Lipid and Protein Oxidation by the Iron Chelate FCP (Sodium Ferricitropyrophosphate)

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GOAL OF THE STUDY: This research critically examined the stability of a new iron chelate (FCP) in serum. Experimental results were compared with data related to representative IV iron drugs under the same conditions.

RESULTS: Both the unique attributes of FCP and data from these and related studies indicate that FCP, appropriately formulated for intravenous administration, may effectively treat iron deficiency anemia, including anemia of inflammation.

DESCRIPTION OF FCP: Sodium ferricitropyrophosphate sodium (FCP) is a water-soluble, 1,000-2,000 Dalton ferric iron chelate in which ferric ion is stably embedded in and chelated to three ligands (citrate, pyrophosphate, and phosphate) and is a candidate for iron repletion intravenously. Our preliminary studies have shown that the ligands stabilize FCP until its Fe^{3+} core is transferred to serum transferrin, a transfer process that saturates transferrin within a few minutes.

DESCRIPTION OF THE EXPERIMENTS: Solutions of FCP (test solutions) or ferrous sulfate (control solutions) in pooled human serum (sera) were maintained at 37 °C and periodically sampled to monitor chelate stability. In separate experiments, we compared serum lipid and protein oxidation that resulted from adding FCP (5.6 mM iron) to sera with the oxidation that resulted from addition of similar concentrations of ferrous sulfate (5.6 mM iron) and copper/ascorbate to sera. In each experiment at defined intervals over a 48-hour period, aliquots were withdrawn and frozen for subsequent analysis of iron, malondialdehyde (MDA), and oxidized proteins. Subsequently, the samples were thawed and assayed for iron, protein carbonyls, or lipid peroxidation products (as MDA). The resulting data provided an assessment of the potential for the iron compound to initiate oxidative stress in serum in which transferrin is fully saturated.

Iron Stability in Human Serum

The iron in each sample was determined as described in the instructions for a commercial iron assay kit using increased volumes of hydrochloric acid and reducing agent. As Figure 1 shows, there was no trend in the iron content of FCP-spiked serum over 48 hr of storage at 37 °C, indicating that FCP is stable in human serum. As Figure 2 shows, the citrate and phosphate concentrations remained constant over 48 hours, whereas the pyrophosphate concentration steadily decreased. Previous studies have shown that the pyrophosphate ligand on FCP undergoes hydrolysis to two phosphate ligands, which remain bound to the ferric iron. The experimental data confirm that this hydrolysis occurs in serum.

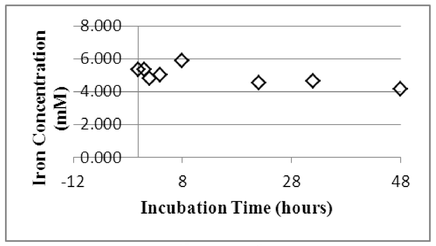

Figure 1

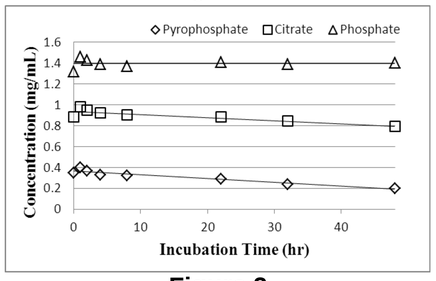

Figure 2

Protein Carbonyl Formation

The protein carbonyl content of pooled human serum and metal-spiked human serum were determined after derivatization with 2,4-dinitrophenylhydrazine, protein precipitation, washing to remove residual supernatant, and resuspension using the components in a commercial protein carbonyl assay kit. The experimental data (Table 1) show that during the 48 hours after addition of FCP to serum there was a small but insignificant increase in the protein carbonyl content. In contrast, addition of ferrous sulfate or addition of copper and ascorbate (a recognized oxidant couple) resulted in 2 and 4 times greater protein carbonylation, respectively, than was observed after addition of FCP.

Table 1: Carbonyl Content of Pooled Human Serum

Sample Identification	Sample Absorbance (AU)	Carbonyl Content (nmol/mL)	Average Carbonyl Content (nmol/mL)
Pooled Human Serum (Control)	0.022	50	56
	0.027	61	
Cu (II)/Ascorbate-spiked Serum (Positive Control)	0.103	234	238
	0.106	241	
FCP-spiked Serum	0.042	95	82
	0.030	68	
Ferrous Sulfate-spiked Serum	0.079	180	183
	0.082	186	

Lipid Peroxidation

Lipid peroxidation in pooled human serum and metal-spiked human serum was determined as malondialdehyde (MDA). Initial attempts to monitor lipid peroxidation as malondialdehyde and 4-hydroxynonenal using a commercial lipid peroxidation assay kit failed to provide reliable results. The assay was repeated using HPLC methodology developed in our laboratories. Under the HPLC conditions, a linear standard curve (0 – 18 µM MDA) was obtained (r^2 = 1.00). Standard recovery was within 95.0% - 105.0% with an RSD of 0.97%. The MDA derivative had a retention time of about 4.8 minutes and was well resolved from other peaks in the chromatogram. Experimental data are summarized in Table 2 and presented graphically in Figure 3.

Table 2: Changes in Malondialdehyde Content in FCP-treated Serum over 48 Hours at 37°C

Incubation Time (Hours)	MDA Content in FCP-Serum (µM)	Average MDA Concentration (µM)
0	3.5	3.4
	3.3	
1	3.5	3.4
	3.3	
2	3.5	3.3
	3.0	
4	3.7	3.7
	3.6	
8	3.9	3.9
	3.9	
22	3.5	3.4
	3.3	
32	3.1	3.2
	3.3	
48	3.1	2.8
	2.6	

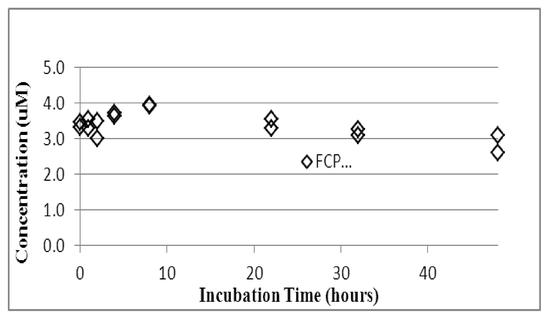

Figure 3

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