Chronic Injections of Iron Dextran Into Peritoneal Cavity of Rats

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Anemia is one of the most serious complications which cannot be improved by dialysis. The use of recombinant human erythropoietin(rHuEPO) to treat anemia in end-stage renal disease has been a major advance in the care of these patients with hemodialysis or peritoneal dialysis[1]. Such erythropoietin replacement therapy is frequently associated with the development of functional iron deficiency due to the massive transfer of bone marrow iron stores to erythroid progenitor cells. So erythropoietin replacement therapy requires long-term maintenance therapy with iron, with frequent monitoring for adequacy of iron stores[2-6].

Peritoneal dialysis patients are less prone to iron deficiency, but they require iron supplementation to compensate for losses of blood through the gastrointestinal tract, menstruation, and phlebotomy for laboratory tests. Available iron delivery routes are intravenous injection[7-9], intramuscular injection[10] and oral administration of iron[11, 12]. Intravenous administration seems to be inconvenient for PD patients because of poor intravenous access. Intravenous iron dextran infusion has been associated with serious reactions including hypotension, respiratory failure and anaphylaxis[13, 14]. Intramuscular iron dextran injection was suggested, but there have been some reports of development of sarcoma at the injection site after chronic intramuscular iron injection[15, 16]. Oral administration of iron may be limited by patient non-compliance, gastrointestinal side effects, and interactions with other oral medications[11] . If the intraperitoneal route of iron delivery were available, it would be much more convenient for the PD patients.

Our previous acute studies[17, 18] with the 6hours cycle showed that more than 70% of iron dextran injected into the peritoneal cavity of rats is absorbed from the peritoneal cavity. But there was no significant change of serum iron. There was no evidence of macroscopic change of the peritoneum or microscopic iron deposition on the peritoneum in acute study. The goal of this chronic study was to determine the influence of chronic iron dextran injections into the peritoneal cavity of rats on function and anatomy of the peritoneal membrane.

Materials and Methods

1. Animal Treatment

Thirty six(36) male Sprague-Dawley rats weighing 300-350g were divided into 3 groups (n=12). Group 1(Control group) was given standard 1.5% Dianeal PD-2(Baxter Health Care Inc., IL, USA). Group 2(Low dose group) was given 1.5% Dianeal with 2mg/L concentration of iron dextran. Group 3(High dose group) was given 1.5% Dianeal with 10mg/L concentration of iron dextran.

For the experimental groups the iron in the

form of iron dextran injection(InFeD - Schein Pharmaceutical, Inc.) was added to the dialysis solution. Iron dextran is available in 2ml ampules containing 50mg of elemental iron per ml. The solution contains approximately 20% of dextran with molecular weight 160-180 KD. For Low dose group 50g/25ml(2mg/L) of elemental iron was added. For High dose group 250g/25ml(10 mg/L) of elemental iron was added. The iron was delivered by infusion of a solution through the intraperitoneal catheter.

2. Catheter Implantation[19]

The animals were placed supine on a 37℃ heating pad in a large jar and anesthetized with methoxyflurane(Metofane, Pitman-Moore, Inc., NJ, USA). The anesthesia was maintained with a nose cone. The abdomen was shaved and disinfected with Amukin®(Amuchina, Genova, Italy). The area was then covered with Tegaderm®(3-M Corporation, Minnesota). An incision was made in the skin along the midline starting at the xiphoid process and extending caudally approximately 3cm. A blunt dissection was then done on the midline through the abdominal wall approximately 1.5 - 0.75cm below the xiphoid process. Once the cavity was penetrated, the tip of the catheter was advanced into the cavity and secured with a single stitch through the cuff material and the superficial muscle layer. The procedure was performed carefully to avoid bleeding and trauma to the peritoneum. The hole into the peritoneum was large enough to allow the catheter to pass through but small enough that the catheter fits snugly. With the use of the 3mm trocar the other end of the catheter was tunneled under the skin to a point the animals scapulas that had been shaved and disinfected. The trocar was then forced through the skin and the catheter pulled through the tunnel. When the second cuff was next to the exit hole, the catheter was trimmed leaving approximately 1.5-2.0cm exposed. The catheter was capped. The animals were allowed to wake up, placed again their cages and allowed free access to food and water. The animals were given bupremorphine subcutaneously for analgesia with the dose adjusted as needed. This therapy was continued for 72 hours post surgery.

