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Original article

Oxidative effect ferumoxytol and iron dextran on urinary bladder contraction and impact of antioxidants

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ABSTRACT

Introduction: Intravenous (IV) iron compounds enjoy widespread clinical use. No studies have examined the effect of antioxidants on oxidative damage-associated changes in bladder contractility from IV iron compounds.
Objective: To determine if oxidative stress from 2 IV iron compounds effect rat bladder contraction and if coadministered antioxidants are protective.
Methods: Three groups of 6 SD rats were studied. Group 1 received weekly 1 mg/kg doses of ferumoxytol (FX) or iron dextran (ID) for 5 doses. Group 2 received coenzyme Q10 (5 mg/kg) plus alpha lipoic acid (10 mg/kg) daily for 4 weeks before starting IV iron, then 1 mg/kg of the 2 iron compounds weekly for 5 doses. Antioxidant treatment continued until the last iron dose. Group 3 were controls and received saline and half received the antioxidants over the same time period. Seven days after the last iron dose, liver and heart samples were analyzed for nitrotyrosine (NT) and dinitrophenol (DNP) content. The contractile responses of bladder strips to field stimulation (FS) (2, 8, and 32 Hz), carbachol (10 μg), and KCl (120 mM) were determined.
Results: DNP concentrations in heart and liver after treatment with FX were similar to control. NT concentrations in both tissues were increased above control with FX. DNP and NT concentrations of both tissues due to ID were higher than both controls and FX. FX had no effect on bladder contractile responses to any form of stimulation, but ID produced decreases in bladder responses to all stimuli. Antioxidants had no effect on bladder contractility in FX-treated rats, but contractile responses to all forms of stimulation were increased by the antioxidants in ID-treated rats. The antioxidants had no effect on control rats.
Conclusion: FX and ID caused oxidative stress to several organs, but FX was less stressful than ID. Antioxidants attenuated the effects of ID on bladder contractility.
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1. Introduction

Intravenous (IV) iron compounds are widely used in clinical practice for the management of moderate to severe iron deficiency anemia and anemia of chronic disease.[1–4] These preparations have been shown to cause oxidative damage to a variety of cells, tissues and organs in animals and humans.[5–17] Generally, the magnitude of oxidative stress is dependent on the physicochemical robustness of the complex: the less robust products more readily release labile iron into the circulation, increasing the likelihood of production of highly reactive oxygen species.[18,19] We previously demonstrated, unexpectedly, that iron dextran (ID), a highly robust compound, produced the greatest elevations in production of biomarkers for oxidative stress (dinitrophenyl [DNP] and nitrotyrosine [NT]) of the series of IV iron compounds tested, in heart, lung, brain and kidneys of rats, using doses equivalent to clinical practice. A much more attenuated effect was observed with ferumoxytol (FX), which is also considered to be a robust complex (unpublished data).

In previous studies, we have shown that the urinary bladder is very sensitive to oxidative stress and the effects can be quantified by studying the effects of isolated urinary bladder strips in response to specific forms of stimulation.[20–23] Bladder contraction can be caused by field stimulation (FS) via the release of contractile neurotransmitters (acetylcholine and ATP), by carbachol (direct

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stimulation of muscarinic cholinergic receptors) and by potassium chloride (KCl) (direct depolarization of smooth muscle cell membrane). Using the bladder in this manner allows the study of the functional effects of the oxidative stress.

Additionally, previous studies have shown that changes in rabbit bladder contractility due to oxidative stress can be attenuated by pre-administration of antioxidants.[24–26] To our knowledge, no studies have examined the effect of antioxidants on the oxidative damage-associated changes in bladder contractility due to intravenous (IV) iron compounds. Therefore, the objective of this study was to determine if the oxidative stress mediated by two IV iron compounds affect urinary bladder contraction and whether antioxidant treatments would protect the bladder. We chose ferumoxytol (FX) and iron dextran (ID) because of differences observed in our previous unpublished observations. We have chosen coenzyme Q10 (CoQ) and alpha lipoic acid (LA) as the antioxidants based on the prior studies showing the effectiveness of these two specific antioxidants against oxidative bladder damage.[24–27]

