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RAPID COMMUNICATION

HYDROXYL RADICALS AND THE TOXICITY OF ORAL IRON

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Ferrous ions in solution are readily oxidized by molecular oxygen. Products of the overall reaction include the superoxide radical (O_2^-, Equation 1), hydrogen peroxide (Equation 2), and the hydroxyl radical ($\cdot OH$, Equation 3).

$$Fe^{2+} + O_2 \longrightarrow Fe^{3+} + O_2^- \quad (1)$$

$$O_2^- + O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2 \quad (2)$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + \cdot OH + OH^- \quad (3)$$

The hydroxyl radical, in particular, is a highly reactive species, whose formation is generally associated with pathologic consequences [1,2].

We wished to determine if ingestion of iron salts could lead to the formation of $\cdot OH$ in the gastrointestinal tract. These experiments were prompted by the knowledge that poisoning by oral iron preparations is a major cause of accidental death in young children [3]. In addition, intolerance to oral iron can be a serious problem when iron is prescribed for a variety of medical reasons [4]. The mechanisms for the toxicity of oral iron have not been elucidated. If evidence were available for the generation of $\cdot OH$ *in vivo*, then methods to treat acute iron poisoning or to suppress oral intolerance to iron might become apparent.

Almost all oral iron preparations contain a ferrous salt (rather than a ferric salt) as the source of elemental iron [3,5]. The reason is that iron is absorbed from the gastrointestinal tract most efficiently when it is in the ferrous form. Many preparations additionally contain ascorbic acid, which is intended to maintain the iron in the ferrous state. In our experiments, we used a ferrous salt combined with ascorbic acid. It should be noted that ascorbic acid reduces the ferric ion to the ferrous state, but it does not inhibit equation 1. Hence, reactive products of reduced oxygen, including $\cdot OH$, will still be formed.

Female Sprague-Dawley rats (220–250 g) received 100 mg of ascorbic acid (Fisher) and 100 mg of ferrous sulfate ($7H_2O$, Fisher; equivalent to 20 mg elemental iron) in aqueous solution by gastric intubation (Table 1).

The animals had first received an aqueous solution of 100 mg of 2-keto-4-methiolbutyric acid (KMB, a potent •OH scavenger; Sigma), by gastric intubation. KMB reacts vigorously with •OH and generates ethylene, a hydrocarbon gas, as a product (Equation 4) [6,7].

$$CH_3-S-CH_2-CH_2-CO-COOH + \bullet OH \longrightarrow CH_2=CH_2 + \text{other products} \quad (4)$$

KMB has been used in numerous *in vitro* studies to detect •OH [7,8]. In studies conducted with biochemical or chemical systems *in vitro*, ethylene production is blocked by catalase (Cf. Equation 3) or by competitive scavengers for •OH [6-9].

Previous studies from this laboratory described methodology for concentrating volatile hydrocarbons evolved from living animals in order to facilitate analyses by gas chromatography [10]. In the experiments reported here, the animals were placed into sealed rebreathing chambers in which the ethylene accumulated in the chamber atmosphere [10]. Samples of chamber air were removed for assay of ethylene.

In Table 1, the rats receiving KMB alone showed a relatively low background rate of ethylene production. This rate was not significantly different from that for untreated rats (viz. 3.59 ± 1.76 nmoles/kg/2 hr, mean ± S.D., N = 4). Those rats receiving oral iron and ascorbic acid along with KMB showed a marked production of ethylene, which persisted for up to 8 hr. These data indicate the generation of a •OH-like species following the oral administration of iron and ascorbic acid. The low level ethylene production seen in untreated animals is consistent with other reports of endogenous production of ethylene and other hydrocarbon gases by normal animals [11].

Table 1. Hydroxyl radical production from orally administered ferrous sulfate and ascorbic acid: *In vivo* detection with KMB

Time (hr)	Rate of ethylene production (nmoles/kg/2 hr) KMB alone	KMB + Iron + Ascorbic acid
0-2	2.39 ± 0.26	45.59 ± 21.69
2-4	3.52 ± 0.90	58.43 ± 26.09
4-6	2.83 ± 1.20	61.38 ± 35.75
6-8	1.12 ± 2.19	9.38 ± 4.64

Gastric instillations of KMB (100 mg), ascorbic acid (100 mg), and ferrous sulfate ($7H_2O$, 100 mg) were made in volumes of 0.5 ml water each, and an additional 0.5 ml water was used as a rinse. Control rats received 0.5 ml of KMB solution and 1.5 ml of water as a rinse (iron and ascorbic acid were omitted). Data are the mean ± S.D. for N=4 except for KMB alone at 6-8 hr, for which N = 3. The differences between the values for KMB alone versus KMB + iron + ascorbate are statistically significant at all time intervals ($P < 0.01$ for 0-2 hr, 2-4 hr, and 4-6 hr, and $P < 0.025$ for 6-8 hr; two-tailed Student's *t*-test).

Ferrous salts dissolved in phosphate-buffered solution at neutral pH are oxidized by molecular oxygen at extraordinarily rapid rates (e.g. half-life of less than 15 seconds for 0.1 mM solution in 50 mM phosphate at pH 7.0) [12]. However, higher concentrations (e.g. 10 mM) in pure water are relatively stable to autoxidation. Nonetheless, oxidation of ferrous ions by oxygen does proceed and can be followed by the appearance of yellow color. The stomach exhibits a strongly acidic pH, while the small intestine is mildly alkaline. These experiments show that •OH is observed under naturally-occurring conditions of pH and in the presence of various endogenous biomolecules when the ferrous salt is instilled directly into the stomach.

Although the gastrointestinal tract *per se* is an obvious site of oxygen radical formation after ingestion of a ferrous salt, it is not clear whether ethylene generation from KMB may additionally reflect the formation of a •OH-like species, stimulated by iron, elsewhere in the body. We note that ethylene production continued for up to 8 hrs, whereas KMB in solution may have already cleared from the gastrointestinal tract at the later time points in Table 1. Further studies concerning this aspect are warranted.

Another point of interest is that ascorbate can have both pro- and anti-oxidant actions. The recycling of ferric ions to the ferrous state by ascorbate can promote formation of •OH when the availability of ferrous ions for Equations 1 and 3 is limiting. Indeed, iron-EDTA plus ascorbate is a well-known system for generating a •OH-like species at neutral pH [13]. On the other hand, ascorbate reacts very effectively with •OH [14] and, therefore, high concentrations of ascorbate may be capable of scavenging a portion of the •OH that is formed. These aspects warrant further investigation.

This preliminary report provides experimental evidence for the generation of •OH *in vivo* following administration of oral iron and ascorbic acid. The dose of iron used in Table 1 is at the low end of a range (0.08 to 0.32 mg elemental iron/g) known to result in damage to the stomach and proximal small bowel in rats [15]. The damage parallels similar findings made at autopsy following death from acute ferrous sulfate poisoning in human subjects [3, 16]. We suggest that hydroxyl radicals may be a mediator of damage to the gastrointestinal tract by oral iron.

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