3. Intraperitoneal Injections

The first injection was done on the day of catheter implantation. 25ml of control or one of experimental dialysis solutions had been injected into the peritoneal cavity through the catheter in the morning 7days a week for 6months. The non absorbed dialysate was drained the next morning before the next injection and cultured for bacterial growth. Every effort was made to avoid system contamination.

4. Treatment of Peritonitis

Diagnosis of peritonitis was based on the culture(more than 10 colonies in a blood agar plate). Upon diagnosis of peritonitis the animal was treated intraperitoneally with a loading dose of vancomycin(1g/L) and gentamycin(1.7mg/kg) and was followed with a maintenance dose of 30mg/L and 8mg/L respectively per exchange for a week.

5. Peritoneal Membrane Function

On day eight, at three months, and at the last exchange(six months) the animals were anesthetized as outlined above two hours after the instillation of the exchange. At a two-hour dwell a 5ml dialysate sample was collected through the catheter and divided into chemistry and bacteriology. A 2ml sample of blood was taken by direct cardiac puncture. In case of peritonitis the test was deferred until the membrane inflammation has subsided, i.e., two days after an antibiotic has been discontinued. Peritoneal equilibration was assessed by measuring creatinine and urea con-

centrations in the blood and dialysate, and glucose concentration in the dialysis solution and dialysate. Glucose, urea nitrogen, and creatinine of the samples were determined by autoanalyzer ASTRA-8(Beckman Instruments, Brea, CA, U.S.A.).

6. Laboratory Tests

Serum iron, ferritin, TIBC, and hematocrit were measured in the first, second and third blood samples taken through the cardiac punctures. Serum Fe, TIBC were determined by colorimetric procedure using Iron, UIBC kit(Fisher Scientific Co.). Ferritin was measured by immunoradiometric assays(Quantimune Ferritin IRMA).

7. Tissue sampling

After final PET test at 6 months, the abdomen was opened, the solution completely drained, the peritoneal membrane was evaluated by gross inspection and in light microscopy after staining with Prussian blue. Tissue samples were taken from 3 parts of abdominal wall(both lateral & diaphragm) and the mesentery of small intestine. We terminated the animals which had poor function of PD catheter, and sacrificed to take tissue samples at that time.

8. Iron Dextran Stability in Bags

Iron dextran in doses identical to those used in animal experiments were injected into dialysate solution bags. Iron concentrations were measured after 24, 48, 72 hours and 1, 2, 3, 4 weeks of storage at room temperature. Finally iron concentration was measured after additional 24 hours storage at 38℃. Iron concentration was measured by nuclear absorption spectrophotometry.

9. Statistical Analysis

The results were analyzed by looking at quantitative response regarding eventual morphological changes in the peritoneum including adhesions, fibrosis and iron deposition. Regarding PET test and laboratory data simple t-test for unpaired comparisons were performed by Sigmastat® program. One way ANOVA and Student-Newman-Keuls method were also applied. Variables are

Table 1. Baseline B.W., Hct, BUN, Serum Cr, Blood Glucose and PET test

Group	Number	B.W. (grams)	Hct (%)	Serum Cr. (mg/dL)	BUN (mg/dL)	Blood glucose (mg/dL)	D/Do glu-2hr
Control	12	362.2±21.7	44.8±1.2	0.37±0.07	11.8±1.6	228.3±23.9	0.24±0.06
Low dose	12	332.7±35.1	44.1±2.3	0.41±0.05	12.9±2.0	208.8±23.6	0.26±0.08
High dose	12	335.8±21.8	43.0±2.7	0.39±0.03	12.7±3.0	223.1±30.8	0.23±0.06

