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PHASE II TRIAL OF COPPER ZINC SUPEROXIDE DISMUTASE (CuZn SOD) IN THE TREATMENT OF CROHN'S DISEASE

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Bovine Cu Zn SOD was used during an 8 year period as an antiinflammatory drug in 26 patients with severe Crohn's disease (CDAI 300) usually after failure of corticotherapy or when this drug was discontinued because of side effects or infection.

This was a phase II trial during which doses routes of administration and concomitant therapies were progressively modified.

We obtained 73% good short term responses (judged upon CDAI and anatomic healing) and 82% positive results on long term evolution (the criteria were : i CDAI lower than 100 in between relapses, ii complete healing or notable improvement of lesions, iii no surgery needed, iv return to work. The acceptability was excellent with the free enzyme. Since the above described experience, published in *Free Radical Biology and Medicine* (1989, 7 : 145-151), we used always the same treatment schedule (SOD 8 mg/day associated with Desferroxamine – 500 mg subcutaneous every 2 days). The follow-up during the 87-89 period showed that 12 are in good health without any relapse, 9 experienced one or more relapses, and showed good responses upon resumption of treatment, 5 failed to respond to treatment, all part of the initial group on which SOD treatment had already failed, and among whom 3 were lost for follow-up before 1987, and two others took up another SOD treatment which also failed. 3 new patients (2 females, 1 male) were treated since then, and all 3 had positive results (one with disappearance of ileocoecal mass).

The efficacy of SOD as an antiinflammatory drug in Crohn's disease needs to be confirmed by controlled trials.

KEY WORDS:

As pointed out by McCord, the gastrointestinal tract is a free radical time bomb.[1] The quantity of xanthine dehydrogenase is higher in the GI tract than in any other tissue. The intestine represents an intimate interface between self and an extremely high concentration of microorganisms. Hence vast numbers of phagocytes including macrophages, polymorphonuclear leucocytes and eosinophils reside there.

The response of the intestinal mucosa to insult, be it traumatic infectious or ischemic, is non specific. Thus when a patient presents with new or recurrent manifestations of intestinal inflammation, before he can be classified as having ulcerative colitis or Crohn's disease, alternative pathologic processes must be considered (Table I).[2,3]

Crohn's disease is a subacute and chronic inflammation of the of the digestive tract from mouth to anus, presenting especially in the distal ileum, the colon and the anorectal area. The symptoms include fever, diarrhea, cramping, abdominal pain and weight loss. The inflammation is focal, with microerosions, fissuring ulcerations, proeminent lymphoid aggregates, and a pronounced round cell and polymorphonuclear (PNNS) infiltrate.[3]

TABLE I
Differential Diagnosis of Inflammatory Bowel Disease

Bacterial	Known Etiology	Idiopathic
Campylobacter	Diverticulitis	Ulcerative colitis
Salmonella	Radiation	Crohn's disease
Shigella	Ischemia	Behcet's
Escherichia coli	Vasculitis	
Clostridium difficile	Drug-induced	
Aeromonas hydrophilia		
Plesiomonal shigelloides		
*Neisseria gonorrhea**		
Syphilis		
Parasitic		
Amoeba		
Viral		
Cytomegalovirus*		
Herpes simplex*		
Lymphogranuloma venerum*		

*Gay bowel or immuno-compromised host.

At times the distinction between ulcerative colitis and CD of the colon may be difficult (Table II).[4,5]

To date, there is no unifying explanation regarding the pathogenesis of either UC or CD.[3] Numerous immunologic perturbations have been described in patients with CD, but to date no primary immune event has been identified.[6]

Regardless of the nature of the specific primary event the local immune apparatus now stimulated recruits PNMS lymphocytes, macrophages and mast cells. This accumulation of immune cells releases inflammatory mediators and reactants. Consequently in actively inflamed segments of mucosa, oxygen radicals are produced in abundance,[7] and there is enhanced release of prostaglandins and leucotrienes.[8,9]

