Effects of Iron Polymaltose Complex, Ferrous Fumarate and Ferrous Sulfate Treatments in Anemic Pregnant Rats, Their Fetuses and Placentas

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Abstract: Although oral iron preparations are widely prescribed to prevent and to treat iron deficiency anemia in pregnancy, comparative data on their effects to the mother, fetus and placenta are limited. In this study, the effects of oral iron polymaltose complex (IPC), ferrous fumarate (FF) and ferrous sulfate (FS) were compared in anemic pregnant rats, their fetuses and placentas. Hematological variables and oxidative stress markers in the liver, heart and kidneys of the dams and fetuses as well as the markers for oxidative stress, inflammation and hypoxia in placentas were assessed. Pregnancy outcome was measured by number of fetuses, and by neonate and placental weight. All therapies were comparably effective in correcting anemia. FS and FF, but not IPC, resulted in liver damage in dams and oxidative stress in dams, fetuses and placentas. FS group presented the highest catalase and GPx levels in dams, fetuses and placentas. IPC, but not FF or FS, restored normal TNF-α and IL6 expression levels in placentas whereas FS-treated animals presented the highest cytokine levels, suggesting a local inflammatory reaction. Anemia-induced high levels of HIF-1α were partially lowered by IPC and FF but further elevated by FS. Most of the negative effects associated with IDA were resolved by IPC treatment. Especially FS treatment was found to elicit hepatic damage in the dams, oxidative stress in the dams, fetuses and placenta as well as inflammation and high levels of HIF-1α in the placenta. Pregnancy outcome of FF- and FS-treated animals was worse than that of IPC-treated animals.

Keywords: Ferrous fumarate, ferrous sulfate, fetus, iron deficiency anemia, iron polymaltose complex, oxidative stress, placenta, pregnancy.

INTRODUCTION

The high iron demands during pregnancy increase the requirement for iron absorbed from the diet, which, however, is often inadequate to protect a pregnant woman from developing iron deficiency anemia (IDA) [1]. The presence of IDA during pregnancy is associated with adverse consequences in the mother, fetus [2], and, depending on its severity, placenta [3]. Therefore, WHO recommends iron supplementation throughout pregnancy [4].

The most commonly used oral iron preparations to prevent and to treat mild to moderate IDA during pregnancy are ferrous [Fe(II)] sulfate (FS), ferrous fumarate (FF), and ferrous gluconate [5]. These compounds are frequently associated with significant gastro-intestinal (GI) side effects including nausea, vomiting, heartburn, abdominal pain and constipation [6], all of which may reduce the compliance to therapy, a common issue in pregnant women prescribed iron therapies [7]. These side effects are most likely caused by Fe(II)-induced oxidative stress in the GI system [8]. Moreover, iron from FS is rapidly absorbed in the blood, largely by an uncontrolled way [9], leading to high levels of transferrin saturation and to significant amounts of non-transferrin bound iron (NTBI) [10, 11], which can induce oxidative stress [12].

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IDA in pregnant women has been associated with oxidative stress, attributed to overproduction of reactive oxygen species (ROS) and/or to a deficiency of antioxidant defenses [13]. During pregnancy, oxidative stress has the potential to increase the risk of congenital defects, preterm delivery and low birth weight of the fetus [2]. Thus, it is important that iron therapy during pregnancy does not cause oxidative stress.

Iron(III)-hydroxide polymaltose complex (IPC) is a stable, orally-administered non-ionic Fe(III) preparation, which has been shown to effectively correct IDA during pregnancy [14, 15]. The toxicity of IPC is low, with an LD_{50} value in rats >2,800 mg iron/kg body weight (bw) compared to 255 mg iron/kg bw for FS [16]. The iron from IPC is essentially taken up *via* regulated active mechanism and only very low levels of NTBI have been detected after administration of a therapeutic dose of IPC [10]. Thus, the risk of NTBI-induced oxidative stress is very small.

To date, comparative data on the negative effects of different oral iron preparations during pregnancy are limited [17, 18]. We have developed an animal model to assess the various effects of oral iron treatment by analyzing the oxidative stress markers in the placenta and in various organs of pregnant rats and of their fetuses. We have recently reported the effects of IDA and its treatment with IPC in this model [19]. In the current paper, we compare the effects of IPC, FF and FS treatments in pregnant rats with IDA.