Table 2. The Change of Hematocrit, Serum Iron and TIBC

	Group	Baseline	3rd month	6th month
Hct	Control	44.8± 1.2 (n=12)	43.1± 2.9 (n=10)	44.2± 1.4 (n=5)
	Low dose	44.1± 2.3 (n=11)	45.4± 2.3 (n= 8)	46.4± 0.9 (n=6)*♣
	High dose	43.0± 2.7 (n=11)	47.9± 1.5 (n= 3)*	48.4± 1.8 (n=3)*♣
Serum Fe	Control	172.4±61.7 (n=10)	107.9±42.3 (n= 7)*	159.9± 60.7 (n=5)
	Low dose	157.5±44.3 (n= 8)	131.7±31.8 (n= 8)	197.9±106.5 (n=6)*
	High dose	159.7±40.2 (n=12)	297.0±52.9 (n= 3)*♣	230.8± 42.0 (n=3)*♣
TIBC	Control	489.5±46.7 (n= 9)	478.1±71.5 (n= 7)	535.2± 53.7 (n=5)
	Low dose	439.5±41.3 (n= 8)	522.9±71.1 (n= 8)*	548.3± 57.4 (n=5)
	High dose	486.2±48.2 (n= 7)	479.6±16.3 (n= 3)	500.9± 16.4 (n=3)

*$p<0.05$ vs. baseline ♣$p<0.05$ vs. other groups ♣$p<0.05$ vs. control group

Table 3. The Result of PET test(D/Do glucose-2hr)

	Baseline	3rd month	6th month
Control	0.24±0.06(n=12)	0.33±0.10 (n=7)*	0.25±0.12 (n=5)
Low dose	0.26±0.08(n=12)	0.36±0.07 (n=8)*	0.33±0.07 (n=6)
High dose	0.23±0.06(n=12)	0.37±0.12 (n=3)*	0.38±0.10 (n=3)*

*$p<0.05$ *vs. baseline*

Table 4. Gross Findings of Peritoneum (at 6th month)

Group	Adhesion of Peritoneum	Fibrosis around Catheter	Brown Pigmentaion on Peritoneum
Control (n=5)	1 case (20%)	1 case (20%)	0 case (0%)
Low dose (n=6)	3 cases (50%)	2 cases (33%)	0 case (0%)
High dose (n=3)	3 cases (100%)	3 cases (100%)	2 cases (67%)

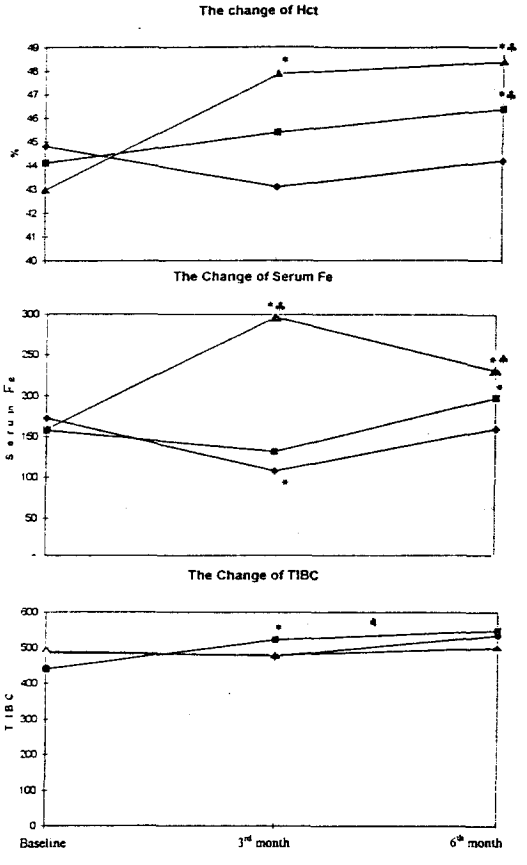


Fig. 1. The change of Hct, Serum Fe, TIBC Symbols are: (◇) control group; (□) low dose group; (△) high dose group; (*) $p<0.05$ vs. baseline; (♣) $p<0.05$ vs. other groups; (♠) $p<0.05$ vs. control group.

shown as mean SD; $p<0.05$ was considered statistically significant.

