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2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potentiates nitrosation of a heterocyclic amine carcinogen by nitric oxide

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Abstract

Although nitrosation plays an important role in initiation of carcinogenesis, the reactive nitrogen oxygen species (RNOS) mediating this reaction by multiple pathways have not been determined. The heterocyclic amine carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was used as a target to investigate RNOS and pathways for potentiation of nitric oxide (NO)-mediated nitrosation. 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (CPTIO) oxidizes NO to NO_2 and was used as a tool to investigate NO_2 potentiation of nitrosation. The IQ nitrosation product, 2-nitrosoamino-3-methylimidazo[4,5-*f*]quinoline (^{14}C–N–NO–IQ), was monitored by HPLC. Autoxidation of NO, generated by spermine NONOate (2.4 μM NO/min) for 7.5 min, did not convert 10 μM ^{14}C–IQ to N–NO–IQ. However, the presence of 15 μM CPTIO resulted in 3 μM N–NO–IQ formation. Potentiation by CPTIO occurred at low and high fluxes of NO, 0.075 to 1.2 μM/min, and over a range of IQ to CPTIO ratios of 0.5 to 10. A significant portion of N–NO–IQ formation was insensitive to azide (10 mM) inhibition, suggesting oxidative nitrosylation. NADH (0.02 mM) did not alter nitrosation by autoxidation, but effectively inhibited potentiation by CPTIO. Ascorbic acid (0.2 mM) and 5,5-dimethyl-1-pyrroline *N*-oxide (30 mM) inhibited nitrosation with or without CPTIO, while superoxide dismutase was not inhibitory. The RNOS produced by CPTIO had a 27-fold greater affinity for IQ than those produced by autoxidation. Results are consistent with NO_2 or a RNOS like NO_2 potentiating IQ oxidative nitrosylation. Nitrosation occurring at both low and high fluxes of NO can contribute to carcinogenesis.

Keywords: Nitric oxide; Nitrosation; 2-Amino-3-methylimidazo[4,5-*f*]quinoline; Heterocyclic amines; Reactive nitrogen oxygen species; 2-(4-Carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide

Introduction

Reactive nitrogen oxygen species (RNOS) elicit nitrosation reactions, which cause deleterious effects that contribute to chronic inflammation and play an important role in many common diseases, including initiation of carcinogenesis (Parsonnet, 1999). By reacting with superoxide to form peroxynitrite anions or with oxygen (autoxidation), nitric oxide (NO) produces RNOS such as nitrogen dioxide radicals ($NO_2^{\cdot}$) and dinitrogen trioxide (N_2O_3). NO-derived RNOS nitrosatively deaminate DNA (Wink et al., 1991) and inhibit DNA repair (Jaiswal et al., 2001; Liu et al., 2002). Nitrite and nitrate, stable end products of NO metabolism, and *N*-nitrosamines, nitrosation reaction products, have increased urinary excretion in individuals suffering from infections (Green et al., 1981; Tricker et al., 1989; Satarug et al., 1996). These increases are due to stimulated cells, i.e., macrophage, upregulating inducible nitric oxide synthase (iNOS) with increased synthesis of NO (Miwa et al., 1987; Grisham et al., 1999). While pathways for production of NO and cellular nitrosation targets are known, nitrosation pathways in biological systems are not well understood.

Chronic inflammation/infection and injury play an important role in colon cancer (Parsonnet, 1999). The incidence of colorectal carcinoma in patients with inflammatory bowel disease, a chronic inflammatory disease which includes colitis, is 20-fold higher and has an average age of onset 20 years younger than the general population (Harpaz and Talbot, 1996). Inflammatory bowel disease is associated with high levels of

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iNOS and 3-nitro-tyrosine, a marker of RNOS (Singer et al., 1996; Hussain et al., 2000). Genotoxic effects of NO, due to nitrosation by RNOS, in both colorectal tumors and colons of individuals with ulcerative colitis correlate with increased iNOS detected in these tissues (Ambs et al., 1999; Hussain et al., 2000). In addition, experiments in humans and mice have demonstrated that colitis produces an increase in fecal *N*-nitroso compounds and dimethylnitrosamine (Mirvish et al., 2003; de Kok et al., 2005). Increased mouse fecal nitrosation products correlated with increased nitric oxide production (increased urinary NO_2^-/NO_3^-) (Lakshmi et al., 2005a). This suggests that chronic inflammation of the colon results in an increased flux of NO that reacts with susceptible compounds in the gut.