The antiinflammatory properties of Cu Zn SOD are well established, even though

TABLE II
Distinguishing Features Of Crohn's Disease and Ulcerative colitis

	Crohn's Disease	Ulcerative Colitis
Cigarette smoking	+	0
Macroscopic		
Small bowel disease	±	0
Perianal disease	±	0
Fistula	±	0
Diffuse, symmetric	0	+
Skip areas	+	0
Rectum	±	+
Linear ulcers	+	0
Aphthous ulcers	+	0
Microscopic		
Focal ulceration	+	0
Aphtous ulcer	+	0
Granuloma	±	0
Transmural inflammation	+	0

it is not precisely known how and when it works in the complex events of inflammation.[10]

Presently steroids and sulfasalazine are considered the mainstays of CD therapy. There is evidence that Sulfasalazine is effective in the treatment of acute attacks of CD when the disease is confined to the colon. Its active component 5 amino-salicylic acid is assumed to have the same effect.[11]

Corticosteroids have demonstrated efficacy in CD as remission inducing agents. Steroids while effective are not the antiinflammatory drugs of choice because of the many, often serious side effects associated with their use.[12]

The limitation of surgical therapy is the high recurrence rate and malabsorption secondary to the short bowel syndrome.

For these reasons, SOD appeared to be a drug to experiment as an antiiflammatory in the treatment of CD.

Free iron seems to play an important role in inflammation via the Fenton reaction and the chelation of iron partly inhibits the inflammatory reaction. In light of these findings we employed SOD in conjunction with Desferroxamine, during the 1985–1989 period.[13]

MATERIAL AND METHODS

The results obtained during the 1980–1987 period, are published in FRBM.[14] 26 patients were treated. The treatment was started at an average of 6.6 years following the diagnosis of CD. 7 patients (26%) had previous surgery and 13 had previous corticotherapy. The SOD treatment was started usually after failure of corticotherapy or when this drug was avoided because of side effects or abcesses.

The GI involvement by CD was small intestine in 5 cases (4 ileal, 1 duodenojejunal), colic in 14 cases and ileocolic in 7 cases.

The clinical activity of the disease was assessed by the Crohn's Disease Activity Index (CDAI), that is:

$$\text{CDAI} = 2X_1 + 5X_2 + 7X_3 + 20X_4 + 30X_5 + 10X_6 + 7X_7 + X_8$$

where X_1 = number of soft/liquid stools in one week, X_2 = sum of abdominal pain ratings (0–3) for one week, X_3 = sum of well-being ratings (0–4) for one week, X_4 = number of types of extraintestinal findings (fistula, arthritis, uveitis, etc.) X_5 = opiates or lomotil (O-one, 1- used), X_6 = abdominal mass (O-one, 2-questionble, 5-present), X_7 = (47 - hematocrit) in males; (42 - hematocrit) in females, and X_8 = 100 - (percentage of standard weight).

The CDAI was over 300 in 22 patients and over 150 in 4 patients, prior to their first SOD treatment.

Cu Zn SOD treatment

The SOD used was Bovine erythrocyte Cu Zn SOD. Treatments used before are described in FRBM. The dose administered during the 86/89 period was 8 mg/day in 2 IM injections.

Treatment was interrupted when the clinical, biochemical and radio-endoscopic signs of the disease disappeared. The average time for the disappearance was one month (range 10 days–4 months). The clinical signs were the first to disappear.

Concomitant therapy

Cu Zn SOD was never used in combination with other anti-inflammatory drugs (corticoids or sulfasalazine). However, the simultaneous use of metronidazole was frequent, particularly in patients with a CDAI over 300. The drug was used I.V. (150 g/day) and/or orally (1 g/day). The rationale for using metronidazole was to suppress the activity of anaerobs which perhaps exacerbate the disease.

In the period from 1985–1989, concomitant desferroxamine treatment was used for 15 days (slow subcutaneous injection of 500 mg in 12 hours every two days).

Evaluation criteria

Short term efficacy was judged upon: i) The clinical and biological responses as assessed by CDAI and ii) the anatomic healing as assessed by radiologic and endoscopic examinations.