Results

Baseline data of the groups are listed in Table 1. At baseline there was no significant difference among 3 groups in mean hematocrit, serum creatinine, BUN, blood glucose and baseline PET test. At 3 months, mean hematocrit was significantly higher than baseline in high dose group. At 6 months, mean hematocrits were significantly higher in high & low dose group than in control group(48.4±1.8, 46.4±0.9 44.2±1.4, respectively; $p<0.05$). There was also significant difference of hematocrit between low dose group and high dose group(Table 2, Fig. 1).

There was no significant difference in mean serum iron among 3 groups at baseline. At 3 months and 6 months, serum iron levels were significantly higher in high dose group. At the end of the 6 months study period, mean serum iron level of high dose group was 230.8±42.0 compared with 159.9±60.7 in control group($p<0.05$) (Table 2, Fig. 1).

Among three groups, there was no significant difference of TIBC at baseline. Although mean TIBC was increased in low dose group at 3 months, there was no significant difference among

3 groups at 6 months(Table 2, Fig. 1).

There was no significant difference of D/Do ratio of 2 hour glucose among 3 groups at baseline. At 3 months, mean D/Do ratio of glucose were significantly increased than baseline in all 3 groups($p<0.05$). Comparing to other groups, D/Do ratio of glucose was still high in high dose group at 6 months($p<0.05$). D/Do ratio of 2 hour PET test in high & low dose and control group were 0.38 ± 0.10, 0.33 ± 0.07, 0.25 ± 0.12 respectively(Table 3, Fig. 2).

While 1 case(20%) of control group and 3 cases (50%) of low dose group revealed adhesion of peritoneum, all cases of high dose group revealed adhesion of peritoneum. Fibrosis around catheters were noted in 1 case(20%) of control group, 2 cases(33%) of low dose group and all cases (100%) of high dose group. Comparing to control and low dose group which didn't show any pigmentation on peritoneum, 2 cases of high dose group showed dark brown pigmentation on peritoneum of mesentery as well as abdominal wall by gross inspection(Table 4, Fig. 3).

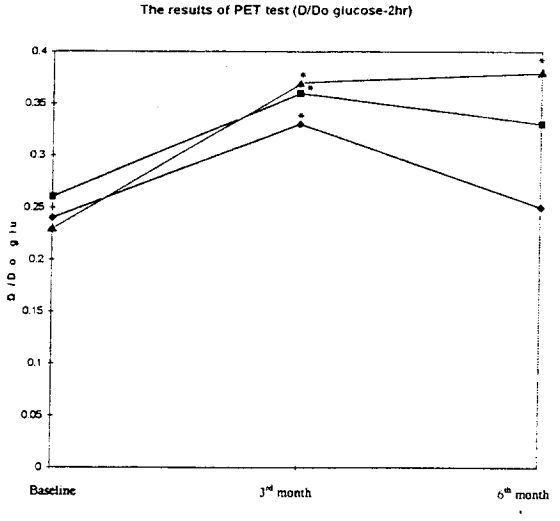


Fig. 2. The results of PET test(D/Do glucose-2hr). Symbols are:(◇) control group; (□) low dose group; (△) high dose group; (*) $p<0.05$ vs. baseline.

There was no iron deposition on peritoneum of mesentery as well as abdominal wall in microscopic finding after Prussian blue staining in control group at 6 months. In low dose group at 6 months, there were countable blue colored iron particles on peritoneum of abdominal wall and mesentery. In high dose group at 6 months, there were uncountable iron aggregates which revealed marked iron deposition on peritoneum of mesentery as well as abdominal wall. Microscopic finding of brown pigmentation on gross inspection showed deep infiltration of iron aggregates. There was no iron deposition in low dose group at 1.5 months & 3 months, but there was mild iron deposition in high dose group at 1.5 months.

The concentration of iron dextran in dialysate bags were stable after storage for 1 month in room temperature in low and high dose groups. There was no significant change of iron dextran level in both groups after storage for 24 hours in an incubator(Table 5).