2. Methods

Three groups of 6 adult SD rats were studied. Group 1 received 1 mg/kg of ferumoxytol (FX) or 1 mg/kg high molecular weight iron dextran (ID), weekly for 5 doses. The dose of 1 mg/kg was chosen to mimic doses of IV iron that typically are observed with clinical use (up to 1000 mg in adults of 50–100 kg). Seven days following the last dose, animals were euthanized using CO_2 followed by cardiac puncture. Rats in group 2 received two naturally occurring antioxidants, coenzyme Q10 (5 mg/kg) plus alpha lipoic acid (10 mg/kg), daily for 4 weeks before starting IV iron doses. These doses of antioxidants were chosen as they have proven effectiveness in significantly reducing the oxidative stress to the bladder caused by partial outlet obstruction and *in vitro* ischemia.[12,13] Thereafter group 2 received 1 mg/kg of the two IV compounds weekly for 5 doses and antioxidant treatment continued until the end of the experiment. Seven days following the last iron dose, animals were euthanized as described above. Group 3 rats were controls and received saline. Half of the control rats received the antioxidants over the same time period. Antioxidant treatment continued until the end of the experiment. Seven days following the last IV iron dose, animals were euthanized. After euthanasia, samples of liver and heart were removed for analysis of NT and DNP as markers of oxidative stress. The bladders were removed, cut into two strips and placed in separate isolated baths containing oxygenated Tyrode's solution containing 1 mg/ml glucose. The contractile responses of the strips to FS (2, 8, and 32 Hz), carbachol (10 μg), and KCl (120 mM) were determined.

3. Results

Fig. 1 shows the responses of the heart and liver to the IV iron compounds. The DNP concentrations of both tissues in FX-treated animals were similar to those in the saline controls. The concentrations of NT in both tissues were significantly increased above control with FX. The concentrations of both DNP and NT of both tissues to ID were significantly higher than both controls and FX.

Fig. 2 shows the effect of the antioxidants as the percent inhibition of the DNP and NT concentrations in heart and liver tissues. The antioxidants showed little inhibition of the DNP or NT concentrations in saline control animals in both heart and liver. There was no significant inhibition of DNP concentrations in FX-treated animals in either heart or liver. However, there were significant inhibitions of NT concentrations in FX-treated rats in both heart and liver and of both DNP and NT concentrations in ID-treated animals in both heart and liver.

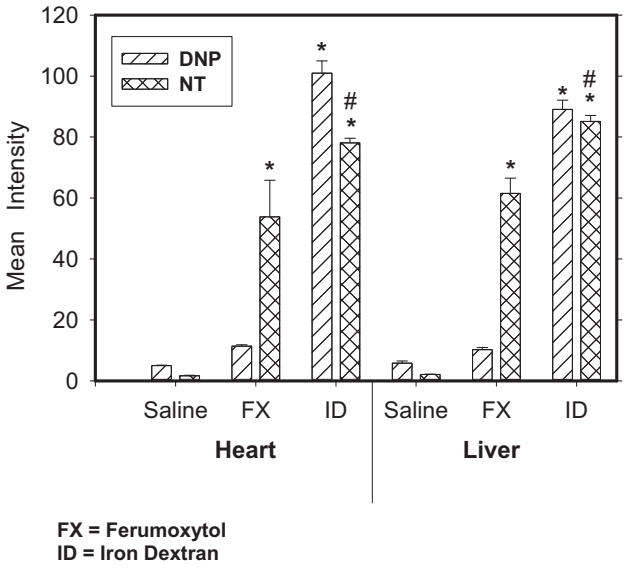

Fig. 1. Relative densities of DNP and NT of the heart and liver in animals treated with ferumoxytol and iron dextran. Each bar is the mean ± SEM of 6 individual rats. * = Significantly different from control ($p < 0.05$); # = Mean intensity times 10.

The contractile responses of the bladder strips to both FX and ID are presented in Fig. 3 (due to FS) and Fig. 4 (due to KCl and carbachol). FX had no effect on the contractile responses to any form of stimulation, whereas ID resulted in significant decreases in the responses to all stimuli.