Long term efficacy was judged upon: i) A CDAI lower than 100, in between relapses; ii) complete healing or notable improvement of lesions; iii) no surgery needed; iv) shortening of the duration of relapses and resumption of normal activity - return to work.

The study referred to in FRBM ended late 1987. During 87–89 period, the treatment was 8 mg/day in 2 injections with Desferroxamine for a 15 day period. Nine of the 26 patients experienced relapses and this treatment schedule was applied. New patients (2 females and 1 male) were included in this study, all with a CDAI lower than 300 and with previous corticotherapy.

RESULTS

During the 82–87 period, we observed:

– short term responses: all 26 patients responded to the treatment. The clinical response was very satisfactory in 18 patients (64.2%) (CDAI below 100). Further endoscopic and/or radiographic investigations showed complete healing or significant improvement in 17 patients.

– long term responses: Four groups of patients could be distinguished: i) 16 patients (61.5%) fulfilled all the criteria of long term efficacy, ii) 5 patients (19.2%) fulfilled all these criteria except the fact that surgery was needed to remove quiescent lesions, iii) 4 patients for whom SOD treatment was considered a failure (there was an initial response to treatment in 2 patients (11.7%), while the other 2 experienced no response), iv) 1 patient (3.2%) dropped out and was unavailable for follow-up.

The follow-up during the 87–89 period showed that:

– 12 are in good health without any relapse;

– 9 experienced one or more relapses, showed good responses upon resumption of treatment;

– 5 failed to respond to treatment, all part of the initial group on which SOD treatment had already failed, and among whom 3 were lost for follow-up before 1987, and two others took up another SOD treatment which also failed.

– For the 3 new patients (2 female, 1 male), the following results were observed.

In one female patient suffering from ileocolitis with mass, the mass disappeared completely after 3 months of SOD treatment (1 year follow-up). The second female patient recovered from colitis following 4 months of SOD treatment. The male patient