Discussion

Recombinant human erythropoietin(rHuEpo) has been associated with clinical and symptomatic improvements as well as improvements in quality

Table 5. Iron Dextran Stability Test

Group (n=6)	baseline	24hrs	48hrs	72hrs	1wk	2wks	3wks	4wks	24hrs (38℃)
Low dose (mg/L)	2.06±0.03	2.04±0.06	2.02±0.18	2.05±0.08	1.97±0.08	2.05±0.03	2.01±0.06	2.02±0.05	2.04±0.10
High dose (mg/L)	10.40±0.44	10.10±0.52	10.16±0.33	10.18±0.41	9.91±0.31	9.93±1.21	9.95±0.25	10.01±0.82	10.03±0.24

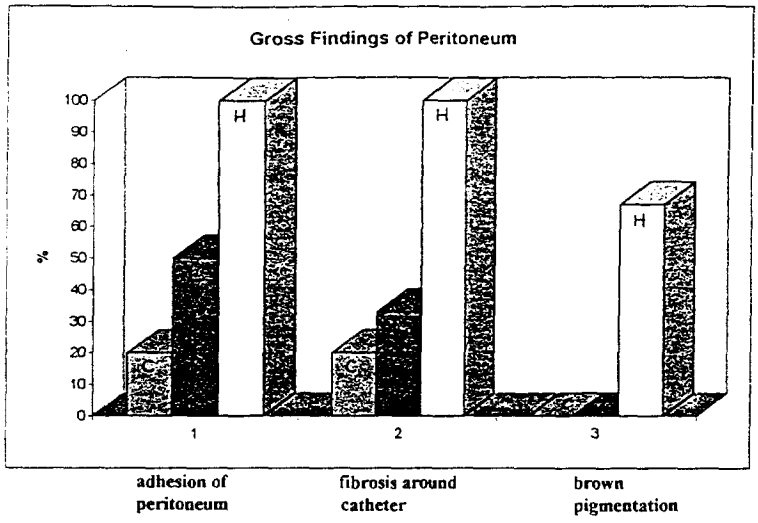


Fig. 3. Gross Findings of Peritoneum. ▒ Control group; ■ low dose group; □ high dose group.

of life in patients with anemia of chronic renal failure[1]. However, resistance to rHuEpo therapy, related to insufficient iron stores, occurs often. Although many patients treated with rHuEpo take oral iron, patients continue to develop iron deficiency[20]. Parenteral iron is a safe and effective alternative in replacing iron stores to sustain erythropoiesis in hemodialysis patients treated with rHuEpo[10]. But, intravenous iron administration as well as intramuscular iron injection have been associated with serious side effects including anaphylaxis[13, 14] and sarcoma[15, 16]. In peritoneal dialysis patient, intravenous iron administration is inconvenient due to poor vascular access. If the intraperitoneal route of iron delivery were available, it would be much more convenient for peritoneal dialysis patients. The purpose of this chronic study is to determine the influence of chronic intraperitoneal iron injections into the peritoneal cavity of rats on function and anatomy of the peritoneal membrane.

Iron dextran solution contains ferric iron which is combined with dextran with molecular weight 160-180 KD. It is commercially available and is widely used for dialysis patients in spite of known anaphylactoid reactions to dextran[13, 21]. Iron dextran stability test shows the iron dextran is very stable in dialysate solution bags in 38℃ as well as room temperature. It might suggest that we can use dialysate mixed with iron dextran as a good source of iron.

The improved erythropoiesis observed with high dose of intravenous iron compared with low dose of iron or no iron in this chronic study was most likely due to an improvement in iron stores induced by intraperitoneal iron injection. Previous our acute study[18] suggested that more than 70% of the iron dextran is absorbed from the peritoneal cavity of the rats during a 6-hour peritoneal dialysis exchange. Hematocrit levels increased in the groups which were instilled dialysates mixed with iron dextran. It means that chronic intraperitoneal iron injections increase iron store by absorption through peritoneal membrane. Serum iron level decreased significantly at 3 months in control group. It can be related to blood sampling by cardiac puncture. While serum iron level was not changed at 3 months in low dose group,

serum iron level increased markedly at 3 months in high dose group. It suggests that chronic intraperitoneal iron injections increase serum free iron as well as iron storage. At 6 months, the graph of serum iron level in high dose group shows a trend to decrease, it can be caused by transformation from serum iron to iron storage including ferritin or hemosiderin[22, 23]. TIBC increased at 3 months in low dose group, but there was no significant difference of TIBC among 3 groups at 6 months.

