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Effects of the non-peptidyl low molecular weight radical scavenger IAC in DNBS-induced colitis in rats

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ABSTRACT

Intestinal inflammation is accompanied by excessive production of reactive oxygen and nitrogen radical species because of the massive infiltration of polymorphonuclear and mononuclear leukocytes. Antioxidant compounds seem to protect against experimental colitis. Here we investigated the effects of the innovative non-peptidyl, low molecular weight radical scavenger bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl) decandioate (IAC), which is highly reactive with most oxygen, nitrogen and carbon centred radicals and is easily distributed in cell membranes and intra-extra cellular compartments, in the DNBS model of colitis. Colitis was induced in male SD rats by intrarectal administration of DNBS (15 mg/rat). IAC (30 mg/kg b.w., hydrophilic or lipophilic form) was administered daily (orally or i.p.) starting from the day before the induction of colitis for 7 days ($n=6$–8 per group). Colonic damage was assessed by means of macroscopic and histological scores, myeloperoxidase activity (MPO) and TNF-α tissue levels. Colitis impaired body weight gain and markedly increased all inflammatory parameters. IAC significantly counteracted the reduction in body weight gain, decreased colonic damage and inflammation and TNF-α levels in DNBS-colitis. The antioxidant IAC significantly ameliorates experimental colitis in rats. This strengthens the notion that antioxidant compounds may have therapeutic potential in inflammatory bowel disease.

1. Introduction

Inflammatory bowel disease, including Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of unknown origin: multiple factors, including genetic and environmental factors, probably contribute to inflammatory bowel disease. Intestinal inflammation is accompanied by excessive production of reactive oxygen and nitrogen radical species, such as superoxide ($O\cdot_2^-$), nitric oxide ($NO\cdot$), peroxynitrite ($ONOO\cdot^-$) and hydroxyl radicals ($\cdot OH$) (Grisham, 1994; Grisham and Granger, 1988; Middleton et al., 1993; Rachmilewitz et al., 1993) because of the massive infiltration of polymorphonuclear and mononuclear leukocytes which may produce large amounts of free radicals (Kitahora et al., 1988; Kruidenier et al., 2003a; Mahida et al., 1989; Verspaget and Beeken, 1985). Large numbers of peripheral neutrophils (Crama-Bohbouth et al., 1988) producing oxygen-derived free radicals (Verspaget et al., 1988), migrate into the intestinal wall of inflammatory bowel disease patients. In addition, nitric oxide ($NO\cdot$) overproduction, due to the expression of the inducible isoform of $NO\cdot$ synthase (iNOS), plays an important role in several animal models of inflammation (Cuzzocrea et al., 1998). Inflammatory bowel disease patients show high levels of nitrite (metabolite of $NO\cdot$ in water) and increased iNOS activity(Ikeda et al., 1997; Middleton et al., 1993): inhibition of iNOS activity exerts beneficial effects in animal models of experimental colitis (Aiko and Grisham, 1995; Mourelle et al., 1996). NO-induced damage is believed to be mediated, at least in part, by peroxynitrite ($ONOO\cdot^-$), a highly reactive oxidant produced by the combination of $O\cdot_2^-$ and $NO\cdot$ at rates approaching the diffusion limit (Aiko and Grisham, 1995; Ischiropoulos et al., 1992). $ONOO\cdot^-$ can induce cytotoxicity by initiating lipid peroxidation, inactivating various enzymes (mitochondrial respiratory enzymes and membrane pumps) (Beckman, J.S., 1996) and by depleting glutathione (Phelps et al., 1995). Peroxynitrite can also cause DNA damage (Inoue and Kawanishi, 1995) resulting in the activation of the nuclear enzyme poly (ADP-ribose) synthetase and poly (ADP-ribose) synthase-driven cell death (Szabo and Dawson, 1998). Furthermore, $ONOO\cdot^-$ inhibits the activity of the endogenous superoxide dismutase enzymes, contributing to an increased $O\cdot_2^-$ production (Yamakura et al., 1998).