The effects of the antioxidants on the contractility of bladder strips in iron-treated animals are presented in Fig. 5 (FX treatment) and Fig. 6 (ID treatment). Antioxidants had no effect on the contractile responses to any stimuli in the FX-treated rats (Fig. 5). However, antioxidants caused a significant increase in the contractile responses to all forms of stimulation in the rats treated with ID (Fig. 6).

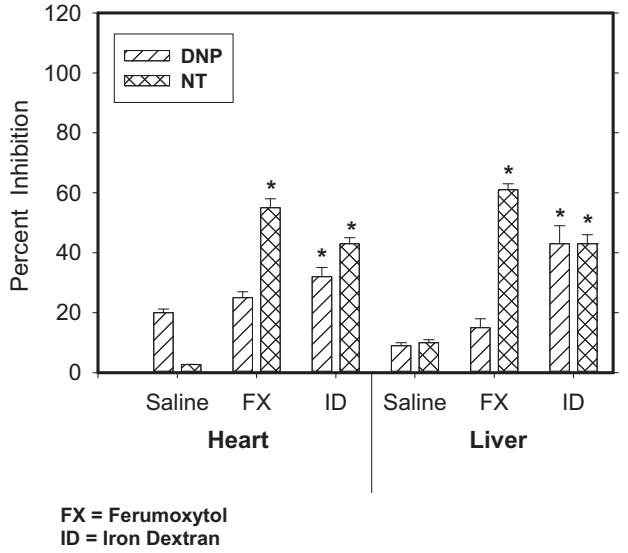

Fig. 2. Percent inhibition of DNP and NT by antioxidant treatment. Each bar is the mean ± SEM of 6 individual rats. * = Significantly different from saline control ($p < 0.05$).

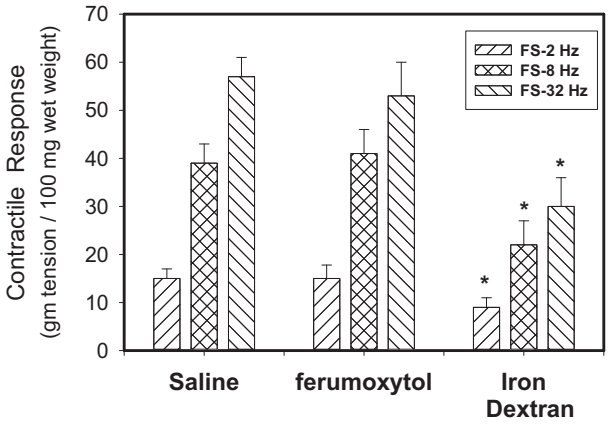

Fig. 3. Contractile responses of bladder strips to field stimulation in animals treated with ferumoxytol and iron dextran. Each bar is the mean ± SEM of 6 individual rats. * = Significantly different from ferumoxytol and control ($p < 0.05$).

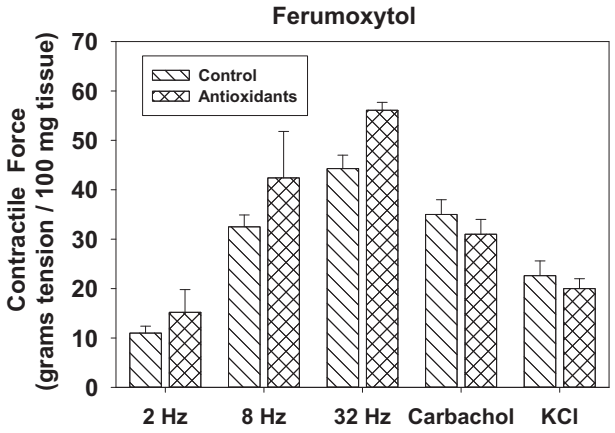

Fig. 5. Effect of antioxidants on contractile responses of bladder strips to field stimulation, KCl and carbachol in animals treated with ferumoxytol. Each bar is the mean ± SEM of 6 individual rats.