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METHODS

Animals and Housing

All experiments were approved by the Animal Care Committee of Hospital Alemán, Buenos Aires, Argentina, and were undertaken according to the NIH Guide for the Care and Use of Laboratory Animals. All rats were housed in a temperature-controlled room (23 ± 2 °C) with free access to tap water.

There were five experimental groups, each of which included eight pregnant female Sprague-Dawley rats: untreated non-anemic animals (control group), untreated anemic animals (anemic group), anemic animals treated with IPC [Maltofer-Fol®, Vifor (International) Ltd., St. Gallen, Switzerland] (IPC group), anemic animals treated with FF (Anemidox Ferrum®, Merck Sharp & Dohme, Inc, Buenos Aires, Argentina) (FF group), and anemic animals treated with FS (Fer-In-Sol, Mead Johnson, Buenos Aires, Argentina and Acifol, Laboratorios Dominguez, Buenos Aires, Argentina) (FS group). The FF-treated animals were studied in an identical study, in which the control, anemic and IPC group had identical results as in this study but they were not included in the original publication [19]. Instead, the results for the FF group are presented in this article to enable direct comparison between IPC, FF and FS.

To obtain the control group, 21-day old male and female rats were fed on Universal Basal Diet (TestDiet® Formula #5755, PMI International, Richmond, IN, USA) with normal iron content (60 ppm) for eight weeks, after which male-female couples were housed together. To obtain the anemic groups, 21-day old male and female rats were fed with Low-Iron Purified Diet (10–20 ppm; TestDiet®, Formula #5859, PMI International, Richmond, IN, USA) for eight weeks. Male-female couples with hemoglobin (Hb) ≤ 9 g/dL [evaluated with HemoCue (HemoCue, Ängelholm, Sweden)] were then housed together. Mating was confirmed by detection of a vaginal plug, and this day was denoted day 0.

Treatments and Sampling

Treatment groups received oral IPC, FF and FS, at a dose of 2 mg iron/kg body weight (bw) + 7 μg folic acid/kg bw daily by gavage with a feeding tube. Treatment was initiated on day 0 and was adjusted weekly based on the bw of each animal.

Blood samples were obtained before and at the end of pregnancy. On day 21 of gestation, dams underwent a Cesarean surgery to obtain fetuses and placenta. After obtaining blood samples, dams were sacrificed and the organs, fetuses and placentas were processed as described previously [19].

Biochemical Procedures

Maternal Hb was determined by SYSMEX XT 1800i (Roche Diagnostic GmbH, D-68298 Mannheim, Germany). Serum iron, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) levels were measured with Autoanalyzer Modular P800 (Roche Diagnostic) with the corresponding reagent. Serum transferrin was determined by radial immunodiffusion (Diffu-Plate, Biocientifica, S.A., Buenos Aires, Argentina). Transferrin saturation (TSAT) was obtained by chemical methods.

Oxidative Stress Markers

Fractions of the whole placenta and the liver, heart and kidneys from mothers and fetuses were individually homogenized (1:3, w:v) in ice-cold 0.25 M sucrose. The ratio of reduced glutathione to oxidized glutathione (GSH:GSSG) was determined as previously described [20-22] in the supernatant obtained after centrifuging the homogenate fractions at 10 000 × g for 30 min. Glutathione peroxidase (GPx) and Cu,Zn superoxide dismutase (Cu,Zn-SOD) activities were measured as previously described [23, 24] in the supernatant obtained after centrifuging the homogenate fractions at 105,000 × g for 90 min.

Further fractions of the corresponding perfused tissues were homogenized (1:10, w:v) in 0.05 M sodium phosphate buffer (pH 7.4). Part of the homogenates were used directly to determine malondialdehyde to evaluate lipid peroxidation by thiobarbituric acid reactive species (TBARS) [25] and part was centrifuged at 4 °C for 15 min at 9,500 × g. Catalase activity was measured in the supernatant as previously described [26].

Enzyme units (U) were defined as the amount of enzyme producing 1 nmol of product or consuming 1 nmol of substrate (catalase) under standard incubation conditions. Specific activity was expressed as U/mg protein.

Light Microscopy and Immunohistochemical Study

Portions of placenta and liver from dams were fixed in phosphate-buffered 10% formaldehyde (pH 7.2) and embedded in paraffin. Three-micron sections of dam livers and placentas were cut. All observations were performed with a light microscope (Nikon E400, Nikon Instrument Group, Melville, NY, USA).