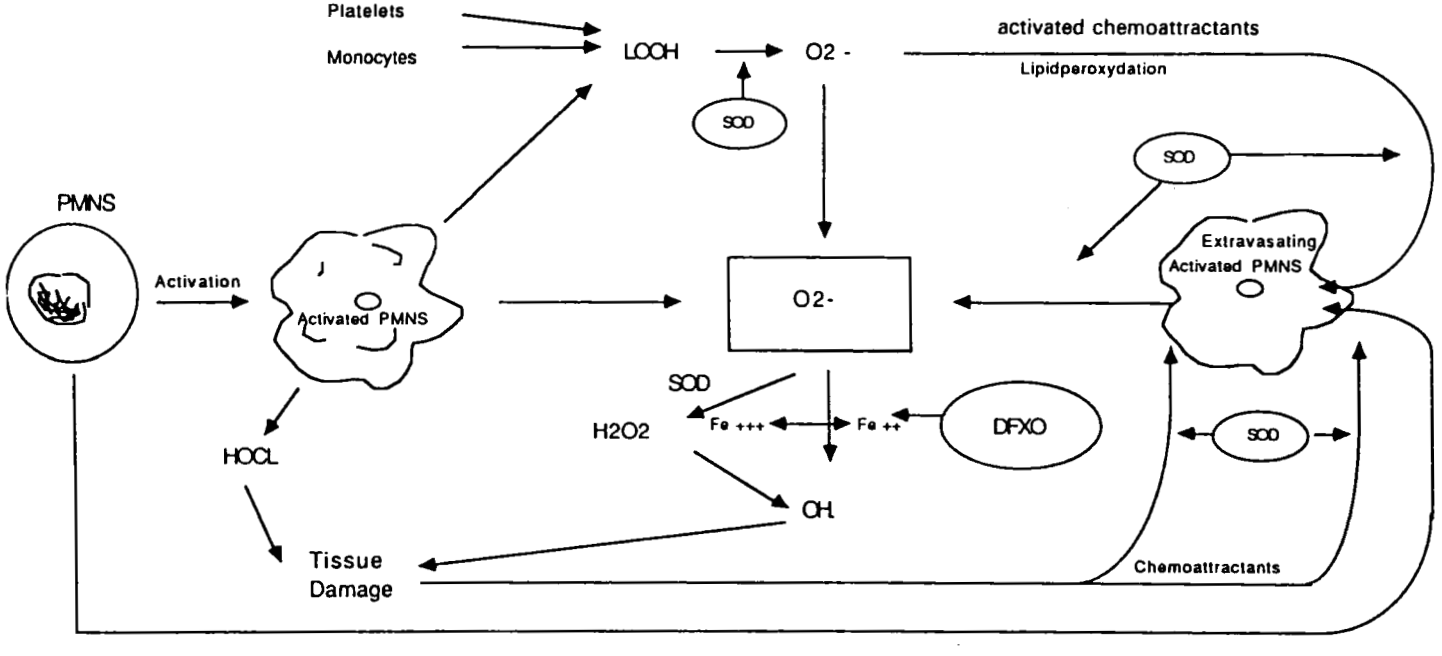

FIGURE 1 *The activation of PMNS and tissue damage.* The presence of lipid chemotactic factors causes neutrophils to stick to the endothelium and extravasate, resulting in tissue damage or injury. SOD can interrupt this course, both by dismutation of O2− and inhibition of chemotaxis. The chelation of iron by desferroxamine (DFXO) slows down the production of OH. radicals.

suffered colitis with spondiloarthritis for which he had been treated during 10 years; both disappeared with SOD treatment (3 months follow-up).

DISCUSSION

In this study, Cu Zn SOD was used as an antiinflammatory drug for the treatment of Crohns disease, usually after failure of corticotherapy. The promising results are clearly preliminary and need to be confirmed by controlled trials.

The acceptability of the drug was good and no adverse effect was observed with the free enzyme (supplied by PCH) used intramuscularly over 8 years of experience.

The efficacy of treatment during this 10-year study appears excellent. The satisfactory short term (75%) and long term results (82%) obtained during the 80-87 period were confirmed during the 87–89 period (9/11 patients who experienced relapses recovered quickly upon resumption of treatment). Three new patients showed excellent results.

Such results, obtained over a 10-year period, represent an impressive score in Crohns disease, where an improvement rate of 60% is usually considered as satisfactory – in a controlled trial with cyclosporine.[16] The efficient SOD (8 mg/day) and a combination with Desferroxamine was applied only during the last five years. Intramuscular injections proves a good mode to administer SOD in the treatment of Crohns disease. Initially, the IV injection of SOD for 1 or 2-day periods may have yielded quicker results but we avoided this mode of administration because of the pyrogenicity of the PCH product.

Based on our observations, SOD has worked on our patients as an antiinflammatory drug. The assumed mechanism of action of the enzyme is probably the protection of tissue from direct cytotoxicity of superoxide radicals generated by neutrophils.[16] Studies in animal models suggest that neutrophils may be involved in the pathogenesis of inflammatory bowel disease.[17] Indeed, "patients with Crohns disease show abnormalities of neutrophils suggestive of activation of these cells".[18]

The action of CuZn SOD in inflammation models is related not only to the dismutation of O_2^- but also to the prevention of formation of superoxide-dependent chemotactic activity, especially of lipid peroxides.[19] The action prevents the accumulation of inflammatory cells at the site of lesion. Free iron may potentiate tissue damage and this could explain the antiinflammatory action of desferroxamine (DFXO). In Figure 1, we present a diagram to explain the action process of both CuZn SOD and DFXO in the protection against tissue damage mediated by activated PMNS.

In conclusion, SOD appears as a useful antiinflammatory drug in the treatment of Crohns disease, especially when administered in conjunction with DFXO. A controlled trial is needed to confirm these findings.

