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Naphthazarin and methylnaphthazarin cause vascular dysfunction by impairment of endothelium-derived nitric oxide and increased superoxide anion generation

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Abstract

The effects of the naphthoquinone analogue, naphthazarin (Nap), and its derivative, methylnaphthazarin (MetNap), on vascular reactivity were studied using isolated rat aortic rings and human umbilical vein endothelial cells (HUVECs). In this study, we determined vessel tension, nitric oxide (NO) formation, endothelial nitric oxide synthase (eNOS) activity, eNOS protein expression, and superoxide anion ($O_2^{\bullet-}$) generation in an effort to evaluate the effect of Nap and MetNap on the impairment of the NO-mediated pathway. Lower concentrations of Nap (0.01–1 μM) and MetNap (1–10 μM) concentration-dependently enhanced phenylephrine (PE)-induced vasocontraction and abrogated acetylcholine (ACh)-induced vasorelaxation in an endothelium-dependent manner. On HUVECs, both Nap and MetNap concentration-dependently inhibited NO formation induced by A23187, and also partially inhibited nitric oxide synthase (NOS) activity. eNOS protein expression by HUVECs was not affected by treatment with Nap or MetNap, even within 24 h. These data suggest that Nap and MetNap might act as inhibitors of nitric oxide synthesis in the endothelium. In addition, Nap and MetNap were also shown to generate $O_2^{\bullet-}$ on HUVECs with short-term treatment. We concluded that Nap and MetNap inhibited agonist-induced relaxation and induced vasocontraction in an endothelium-dependent manner, and these effects might have been due to modification of the NO content by inhibition of NOS activity and bioinactivation through $O_2^{\bullet-}$ generation.

Keywords: Naphthazarin; Nitric oxide synthase; Nitric oxide; Superoxide anion

1. Introduction

Quinoid compounds are widespread in nature (Thomson, 1991) and have several different roles in organisms; ubiquinone and vitamin K1 act as defensive compounds in biochemical systems. The pigments, alkannin, shikonin, and naphthazarin, have been proven to possess wound healing, antibacterial, anti-inflammatory, and anticancer activities (Papageorgiou, 1980). In addition, some quinoid-like chemicals are used as dyes or are environmental pollutants (Schuetzle, 1983). Although, as quinoid compounds, anthracyclines or anticancer agents (doxorubicin, idarubicin, aclarubicin, and naphthazarin) have limited clinical uses because the side effect of life-threatening cardiomyopathy upon chronic administration, they are still under

Abbreviations: Nap, naphthazarin; MetNap, methylnaphthazarin; PE, phenylephrine; ACh, acetylcholine; HUVECs, human umbilical vein endothelial cells; NO, nitric oxide; eNOS, endothelial nitric oxide synthase.

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consideration for their anti-inflammation or antioxidation functions (Anufriev et al., 1998; Kourounakis et al., 2002) in pharmacochemical investigations. This fact should be taken into consideration and rise the important of unwanted toxicity in the use of this potential pharmaceutical drug. Exposure of cells to quinoid compounds may lead to several deleterious cellular consequences. Acute exposure to these compounds may induce cellular damage and even cell death. Furthermore, chronic exposure to various quinoid compounds may cause carcinogenic and mutagenic insults (Price et al., 1975; Tikkanen et al., 1983; Chesis et al., 1984). During recent decades, considerable effects have been made to understand the mechanism underlying the biotoxicity of quinoid compounds.

Naphthazarin, a natural redox-cycling quinoid, and its structural analogue, doxorubicin, were found to have cardioactive effects under in vivo ischemic-reperfusion (Anufriev et al., 1998), to induce antiplatelet aggregation (Ko et al., 1990), formation of glutathione conjugates (Ollinger et al., 1989), and inhibition of DNA topoisomerase-I activity (Song et al., 2000), and to have antibacterial (Papageorgiou, 1980) and anticancer activities (Kyong-Up et al., 1997). In addition to the desired pharmacological actions, naphthazarin has been shown to produce some adverse effects on cellular systems. For example, this natural compound can induce intracellular oxidative stress (Cohen and d'Arcy Doherty, 1987; Ollinger and Brunmark, 1991), cytotoxic effects in hepatocytes (Ollinger and Brunmark, 1991), apoptotic cell death by damaging the lysosomal membrane (Ollinger and Brunk, 1995; Roberg et al., 1999), and genotoxic action on the Ames test (Tikkanen et al., 1983).

The vascular endothelium is important in the maintenance of vascular homeostasis. Endothelial NO plays an important role in the regulation of vascular tone and blood pressure (Luscher and Vanhoutte, 1999). However, NO reacts rapidly with superoxide anions leading to inactivation and loss of its vasodilator activity (Gryglewski et al., 1986). Consequentially, its derived products, including peroxynitrite ($ONOO^-$) and hydrogen peroxide, as permeants and potent oxidants can also cause vasculopathies such as vascular cell injury (Berry et al., 2001; Cai and Harrison, 2000). In particular, vasculotoxic compounds such as homocysteine have been identified as endothelial toxins; their action may involve superoxide-induced impairment of NO-mediated vasorelaxation and oxidative damage to the vascular endothelium (Weiss et al., 2003). On the other hand, indomethacin was recently found to have the vascular action of augmenting ACh-induced vasorelaxation through an increase in reactive oxygen species, particularly $ONOO^-$ (De Angelis et al., 2004).

In the present study, the effects of Nap and MetNap on vascular rings and human umbilical vein endothelial cells (HUVECs) were studied. We found that Nap and MetNap augmented PE-induced vasocontraction and suppressed ACh-induced relaxation in the aorta at lower concentrations in an endothelium-dependent manner. These effects might have been due to the inhibition of nitric oxide synthase activity and to $O_2^{\bullet-}$ generation.

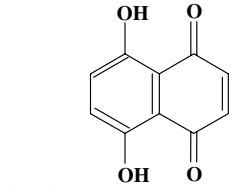
2. Materials and methods

2.1. Chemicals

Nap and MetNap (see Fig. 1) were isolated following a procedure previously described by Cheng et al. (1995). Cell culture reagents including M-199 medium, L-glutamine, penicillin, streptomycin, and fetal bovine serum were obtained from Gibco BRL (Grand Island, NY, USA). All other chemicals were purchased from Sigma (St. Louis, MO, USA) unless specified.

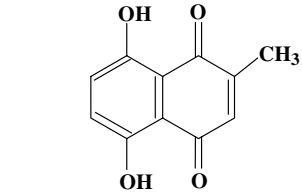
2.2. Thoracic aortic ring preparation

Wistar rats (weighing 200–300 g) were purchased from the Animal Center of the College of Medicine, National Taiwan University, Taipei, Taiwan. Rats were sacrificed, and the descending thoracic aorta of each rat was carefully removed in normal Krebs solution with the following composition (mM): NaCl 118.5, KCl 4.8, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11.1, and $CaCl_2$ 2.5 (pH 7.4). After excess fat and connective tissue were removed, the aorta was cut into rings (about 5 mm in length) in 10-ml organ baths containing Krebs' solution (constantly gassed with $95\%O_2 + 5\%CO_2$ at 37 ± 0.5 °C) and attached to a force transducer (Grass FT.03) to measure the isometric contraction as previously described (Cheng et al., 2003). The aortic rings were equilibrated in Krebs solution and maintained



5, 8-dihydroxy-1, 4-naphthalenedione

(Naphthazarin)



(Methylnaphthazarin)

Fig. 1. Chemical structures of naphthazarin and methylnaphthazarin.