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Short communication

Nordihydroguaiaretic acid induces Nrf2 nuclear translocation *in vivo* and attenuates renal damage and apoptosis in the ischemia and reperfusion model

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ABSTRACT

It has been shown that the pretreatment with nordihydroguaiaretic acid (NDGA), a lignan with direct and indirect antioxidant properties, protects against the ischemia-reperfusion (I/R)-induced renal oxidant damage. Although it has been shown that NDGA induces Nrf2 nuclear translocation in renal epithelial LLC-PK1 cells in culture, it is unknown if NDGA may induce Nrf2 translocation *in vivo*. In this work was explored if NDGA is able to induce *in vivo* Nrf2 nuclear translocation in kidneys of rats submitted to uni-nephrectomy (U-NX) or I/R injury. Four groups of male Wistar rats were used: U-NX, NDGA, I/R, and I/R + NDGA. NDGA was injected i.p. (10 mg/kg/day) starting 48 h before I/R. Kidney samples were obtained at 3 h of reperfusion after to measure Nrf2 translocation. Additional groups of rats were studied at 24 h of reperfusion to measure histological damage and apoptosis. NDGA was able to induce Nrf2 translocation *in vivo* in kidneys of rats submitted to both U-NX and I/R injury and to protect against renal histological damage and apoptosis. It is concluded that the pretreatment of NDGA is able to induce *in vivo* nuclear Nrf2 translocation in kidney of rats suggesting that this may be involved in the renoprotection against I/R.

Introduction

The naturally occurring antioxidant nordihydroguaiaretic acid (NDGA, Fig. 1A) is a phenolic lignan containing two catechols. It was originally isolated from the creosote bush, *Larrea tridentata*, which grows in some desert areas of southwest United States and northern Mexico as well as in some areas of Argentina (Arteaga et al. 2005). Creosote bush tea has been used in the folk medicine for the treatment of more than 50 ailments including, arthritis, diabetes, and inflammation (Arteaga et al. 2005). It has been found that NDGA, given as a pretreatment, exerts protective effect in several experimental models *in vivo* (Ansar et al. 1999; Yam-Canul et al. 2008; Liu et al. 2012) and *in vitro* (Cárdenas-Rodríguez et al. 2009; Guzmán-Beltrán et al. 2008; Rojo et al. 2012). In a previous study of our group, Zúñiga-Toalá et al. (2012) found that NDGA, given as a pretreatment, was able to reduce renal ischemia and reperfusion (I/R)-induced injury and oxidant stress. In fact, it is well recognized that NDGA is an effective scavenger of reactive oxygen species (ROS) (Floriano-Sánchez et al. 2006; Galano et al. 2010; Lü et al. 2010). In addition to the direct antioxidant properties, it has been found that NDGA is able to induce the nuclear factor erythroid 2-related factor 2 (Nrf2) in primary cultures of cerebellar granule neurons (Guzmán-Beltrán et al. 2008) and in renal epithelial LLC-PK1 cells (Rojo et al. 2012). Nrf2 is the master regulator of the cellular stress response that regulates the induction of antioxidant gene expression and phase II antioxidant enzymes. The induction of Nrf2 secondary to NDGA exerts a cytoprotective effects when these cells are submitted to a further oxidant damage in absence of NDGA in the culture medium (Guzmán-Beltrán et al. 2008; Rojo et al. 2012) suggesting that the protection is mediated by indirect actions (Nrf2 induction) of NDGA. Based on the above information, it is known that NDGA exerts direct (as a ROS scavenger) and indirect (inductor of Nrf2) antioxidant