We investigated the effect of chronic intraperitoneal iron injection on the peritoneum through peritoneal equilibration test(PET) in rats. Baseline PET test shows that mean 2-hour D/Do ratio of glucose are 0.24, 0.26, 0.23 in control, low dose and high dose group respectively. It is consistent with other studies in PD of rats. D/Do ratio increased significantly in all 3 groups at 3 months. Suzuki et al[24]. studied alteration of peritoneal function of rats on 9 weeks of PD, 2-hour D/Do ratio of glucose was 0.39 at 9 weeks. They found mesothelial hyperplasia and fibrotic change of the mesentery at 9 weeks. It may suggest that adaptation of peritoneal membrane to peritoneal dialysis takes several months. Comparing to D/Do ratio of control group at 6 months which returned back to baseline level, D/Do ratio of high dose group was still high at 6 months. According to D/Do ratio, peritoneal function was worst in high dose group, worse in low dose group at 6 months than control group. It might be an evidence that chronic intraperitoneal injection of iron dextran effects the function of peritoneum.

Gross findings of peritoneum shows us meaningful information. While only one case of control group revealed adhesion of peritoneum and fibrosis around catheter, all cases of high dose group showed adhesion of peritoneum and fibrosis around catheter. Brown pigmentation was noted in high dose group only. It means that chronic intraperitoneal injections of high dose of iron dextran can cause anatomical changes including adhesion, fibrosis and pigmentation.

Histologic findings show that there are strong iron depositions on peritoneum of mesentery as well as abdominal wall especially in high dose group. Chronic injection of iron dextran can do damage to some organs including the peritoneum. The main target organs of iron deposition are liver, bone marrow, spleen and other tissues[25]. Schwartz et al.[26] studied a guinea pig model of iron overload toxicity by intraperitoneal iron dextran administration. They found marked iron deposition including iron particles and aggregates in liver and heart as well as bone marrow. If iron particles were noted on the peritoneum, it would be possible that iron deposits in the other organ including liver, bone marrow and spleen. Mild iron deposition was noted in high dose group at 1.5 months as well as low dose group at 6 months. Total amount of iron dextran was 9mg for 6 months in low dose group, and 11mg for 1.5 months in high dose group. Total amount of iron dextran was 45mg for 6 months in high dose group which revealed marked iron aggregates of iron particles. These facts support the hypothesis that the degree of iron deposition is directly proportional to the amount of iron dextran instilled intraperitoneally. Because iron deposition in the liver, heart and other organs leads to tissue injury and ultimately to organ failure, we should consider the optimum dose of iron dextran.

In this chronic study, more than half animals were terminated earlier due to poor catheter function. There are several possible causes of poor catheter function. The first possible cause is low frequency of exchanging dialysates. We exchanged dialysates once a day. It might be a cause of peritoneal adhesion or fibrosis around catheter.

Another possible cause is frequent peritonitis. Incidence of peritonitis is 0.55/month/rat in control group, 0.56/month/rat in low dose group and 0.52/month/rat in high dose group. There was no significant difference of incidence of peritonitis among 3 groups. Adjustment of maintenance dose of antibiotics and more careful handling should be considered. The other possible cause of poor catheter function might be related to instilled iron dextran.

In conclusion, this study suggests that intraperitoneal injection of iron dextran might be a convenient route of iron delivery in peritoneal dialysis patients. But we should consider the optimal dosage of iron dextran because iron depositions can cause damage to the function as well as the anatomy of peritoneum. Future studies must be performed to determine whether chronic intraperitoneal injection of lower doses of iron dextran would improve erythropoiesis without causing potential organ iron overload.

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