In order to evaluate iron stores in dam livers, Perls Prussian blue staining was performed. Immunolabeling of specimens was carried out by a modified avidin-biotin-peroxidase complex technique Vectastain ABC kit (Universal Elite, Vector Laboratories, CA) as described previously [27]. All samples of placenta were pre-incubated with Ultra Tech HRP (protein blocking agent PN IM2391, Immunotech SAS, Beckman Coulter Co. Marseille Cedex, France), and then with the corresponding antibody. For the negative control, the incubation was performed with non-immune serum, as recommended by the manufacturer. Hypoxia and inflammatory markers were quantified with monoclonal antibodies against rat hypoxia-inducible factor 1-alpha (HIF-1α; Novus Biologicals, Inc., Littleton, CO), tumor necrosis factor-alpha (TNF-α; R&D Systems, Minneapolis, MN) and interleukin-6 (IL6; Santa Cruz Biotechnology, Santa Cruz, CA) at concentrations of 1:1000 (2.2 μg/ml), 1:50 (2 μg/ml) and 1:100 (2 μg/ml), respectively (phosphate-buffered saline as diluting agent).

Morphometric Analysis

Histological sections were studied in each animal with an image analyzer (Image-Pro Plus version 4 for Windows, Media Cybernetics LP, Silver Spring, MD, USA). Morphological analyses were performed at a magnification

of ×400. In the placentas, tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL6) and hypoxia inducible factor-1 alpha (HIF-1α) expression levels were evaluated by the percentage of positive immunostaining per square millimeter using light microscopy. Median percentage values were calculated for each rat.

Statistical Methods

Values were expressed as median and range. All statistical analyses were performed with absolute values and processed through GraphPad Prism®, version 5.0 (GraphPad Software, Inc., San Diego, CA). The assumption test to determine the Gaussian distribution was performed by the Kolmogorov and Smirnov method. For parameters with non-Gaussian distribution comparisons were performed by Kruskal-Wallis test (nonparametric ANOVA) and Dunn's multiple comparison test. A value of $p < 0.05$ was considered significant.

RESULTS

Hb, Serum Iron and TSAT

Hb, serum iron and TSAT values in the four groups fed with the low-iron diet confirmed the presence of IDA prior to pregnancy. No significant differences were observed between these groups (Table **1**). At the end of pregnancy, the IPC, FF and FS groups showed significantly higher ($p < 0.01$) values for Hb, serum iron and TSAT versus untreated anemic animals although the values remained significantly lower ($p < 0.01$) than those in the non-anemic control group (Table **1**).

Liver Enzymes and Microscopy Findings of the Liver

Levels of the liver enzymes AST, ALT and ALP at the end of pregnancy did not differ significantly between the control, anemic and IPC groups (Fig. **1**). However, the liver enzyme levels in the FF group were slightly higher and in the FS group significantly higher ($p < 0.01$) compared to the non-anemic control, anemic and IPC groups (Fig. **1**).

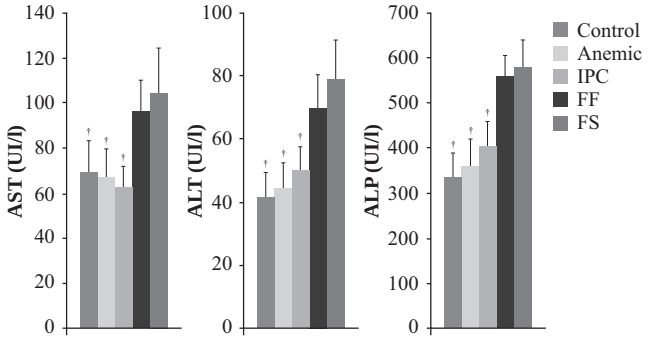


Fig. (1). Levels of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) in control, anemic (Hb ≤ 9 g/dL), IPC-treated, FF-treated and FS-treated dams (daily administration of 2 mg iron/kg + 7 µg folic acid /kg bw) at the end of pregnancy (day 21).

Prussian blue staining in the dam livers revealed that control, IPC, FF and FS groups presented equivalent iron levels, superior to the level observed in the anemic group [Results as median (range) (% positive staining/mm^2): control: 3.6 (2.3–4.6); anemic: 0.5 (0.4–0.3); IPC: 3.5 (2.9–4.5); FF: 3.5 (3.0–4.6); FS: 3.7 (2.9–4.9)].