We have demonstrated that NO-derived RNOS produced during autoxidation convert 2-amino-3-methylimidazo[4,5-*f*] quinoline (IQ) to 2-nitrosoamino-3-methylimidazo[4,5-*f*]quinoline (N–NO–IQ) (Lakshmi et al., 2002). N–NO–IQ may be an alternative to *N*-hydroxy–IQ in initiation of carcinogenesis. IQ is one of a number of heterocyclic amines derived from well-done red meat (Sugimura, 2000) and thought to initiate colon cancer in humans (Kristiansen et al., 1997; Sinha et al., 1999). N–NO–IQ formation from IQ links two processes related with colon cancer risk, well-done red meat from the diet and inflammation of the colon. IQ might also be useful for studying RNOS-mediated nitrosation reactions. Studying RNOS requires their measurement, made difficult by their high reactivity and short-lived nature (<1 s for $ONOO^-$). However, N–NO–IQ is a stable RNOS-derived end product, whose analysis can provide information about it's mechanism of formation, including the RNOS involved. It has not been determined whether RNOS-induce a nitroso (NO^+) and/or a nitrosyl (NO radical) addition to IQ.

Autoxidation is slow and considered unlikely to occur in biological systems because NO can be rapidly inactivated (Espey et al., 2001). Thus, alterative pathways for nitrosation most exist to account for the significant amounts of S- and *N*-nitros(yl)ation detected in vivo (Bryan et al., 2004). We have addressed this issue in our recent work and demonstrated enhancement of NO-mediated nitrosation of IQ by peroxidases, myeloperoxidase and hemin, and stimulated human neutrophils (Lakshmi et al., 2005a,b). Because myeloperoxidase is present at sites of inflammation and hemin or methemoglobin is present in fecal contents of the colon or at sites of tissue damage, peroxidases might be responsible for the same nitrosation observed in vivo. Our studies suggested several potential mechanisms for peroxidase mediated N–NO–IQ formation with $NO_2^{\cdot}$ thought to play a predominant role. $NO_2^{\cdot}$ can be formed in biological systems by several mechanisms. We have selected 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide (CPTIO) to generate $NO_2^{\cdot}$. CPTIO is an oxidant that converts NO directly to $NO_2^{\cdot}$ in an oxygen transfer reaction (Akaike et al., 1993). CPTIO inhibition of NO-mediated biological effects is attributed to its high binding affinity for NO, preventing stimulation of guanylate cyclase and increases in cyclic GMP (Crawford et al., 2006). Prevention of physiological and biological effects of NO has suggested that this type of agent might have therapeutic value. We used CPTIO as a tool to further investigate a role for $NO_2^{\cdot}$ in N–NO–IQ formation. Knowledge of the mechanisms by which nitrosation can occur in biological systems will provide a better understanding of this process and ways in which the process might be modified.

Methods

Experimental materials

[2-^{14}C]–IQ (10 mCi/mmol, >98% radiochemical purity) was purchased from Toronto Research Chemicals (Toronto, ON) and CPTIO from Calbiochem (San Diego, CA). The structural characterization of N–NO–IQ has been previously determined by electrospray ionization mass spectrometry and NMR (Lakshmi et al., 2002). Other supplies and material were provided by the same sources and conditions as previously described (Lakshmi et al., 2005a,b).

Nitrosation of IQ

To assess NO-mediated nitrosation, ^{14}C–IQ (0.01 mM) was incubated in 100 mM sodium phosphate buffer pH 7.4, containing 0 to 0.3 mM spermine NONOate (SpN), 0.1 mM DETAPAC, and 0 to 20 μM CPTIO in a total volume of 0.1 mL for the indicated time at 37 °C. The reaction was started by the addition of SpN. Blank values were obtained in the absence of SpN. The reactions were stopped by adding a stock solution of 10 mM ascorbic acid in dimethylformamide (0.025 mL). Samples were frozen at −70 °C and made stable for analysis by HPLC (Lakshmi et al., 2002).

HPLC analysis of metabolites

Reaction products were assessed using a Beckman HPLC with System Gold software and a 5 μm, 4.6×150 mm C-18 ultrasphere column attached to a guard column. The mobile phase contained 20 mM ammonium formate pH 3.1 buffer in 8% acetonitrile, 0–2 min; 8–16%, 2–10 min; 16–21%, 13–18 min; 21–35%, 18–23 min; 35–8%, 30–35 min; flow rate 1 mL/min. Radioactivity in HPLC eluents was measured using a FLO-ONE radioactive flow detector. Data are expressed as a % of total radioactivity or pmol recovered by HPLC.

Statistical analysis

Data are expressed as a mean±SEM and significant differences ($p<0.05$) evaluated using the unpaired Student's *t* test.

Results

IQ nitrosation

Nitrosation of IQ (10 μM) due to autoxidation was not detected during a 7.5-min incubation of IQ with 2.4 μM NO/min, 0.1 mM SpN. In contrast, addition of 15 μM CPTIO resulted in a 30% conversion of IQ to N–NO–IQ (3 μM). N–NO–IQ was the only product observed. The increase in N–NO–IQ, 3 μM, was linear during the 7.5 min incubation. In the