References

1. J.M. McCord (1987) Radical explanations for old observations. *Gastroenterology*, **92**, 2026-2027.
2. F.W. Nugent and P.F. Kolack (1988) Differential diagnosis of chronic ulcerative colitis and Crohn's disease of the colon: in: *Inflammatory Bowel Disease:* Third Edition. Shorter R.G. Kirsner J.B., editors. Lea & Febiger Publishers, Philadelphia, PA. Chapter 10: 185–207.
3. J.B. Kisner and R.G. Shorter (1982) Recent developments in "non-specific inflammatory bowel disease". *New England Journal of Medicine*, **306**, 775–785, 837–848.
4. R.G. Farmer *et al.* (1985) Clinical Patterns in Crohn's disease: a statistical study of 615 cases.

Gastroenterology, **68**, 1818–1825.
5. H. Malchow *et al.* (1984) Therapie des Morbus Crohn. *Dtsch Med Wochenschr*, **109**, 1811–1816.
6. C.O. Elson (1988) The immunology of inflammatory bowel disease. In: Inflammatory Bowel Disease: Third Edition. Shorter R.G., Kirsner J.B. editors. Lea & Febiger Publishers, Philadelphia, PA, Chapter 7: 97–164.
7. J. Emerit, M.T. Droy-Lefaix, J. Likforman and M.C. Diemert (1989) Les radicaux libres oxygénés (RLO) et maladies inflammatoires de l'intestin (Mll). *J. Chir (Paris)*, **126**, no 5: 287–293.
8. M. Donowitz (1985) Arachidonic acid metabolites and their role in inflammatory bowel disease. An update requiring addition of a pathway. *Gastroenterology*, **88**, 580–587.
9. P. Sharon and W. Stenson (1984) Enhanced synthesis of leukotrienes by colonic mucosa in inflammatory bowel disease. *Gastroenterology*, **86**, 453–460.
10. L. Flohe, R. Beckmann, H. Giertz and G. Loschen (1985) Oxygen centered free radical as mediators of inflammation. In: Sies H. ed. Oxidative Stress. London: Academic Press. 403–435.
11. R.W. Summers, *et al.* (1979) National Cooperative Crohn's disease study: results of drug treatment. *Gastroenterology*, **77**, 847–869.
12. J.W. Singleton (1980) Medical therapy of inflammatory bowel disease. *Med. Clin. North Am.*, **64**, 1117–1133.
13. B. Halliwell and J.M.C. Gutteridge (1988) Oxygen free radicals and iron in relation to biology and medicine: some problems and concepts. *Archives of Biochemistry and Biophysics*, **246**, 501–514.
14. J. Emerit, S. Pelletier, D. Tosoni-Verlingue and M. Mollet (1989) Phase ll trial of copper zinc superoxide dismutase (CuZnSOD) in treatment of Crohn's disease. *Free Radical Biology and Medicine*, **7**, 145–149.
15. J. Brynskov, L. Freund S.N. Rasmussen *et al.* (1989) A placebo-controlled, double-blind, randomized trial of cyclosporine therapy in active chronic Crohn's disease. *New England Journal of Medicine*, **321**, 13: 845–850.
16. B. Grishamm and D.N. Granger (1988) Neutrophils mediated mucosal injury. *Digestive Diseases and Sciences*, **33**, 65–155.
17. J.F. Chester, J.S. Roos, R.A. Malt and S.A. Weitzman (1985) Acute colitis produced by chemotactic peptides in rats and mice. *American Journal of Pathology*, **121**, 284–290.
18. H.L. Malech and J.I. Gallin (1987) Neutrophils in human diseases. *New England Journal of Medicine*, **317**, 687–694.
19. W.F. Petrone, D.K. English, K. Wong and J. McCord (1980) Free radicals and inflammation. *Proceedings at the National Academy of Sciences USA*, **77**, 1159–1163.
20. J.O. Yoon, J.M. Im, P.N. Manson, G.B. Bulkley and J.E. Hoopes (1987) Amelioration of free radical mediated postischemic reperfusion injury by iron chelation in skin flaps. *Surgical Forum*, **38**, 585–587.

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