Pregnancy Outcome

The number of fetuses per dam, neonatal bw and placental weight average were significantly lower ($p < 0.01$) in the anemic group than in the control, IPC, FF or FS group (Table **2**). The number of fetuses did not differ significantly between the non-anemic control and the treatment groups. However, neonatal bw and placental weight were significantly higher ($p < 0.01$) in the non-anemic control and IPC groups versus FF and FS groups (Table **2**).

Oxidative Stress Markers

The levels of oxidative stress markers in the placentas were much lower than in the organs of the dams and fetuses (Fig. **2**). At the end of pregnancy, the anemic group

Table 1. Hemoglobin, Serum Iron and Transferrin Saturation in Control, Anemic (Hb ≤ 9 g/dL), IPC-Treated, FF-Treated and FS-Treated Dams (Daily Administration of 2 mg Iron/kg + 7 µg Folic Acid/kg bw) Before and at the End of Pregnancy (Day 21)

	Control (n = 8)	Anemic (n = 8)	IPC (n = 8)	FF (n = 8)	FS (n = 8)
Hb (g/dL)					
Before	14.0 (13.6–14.6)[a]	8.5 (7.9–8.8)	8.4 (7.9–8.9)	8.7 (8.3–9.0)	8.6 (8.3–8.9)
End	12.5 (11.7–13.2)[a]	7.8 (7.0–8.2)[c]	11.4 (10.1–12.4)	11.5 (10.2–11.9)	11.7 (10.2–12.7)
Serum Iron (µg/dL)					
Before	238.0 (176.0–271.5)[a]	96.5 (65.1–119.0)	99.0 (63.2–121.3)	101.5 (70.4–127.2)	100.5 (64.0–127.5)
End	196.7 (167.3–227.8)[a]	57.4 (30.0–76.2)[c]	131.2 (113.0–175.9)[b]	152.0 (144.2–195.7)	163.6 (151.0–211.8)
TSAT (%)					
Before	39.7 (37.0–44.4)[a]	12.5 (9.1–16.0)	11.7 (8.9–15.8)	11.8 (9.2–16.1)	12.2 (8.9–15.2)
End	37.0 (31.0–42.1)[a]	9.2 (6.4–12.5)[c]	30.4 (22.0–35.9)	30.8 (24.9–36.5)	33.8 (27.0–37.1)

[a] $p < 0.01$ versus all groups.
[b] $p < 0.01$ versus FF and FS.
[c] $p < 0.01$ versus IPC, FF and FS.

Table 2. Outcomes of Pregnancy in Control, Anemic (Hb ≤ 9 g/dL), IPC-Treated, FF-Treated and FS-Treated Dams (Daily Administration of 2 mg Iron/kg + 7 µg Folic Acid/kg bw) at the End of Pregnancy (Day 21)

Control	Anemic	IPC	FF	FS
Fetuses/Dam				
13.5 (12.0–15.0)	9.0 (8.0–11.0)[a]	12.0 (11.0–14.0)	12.0 (10.0–13.0)	11.5 (9.0–14.0)
Neonatal Body Weight (g)				
5.2 (4.9–5.5)[b]	3.7 (3.4–4.1)[a]	5.0 (4.8–5.4)[b]	4.7 (4.4–4.9)	4.6 (4.2–4.8)
Placenta Weight (mg)				
509.5 (489.0–534.0)[b]	387.0 (321.0–402.0)[a]	489.5 (460.0–523.0)[b]	444.0 (422.0–488.0)	437.5 (397.0–473.0)

[a]$p < 0.01$ versus all groups.
[b]$p < 0.01$ versus FF and FS.

exhibited significantly higher ($p < 0.01$) levels of malondialdehyde (MDA) and Cu,Zn superoxide dismutase (Cu,Zn-SOD) activity, and significantly lower ($p < 0.01$) reduced glutathione to oxidized glutathione ratio (GSH:GSSG), catalase and glutathione peroxidase (GPx) activities in all the analyzed organs of the dams and the fetuses, including the placenta, than the control and treatment groups (Fig. **2**). There were no significant differences between any of the oxidative stress marker levels in the IPC and control groups. However, the FF and FS groups (dams, fetuses and placenta) exhibited significantly higher ($p < 0.01$) levels of MDA and Cu,Zn-SOD activity, and significantly lower ($p < 0.01$) GSH:GSSG ratio than the control and IPC groups. Interestingly, catalase and GPx activities were significantly lower ($p < 0.01$) in the FF group than in the FS, IPC and control groups. In the FS group, catalase and GPx activities were significantly higher ($p < 0.01$) than in any other group.