4. Discussion

IV iron preparations are generally classified by the robustness of the complex. Less robust preparations release labile iron more readily than preparations with higher robustness (such as ID and FX).[18,19] ID has previously been shown to promote oxidative stress.[11,15–17] Toblli et al showed that 40 mg/kg doses of ID produced more oxidative damage than the other IV iron preparations.[11] ID produced significant changes in a number of markers of oxidative stress (such as elevations of glutathione peroxidase and CuZn-superoxide dismutase and decreased reduced-to-oxidized glutathione) in heart, liver and kidney tissues. This was also observed with sodium ferric gluconate (FG), as expected in an agent with ready release of reactive labile iron. In addition, very large elevations in immunostaining in all 3 tissues for IL-6 and TNF-α after both FG and ID were demonstrated. Prussian blue staining for free iron was elevated for both FG and ID, suggesting poor utilization of iron from ID and FG, although the authors did not discuss the possible mechanism of these findings.

We have shown that there are differences in the responses to FX and ID in heart, liver and bladder. In both heart and liver, there were elevations in the production of NT, but not DNP in rats that had received a course of FX compared to saline control. However, in animals that were treated with ID, there were significant elevations in the production of both NT and DNP compared to both control and FX-treated rats. Antioxidant treatment showed significant decreases in the concentration of DNP only in the hearts and livers of ID-treated animals, whereas NT was reduced in both tissues in both ID- and FX-treated animals.

In the contractility studies, treatment with FX showed no effect on bladder responses compared to control, but use of ID showed that contractility was attenuated with all forms of stimulation. These results support our earlier findings that ID is associated with more oxidative damage than other intravenous iron products.

Prior studies on oxidative stress on the urinary bladder show that FS is affected to significantly greater degree than the responses to carbachol or KCl.[20–23] Although FS was slightly more sensitive to inhibition by ID, this difference was not statistically significant. Since all contractile functions were depressed by ID, it would seem that the nerves, receptors, and cell membranes were all equally affected. Since FX had no effect on the contraction to any form of stimulation although NT was significantly increased, it would seem that DNP might be more damaging to bladder contraction than NT.

Of interest is our finding that pretreatment and co-administration of the animals with antioxidants CoQ plus LA protected the bladder from the damaging effects of ID. These findings confirm the similar protection observed in prior studies using other forms of oxidative stress, including partial outlet obstruction, *in vivo* and *in vitro* models

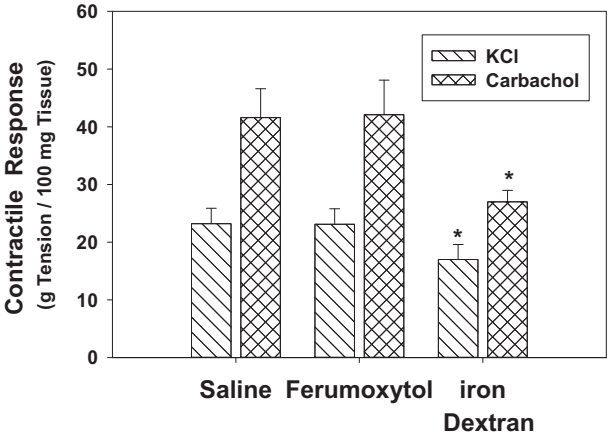

Fig. 4. Contractile responses of bladder strips to KCl and carbachol in animals treated with ferumoxytol and iron dextran. Each bar is the mean ± SEM of 6 individual rats. * = Significantly different from ferumoxytol and control ($p < 0.05$).

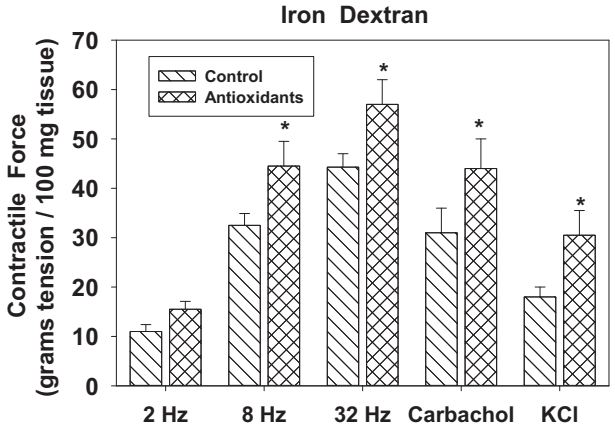

Fig. 6. Effect of antioxidants on contractile responses of bladder strips to field stimulation, KCl and carbachol in animals treated with iron dextran. Each bar is the mean ± SEM of 6 individual rats. * = Significantly different from control ($p < 0.05$).

of ischemia/reperfusion.[24–27] Since these antioxidants were effective in protecting against ID, it would seem logical to conclude that the damaging effects of ID to the bladder was via oxidative stress and free radical generation and damage.