Recent studies indicate that antioxidant compounds, which can reduce the production or the effects of reactive oxygen species, such as melatonin (Cuzzocrea et al., 2001b), natural antioxidants (Korkina

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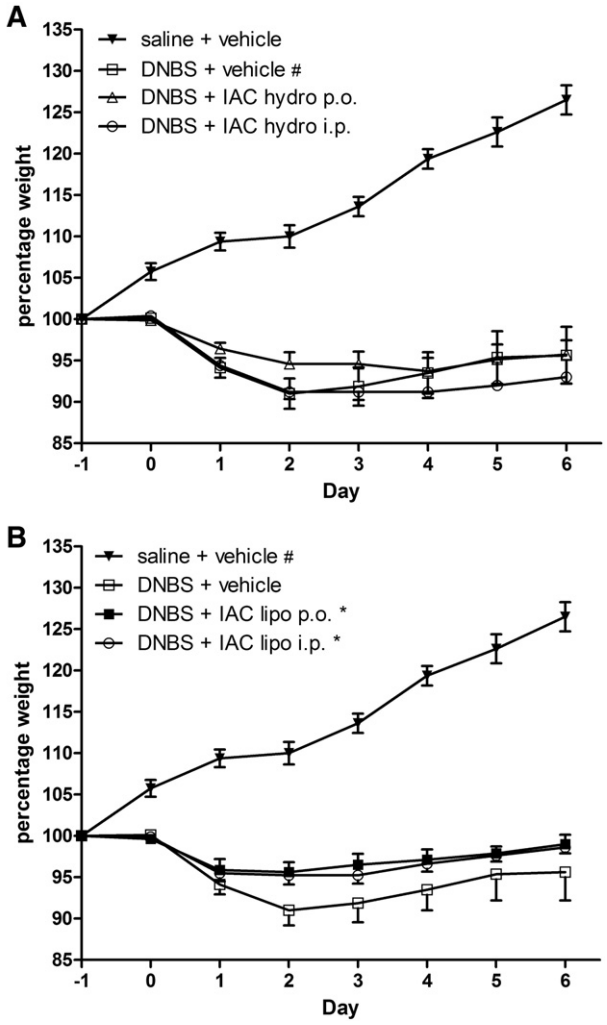

Fig. 1. Panel A: Effect of IAC 30 mg/kg b.w. (hydrophilic form) on body weight gain (%) in the different experimental groups; Panel B: Effect of IAC 30 mg/kg b.w. (lipophilic form) on body weight gain (%) in the different experimental groups. Data are expressed as means ± S.E.M.; $n = 5–8$ rats per group. # $P<0.01$ vs. saline; * $P<0.05$ vs. DNBS; IAC hydro = hydrophilic form of IAC; IAC lipo = lipophilic form of IAC. Group saline (+ vehicle) and DNBS (+ vehicle) in panels A and B represent the same experiments. p.o. = *per os*; i.p. = intraperitoneal administration.

et al., 2003; Oz et al., 2005) glutathione (GSH) precursors or compounds involved in GSH synthesis (Oz et al., 2005) and peroxynitrite decomposition catalysts (Salvemini et al., 1999), protect against experimental intestinal inflammation, including DNBS-induced colitis. Tempol, a well-known low molecular weight radical scavenger, exerted beneficial effects in DNBS-induced colitis (Cuzzocrea et al., 2000) as well as in gastric mucosal damage induced by ischemia/reperfusion (Abdallah et al., 2009). However, cyclic nitroxides such as tempol are very persistent in water or organic solution, but when used *in vivo* or in a biological sample they are reduced to the parent hydroxylamine by several enzymatic processes mainly involving ascorbate or glutathione (Miura et al., 1992).