Microscopy Findings and Immunohistochemical Study of the Placenta

In the placenta, most of the immunohistochemical changes were observed in the murine labyrinth, mainly in the trophoblastic cells. Light microscopy revealed no major differences in placenta morphology between the groups. However, the labyrinth area of placentas of the dams of the anemic group presented a significant increase ($p < 0.01$) in HIF-1α compared to the placentas of the non-anemic control animals (Fig. **3**, arrows indicate cells with positive staining). In the IPC and FF groups the expression of HIF-1α was still significantly higher ($p < 0.01$) than in the non-anemic control group. In the FS group the HIF-1α expression was significantly higher ($p < 0.01$) than any other group, including the anemic group.

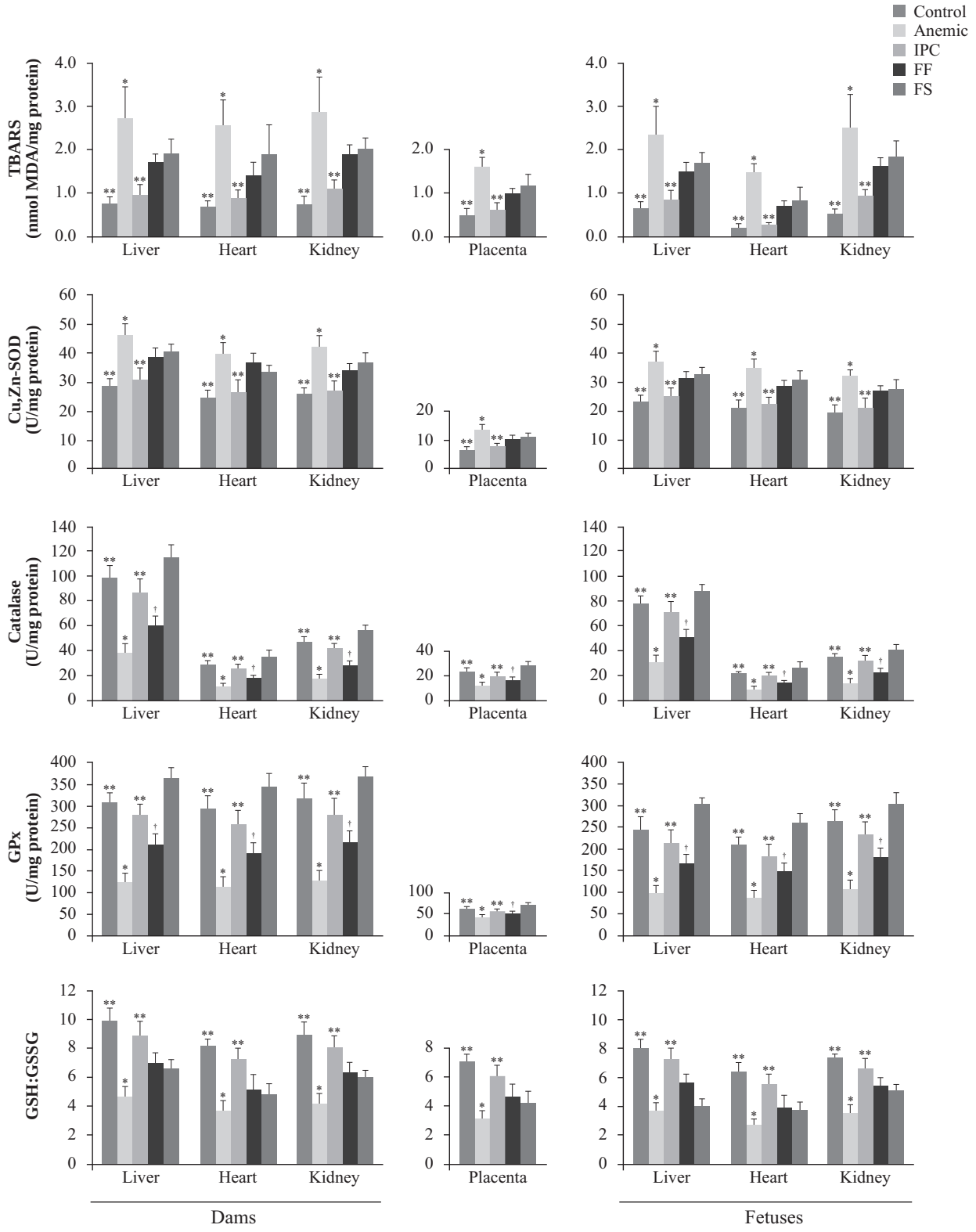
The pro-inflammatory markers, TNF-α and IL6, were significantly higher ($p < 0.01$) in the placentas of the animals of the anemic group than in those of the non-anemic control and IPC groups (Fig. **3**, arrows indicate cells with positive staining). The IPC group presented similar levels of TNF-α and IL6 expression as the non-anemic control group whereas FF and FS presented significantly higher ($p < 0.01$) TNF-α and IL6 expression than the non-anemic control and IPC group. Similarly to HIF-1α, both TNF-α and IL6 expression were significantly higher ($p < 0.01$) in the FS group than in any other group.