There are potential clinical ramifications of these findings. IV iron is used widely, especially in the treatment of anemia of chronic disease (such as that complicating chronic kidney disease [CKD], inflammatory bowel disease, chronic heart failure and some cancers), but also in conditions of iron deficiency (such as during pregnancy and the postpartum period, heavy uterine bleeding and following gastric bypass surgery). In many of these situations, long-term use of intermittent doses of IV iron is common practice, producing cumulative annual doses of several grams. It could be hypothesized that long-term exposure of patients to frequent oxidative insults could be harmful. No hard outcome data are extant. However, recently, the overzealous use of IV iron preparations in randomized clinical trials of anemia correction may be at least partly to blame for the poorer outcomes of CKD patients assigned to higher hemoglobin targets has been suggested.[27] Thus, strategies, such as concurrent use of antioxidants, might be appropriate to ensure less potential long-term toxicity. Our findings are very suggestive that functional changes do occur as a result of oxidative insults, and thus clinical benefits might accrue from antioxidant use in patients receiving IV iron.

5. Conclusions

These studies confirm that IV Fe compounds present a risk of oxidative stress to a variety of internal organs, and that ID was significantly more stressful than FX. ID caused significantly reduced contractile responses of the urinary bladder to all forms of stimulation, and co-administration with antioxidants mitigated the damaging effects of ID on bladder contractility. In addition, the antioxidants coenzyme Q10 + alpha lipoic acid reduced the oxidative stress in a variety of tissues following IV iron administration. Carefully designed clinical studies should be completed to determine the impact of antioxidant use on patients receiving courses of IV iron.

Conflicts of interest

All authors have none to declare.