In the present work we investigated the effects of the non-peptidyl, low molecular weight radical scavenger bis(1-hydroxy-2,2,6,6-tetramethyl-4-piperidinyl)decandioate (IAC) in the DNBS model of colitis, relying on its high reactivity with most oxygen, nitrogen and carbon centred radicals. Due to its peculiar physico-chemical properties, IAC is more stable in physiological solutions, its antioxidant capability is stronger than those of aforementioned cyclic nitroxides (Valgimigli et al., 2000) and, unlike peptidic antioxidants, is slightly affected by the gastric environment (low pH, peptidases) (Paolini et al., 1996). Moreover, due to its log *P* (Valgimigli et al., 2001), it is easily distributed through cell membranes and intra-extra cellular compartments, thus it can directly react with oxidant molecules within the cell, where free radicals are produced.

In the present work, we tested two different forms of IAC, the hydrophilic (protonated, as hydrochloride) and the lipophilic form (for more details on chemical structures see (Valgimigli et al., 2000)). The IAC protonated (hydrophilic) form is completely water-soluble and distributes into the extracellular compartments, but it is also in equilibrium with the free form, which is highly lipophilic (the calculated logP is 4.01) (Crippen, 1987) and readily crosses the cellular membrane, distributing into any compartment. The IAC unprotonated (lipophilic) form permeates the cellular membrane even more easily and the equilibrium between protonated and unprotonated forms is affected by the pH of the compartment, without any loss of activity.

Usually, the hydrophilicity/lipophilicity balance of a radical trap physically confines it to just one compartment and enables it to specifically react only with those radicals produced therein. On the contrary, due to its peculiar physico-chemical properties, IAC readily diffuses through the cellular membrane and can reach virtually any compartment where the production of free radicals occurs. This represents an advantage, because it can directly react with oxidant molecules within the cell in many different compartments.

2. Materials and methods

2.1. Animals

Male Sprague Dawley rats (180–200 g body weight; Harlan Italy, S. Pietro al Natisone, Udine, Italy) were used in this study. Animals were housed in a controlled environment and had free access to food and water throughout the study. Before starting any experimental procedure, in order to minimize the effects of stress *per se* on the parameters to be measured, animals were weighed and gently manipulated in the laboratory environment for 30 min everyday for at least 1 week. All experiments were carried out according to the guidelines set forth by EEC Directive 86/609 on the care and use of experimental animals. The protocol for induction of colitis was reviewed by the Institutional Committee on the care and use of experimental animals of the University of Bologna and was authorized by the Italian Ministry of Health. A persistently hunched posture and laboured respiration, a markedly erected coat and a weight loss of more than 20% were considered as humane end-points to euthanize the animals.

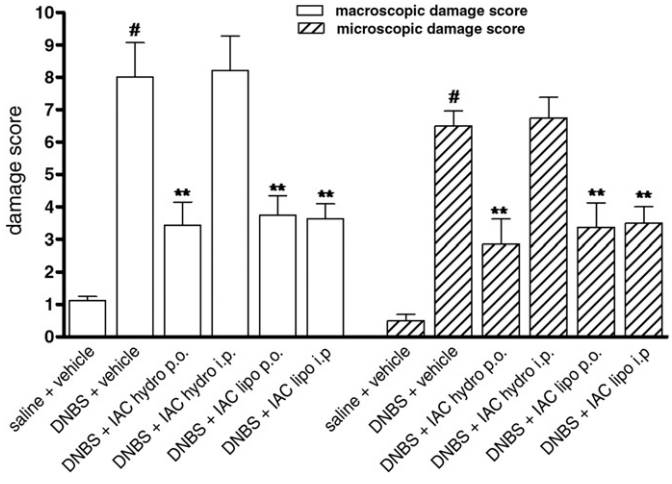

Fig. 2. Effect of IAC 30 mg/kg b.w. (hydrophilic or lipophilic form) on macroscopic and microscopic damage score. Treatment with hydrophilic IAC p.o. significantly decreased colonic damage and inflammation, while i.p. administration failed to protect the colon from DNBS-induced damage. Seven-day treatment with lipophilic IAC p.o. and i.p. significantly reduced intestinal damage induced by inflammation. Data are expressed as means ± S.E.M.; $n = 5–8$ rats per group # $P<0.01$ vs. saline; ** $P<0.01$ vs. DNBS.