosphate cycle supported by the vitamin B group administration that provides more ATP in the erythrocytes. making them more vital. The ascorbic acid content in Agent F, a part of improving iron absorption, can make the vessels more dynamic also.

Two further possibilities have to be considered: the iron mobilization from the reserve and the Hb synthesis. Both these variables were improved.

The increase of transferrin, ferritin, and iron reflect a more efficient iron mobilization.

Agent F was administered before bed time when presumably the duodenum was almost empty. We tried to speculate that unknown factors were triggered in the gut by the presence in duodenal mucosa of a combination of iron and essential amino-acids in the same proportion of Hb.

Mobilization of iron from hemosiderin and ferritin belongs to the capacity of making the ferrohydrite soluble, which is supposed to be determined by the abundance of ascorbic acid and GSH (reduced glutathione). Ferrohydrite is known as the insoluble iron compound strored in both ferritin and hemosiderin. The tGSH increased after treatment with Agent F meaning that this mechanism can support the iron bioavailability within the cells where Hb synthesis takes pace and also protect the Hb from the oxidation within the erythrocytes

Conclusions

The use of Physiological Modulators improves the anemic condition and reduces the blood loss in women characterized by excessive blood loss during menstruation. Additional research is needed to answer all the questions arising from the current study. Some data seem to be consistent, for example the reduction of bleeding in relation to the improvement of cell count, particularly platelets. Other results such as the increase of iron bioavailability for Hb synthesis need further research. The increase of tGSH seems to be in line with the hypothesis of a more efficient iron availability for Hb synthesis.

Furthermore, simple and non-invasive variables such as heart rate and SO2 may give an objective measure of the anemia improvement and can be helpful to monitor anemia as surrogate low cost variables.

Aknowledgments

UC designed the research, analyzed the data and wrote the paper; GB performed the research; JF wrote the paper.

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Competing Interest

The authors declare they have not competing interest.

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