DISCUSSION

IPC, FF and FS were all efficient in correcting IDA during pregnancy, although treated animals did not reach quite the same Hb, serum iron, and TSAT values as those in the control group. As suggested previously [19], oral iron treatment may not have been sufficient to fully correct the initial severe anemia and to keep up with the drastically increasing iron requirements during pregnancy.

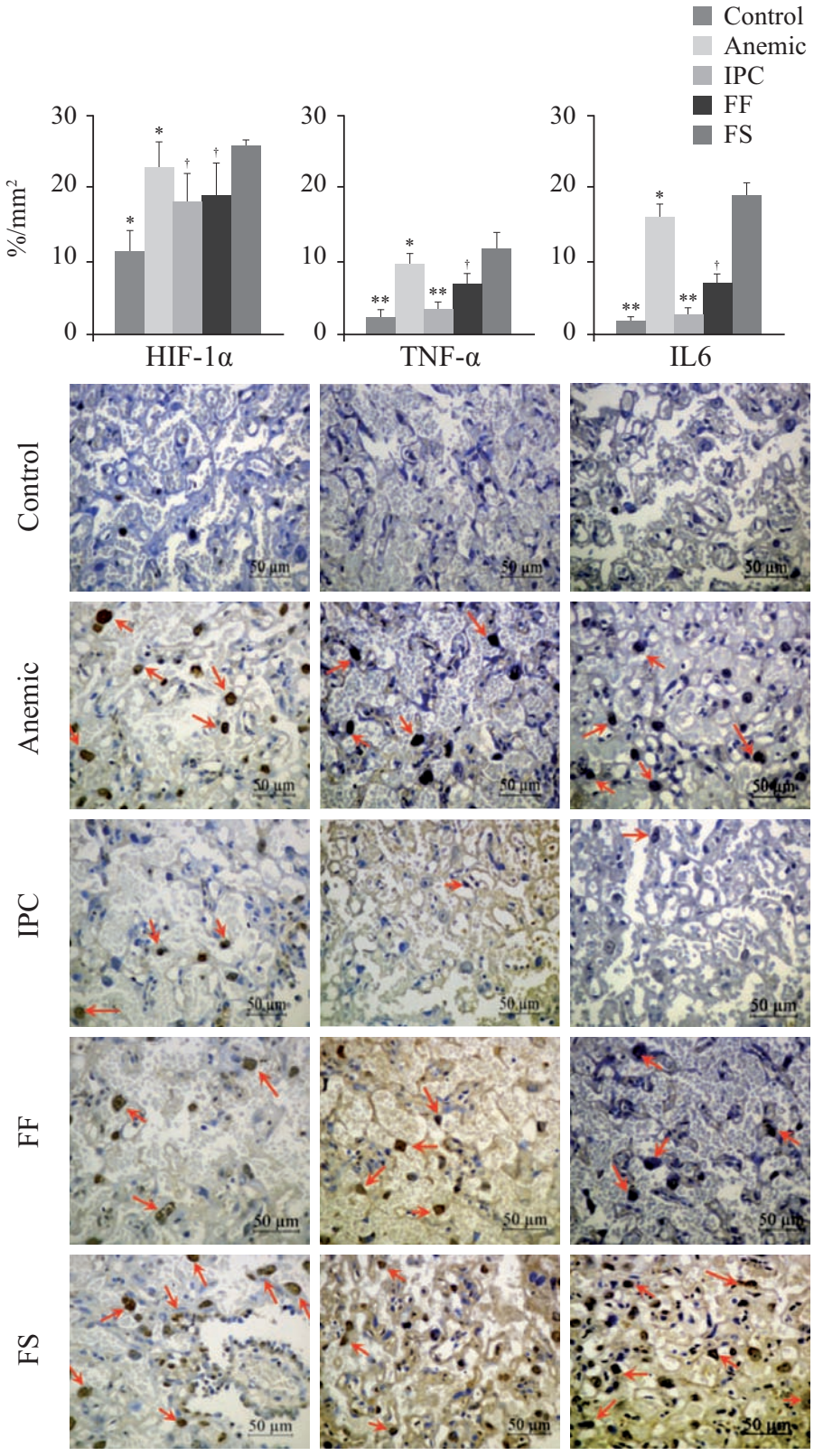
Both IDA and pregnancy have been associated with oxidative stress in humans and rats [28-31]. ROS, produced in mitochondria during normal cellular respiration and in the cytoplasm by xanthine oxidase (XO) and NAD(P)H oxidase, are typically detoxified by antioxidant enzymes such as SOD, catalase and GPx [32]. However, during IDA, the activity of antioxidant enzymes are decreased significantly, leading to increased oxidative stress [28]. As in our previous study, the enzyme activity of catalase and GPx were decreased and MDA levels were increased in animals of the anemic group. Catalase contains heme in its active site and during anemia the lack of iron may cause the reduced catalase activity. GPx is selenium-dependent and decreased GPx expression has been suggested to result from GPx specific pretranslational depression of GPx mRNA levels during ID [33]. As in our previous study [19], the Cu,Zn-SOD values in the anemic group were unexpectedly high compared to the control or IPC group. Since nitric oxide ($NO^{\bullet}$)-production is induced during IDA [34, 35] as well as during pregnancy [36, 37], and the Cu,Zn-SOD gene has been shown to be upregulated by $NO^{\bullet}$ [38, 39], it is possible that the higher levels of Cu,Zn-SOD present in the anemic group are in fact the result of elevated $NO^{\bullet}$ levels.