Acknowledgments

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References

1. Anker SD, Comin Colet J, Filippatos G, et al. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med*. 2009;361:2436–2448.
2. Evstatiev R, Marteau P, Iqbal T, et al. FERGIcor, a randomized controlled trial on ferric carboxymaltose for iron deficiency anemia in inflammatory bowel disease. *Gastroenterology*. 2011;141:846–853. e841–842.
3. Munoz M, Martin-Montanex E. Ferric carboxymaltose for the treatment of iron deficiency anemia. *Expert Opin Pharmacother*. 2012;13:907–921.
4. Qunibi WY. The efficacy and safety of current intravenous iron preparations for the management of iron-deficiency anemia: a review. *Arzneimittelforschung*. 2010;60:399–412.
5. Toblli JE, Cao G, Olivieri L, Angerosa M. Assessment of the extent of oxidative stress induced by intravenous ferumoxytol, ferric carboxymaltose, iron sucrose and iron dextran in a nonclinical model. *Arzneimittelforschung*. 2011;61:399–410.
6. Kamanna VS, Ganji SH, Shelkovnikov S, Norris K, Vaziri ND. Iron sucrose promotes endothelial injury and dysfunction and monocyte adhesion/infiltration. *Am J Nephrol*. 2012;35:114–119.
7. Stefansson BV, Haraldsson B, Nilsson U. Acute oxidative stress following intravenous iron injection in patients on chronic hemodialysis: a comparison of iron-sucrose and iron-dextran. *Nephron Clin Pract*. 2011;118:c249–c256.
8. Gupta A, Zhuo J, Zha J, Reddy S, Olp J, Pai AB. Effect of different intravenous iron preparations on lymphocyte intracellular reactive oxygen species generation and subpopulation survival. *BMC Nephrol*. 2010;11:16. http://dx.doi.org/10.1186/1471-2369-11-16.
9. Toblli JE, Cao G, Oliveri L, Angerosa M. Differences between original intravenous iron sucrose and iron sucrose similar preparations. *Arzneimittelforschung*. 2009;59:176–190.
10. Toblli JE, Cao G, Oliveri L, Angerosa M. Differences between the original iron sucrose complex Venofer and the iron sucrose similar Generis and potential implications. *Port J Nephrol Hypert*. 2009;23:53–63.
11. Toblli JE, Cao G, Olivieri L, Angerosa M. Comparison of the renal, cardiovascular and hepatic toxicity data of original intravenous iron compounds. *Nephrol Dial Transplant*. 2010;25:3631–3640.
12. Toblli JE, Cao G, Olivieri L, Angerosa M. Evaluation of toxicity and oxidative stress induced by intravenous iron isomaltoside 1000 in a nonclinical model. *Arzneimittelforschung*. 2011;61:553–565.
13. Apopa PL, Qian Y, Shao R, et al. Iron oxide nanoparticles induce human microvascular endothelial cell permeability through reactive oxygen species production and microtubule remodeling. *Part Fibre Toxicol*. 2009;6:1. http://dx.doi.org/10.1186/1743-8977-6-1.
14. Kuo KL, Hung SC, Wei YH, Tarng DC. Intravenous iron exacerbates oxidative DNA damage in peripheral blood lymphocytes in chronic hemodialysis patients. *J Am Soc Nephrol*. 2008;19:1817–1826.
15. Martin-Malo A, Merino A, Carracedo J, et al. Effects of intravenous iron on mononuclear cells during the haemodialysis session. *Nephrol Dial Transplant*. 2012;27:2465–2471.
16. Sturm B, Steinkellner H, Ternes N, Goldenberg H, Scheiber-Mojdehkar B. In vitro study on the effects of iron sucrose, ferric gluconate and iron dextran on redox-active iron and oxidative stress. *Arzneimittelforschung*. 2010;60:459–465.
17. Danielson BG. Structure, chemistry and pharmacokinetics of intravenous iron products. *J Am Soc Nephrol*. 2004;15:S93–S98.
18. Geisser P, Baer M, Schaub E. Structure/histotoxicity relationship of parenteral iron preparations. *Arzneimittelforschung*. 1992;42:1439–1452.
19. Juan YS, Lin WY, Kalorin C, Kogan BA, Levin RM, Mannikarottu A. The effect of partial bladder outlet obstruction on carbonyl and nitrotyrosine distribution in rabbit bladder. *Urology*. 2007;70:1249–1253.
20. Levin RM, Agartan CA, Leggett RE, et al. Effect of partial outlet obstruction on nitrotyrosine content and distribution within the rabbit bladder. *Mol Cell Biochem*. 2005;276:143–148.
21. Hsu TH, Levin RM, Wein AJ, Haugaard N. Alterations of mitochondrial oxidative metabolism in rabbit urinary bladder after partial outlet obstruction. *Mol Cell Biochem*. 1994;141:21–26.
22. Lin WY, Chen CS, Wu SB, Lin YP, Levin RM, Wei YH. Oxidative stress biomarkers in urine and plasma of rabbits with partial bladder outlet obstruction. *BJU Int*. 2011;107:1839–1843.
23. Hydery T, Levin RM, Leggett RE, Kogan B. Treatment of obstructive bladder dysfunction by coenzyme Q10 and alpha lipoic acid in rabbits. *LUTS*; 2013 (in press).
24. Juan YS, Levin RM, Chuang SM, et al. The beneficial effect of coenzyme Q10 and lipoic acid on obstructive bladder dysfunction in the rabbit. *J Urol*. 2008;180:2234–2240.
25. Li HT, Schuler C, Leggett RE, Levin RM. Differential effects of coenzyme Q10 and alpha-lipoic acid on two models of in vitro oxidative damage to the rabbit urinary bladder. *Int Urol Nephrol*. 2011;43:91–97.
26. Lin WY, Rehfuss A, Schuler C, Levin RM. Effect of co-enzyme Q10 and alpha-lipoic acid on response of rabbit urinary bladder to repetitive stimulation and in vitro ischemia. *Urology*. 2008;72:214–219.
27. Van Buren P, Velez RL, Variri ND, Zhou XJ. Iron overdose: a contributor to adverse outcomes in randomized trials of anemia correction in CKD. *Int Urol Nephrol*. 2012;44:499–507.