In contrast to FF and FS, treatment with IPC reduced IDA-mediated oxidative stress, i.e. all the analyzed oxidative stress markers returned to normal levels. Upon administration of oral ferrous salts, Fe(II) is rapidly taken up in the gut not only by the physiological, active pathway but also by uncontrolled, passive diffusion directly into the blood through the paracellular route [9, 40]. The rapid iron uptake leads to increased transferrin saturation and marked increase of the NTBI concentration in the plasma [10], which may further contribute to the oxidative stress associated with oral treatment with ferrous salts [2, 41]. Interestingly in this study, although FF treatment resulted in higher lipid



* p<0.01 versus all groups
** p<0.01 versus FF and FS
† p<0.01 versus FS

Fig. (2). Malondialdehyde, Cu,Zn-SOD, catalase, GPx, and GSH:GSSG in control, anemic (Hb ≤ 9 g/dL), IPC-treated, FF-treated and FS-treated dams (daily administration of 2 mg iron/kg + 7 µg folic acid /kg bw) and in corresponding placentas and fetuses at the end of pregnancy (day 21).



* p<0.01 versus all groups
** p<0.01 versus FF and FS
† p<0.01 versus FS

Fig. (3). Bar charts showing HIF-1α, TNF-α and IL6 immunostaining area in the placentas of control, anemic (Hb ≤ 9 g/dL), IPC-treated, FF-treated and FS-treated dams (daily administration of 2 mg iron/kg + 7 µg folic acid /kg bw) and corresponding micrographs in the placenta labyrinth (arrows indicate trophoblastic cells with positive staining).

peroxidation than IPC treatment, FF-treated animals presented significantly lower catalase and GPx levels than IPC or FS-treated animals. As comparative clinical or nonclinical studies of the various effects of different oral iron treatments are still missing to date, it is highly speculative to find an explanation for this difference.

The hepatotoxicity of iron has been widely described [42], as demonstrated by prolonged feeding of excess dietary iron in mice [43]. Treatment with both FF and FS resulted in increased levels of AST, ALT and ALP, indicating a possible hepatic damage. Especially the elevated AST levels of FS-treated animals may suggest a relatively severe form of liver necrosis [44], possibly due to transiently occurring high levels of NTBI after each oral iron dose [12, 45]. NTBI is rapidly taken up by the liver parenchymal cells and has been suggested to exert cellular toxicity by increasing redox-active cytosolic labile iron pool (LIP), which can lead to increased oxidative stress and to accelerated tissue degeneration [46]. In line with our previous results in rats [16, 19], treatment with IPC did not induce liver damage, as indicated by the similar levels of AST, ALT and ALP between the control and the IPC groups at the end of pregnancy. This is not surprising since NTBI levels are not increased after oral administration of IPC, as demonstrated in non-anemic healthy men [47].

Placental function is regulated by different cytokines [48]. Elevated TNF-α and IL6 are associated with various complications for pregnancy outcome [49-51]. ID has been shown to increase TNF-α level in rat placenta [48] and we recently reported that IDA elevated both TNF-α and IL6 expression in rat placenta [19]. IPC treatment of IDA restored normal expression levels of both pro-inflammatory markers in the placenta, whereas FF treatment resulted only in partial normalization. In contrast, FS-treated animals presented higher levels of TNF-α and IL6 expression in the placenta than animals of the anemic group, suggesting the presence of a local inflammatory reaction, in agreement with previous reports in animal models [52] and healthy volunteers [53].

Elevated HIF-1α levels have also been shown to be associated with various complications during pregnancy in mice as well as in humans [54]. The elevated HIF-1α levels in the placentas of anemic rats were not fully corrected by IPC or FF treatment. In agreement with higher oxidative stress and pro-inflammatory marker levels, FS-treated animals presented in their placentas also higher levels of HIF-1α than non-treated anemic animals. The HIF-1α level is regulated post-translationally by the HIF-prolyl 4-hydroxylases (PHDs), a class of enzymes that require iron, dioxygen, 2-oxoglutarate and ascorbic acid to catalyze the hydroxylation of HIF-1α proline residues allowing it to bind to the von Hippel-Lindau-E3 ubiquitin ligase complex to induce proteasomal degradation of HIF-1α [55]. Thus, high HIF-1α levels in the placentas of pregnant rats may be due to reduced activity of PHD caused by hypoxia and/or by lack of iron during anemia and, consequently, reduced degradation of HIF-1α. It is conceivable that PHD activity is inhibited by oxidation of Fe(II) to Fe(III) by ROS resulting in HIF-1α stabilization [55]. Thus, the very high levels of HIF-1α in the placentas of FS-treated animals are possibly the result of the higher oxidative stress observed in the FS-treated animals.

CONCLUSION

This study in anemic pregnant rats showed that IPC, FS, and FF were similarly efficient in the treatment of maternal anemia. In contrast, the three studied oral iron therapies were different in terms of correcting oxidative stress induced by IDA in pregnant rats, fetuses and placentas. Most of the negative effects associated with anemia were resolved by IPC treatment although not all parameters returned to normal levels, possibly due to the severity of anemia prior to pregnancy. On the contrary, treatment with ferrous salts, in particular with FS was found to elicit hepatic damage and oxidative stress in dams, fetuses and placentas as well as inflammation and high levels of HIF-1α in the placenta. Therefore, it is not surprising that pregnancy outcome, measured by number of fetuses, and the neonate and placental weight, of FF- and FS-treated animals was also worse than that of IPC treated animals, possibly due to an unfavorable environment (inflammation/oxidative stress) in the maternal-placental unit induced by FF and FS treatments.

The results also suggest that the oxidative stress caused by IDA is due to decreased antioxidant enzyme levels leading to increased levels of plasma lipid peroxidation products such as malondialdehyde. However, FS- and FF-induced oxidative stress is likely to be caused, at least in part, by NTBI, which triggers increased formation of ROS and thus increased malondialdehyde levels and increased antioxidant enzyme levels.

ABBREVIATIONS

bw	=	Body weight
Cu,Zn-SOD	=	Cu,Zn superoxide dismutase
FF	=	Ferrous fumarate
FS	=	Ferrous sulfate
GPx	=	Glutathione peroxidase
GSH	=	Glutathione
GSSG	=	Oxidized glutathione
HIF-1α	=	Hypoxia-inducible factor 1-alpha
ID	=	Iron deficiency
IDA	=	Iron deficiency anemia
IL6	=	Interleukin-6
IPC	=	Iron polymaltose complex
IUGR	=	Intrauterine growth restriction
NTBI	=	Non-transferrin bound iron
PHD	=	Hypoxia-inducible factor prolyl 4-hydroxylase
ROS	=	Reactive oxygen species
TBARS	=	Thiobarbituric acid reactive species
TNF-α	=	Tumor necrosis factor-alpha
TSAT	=	Transferrin saturation

CONFLICT OF INTEREST

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