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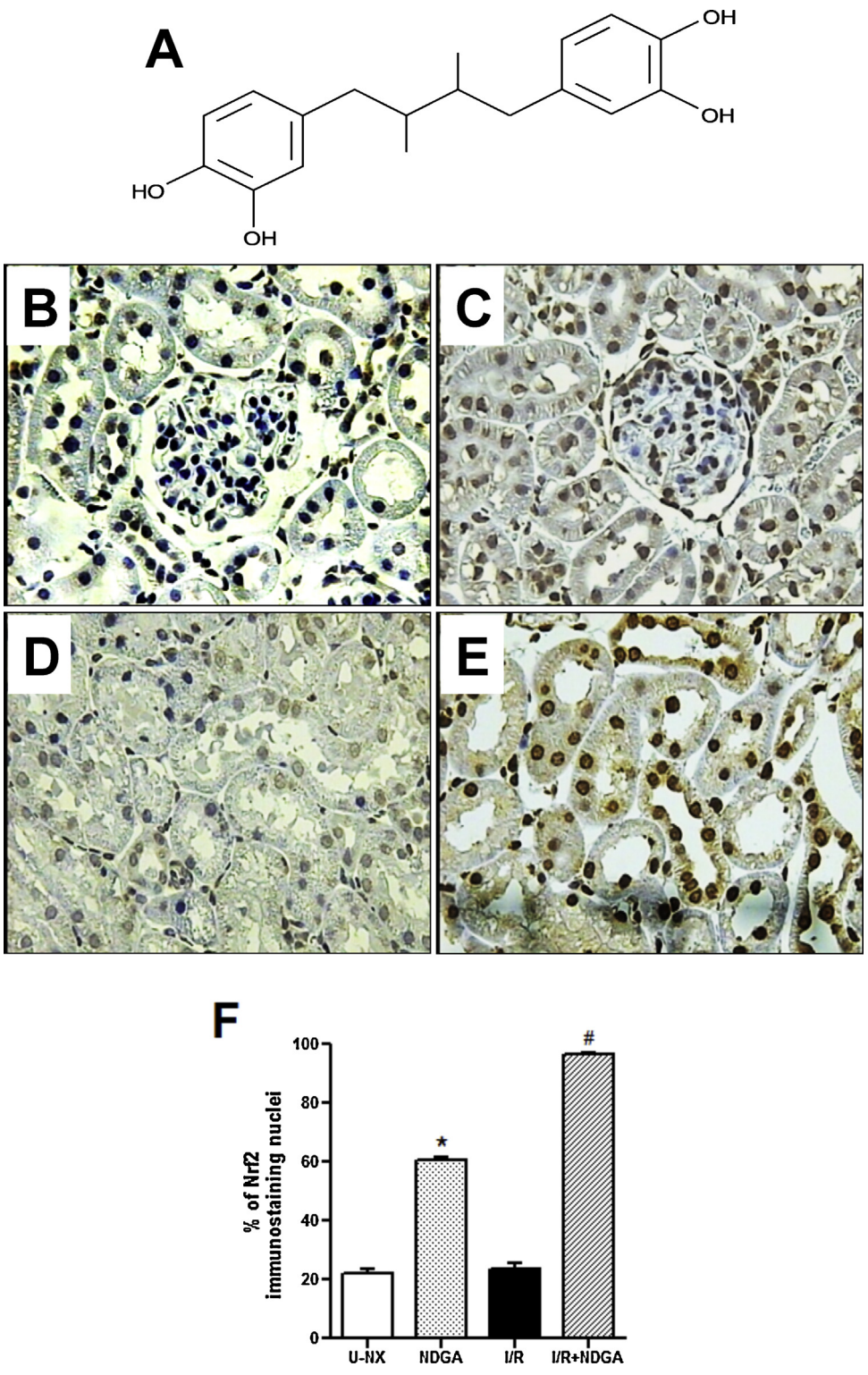

Fig. 1. (A) Structural formula of NDGA. (B)–(F) Representative immunohistochemistry and automated morphometry of Nrf2 immunostaining in the studied groups. (B) U-NX, (C) NDGA, (D) I/R, (E) I/R + NDGA. (F) Automated morphometry. Data are mean ± SEM, $n = 3$, $^{*}p < 0.001$ vs. U-NX, $^{\#}p < 0.001$ vs. I/R and NDGA.

effects that may be involved in the cytoprotective effect of this compound. The antioxidant compounds with direct and indirect actions are called bifunctional antioxidants (Dinkova-Kostova and Talalay 2008).

Although it has been postulated that the direct antioxidant properties of NDGA are involved in the protection in experimental models *in vivo*, the potential involvement of Nrf2 has not been explored. In fact, it is unknown if NDGA may induce Nrf2 translocation *in vivo*. In this work was explored if the pretreatment with NDGA is able to induce *in vivo* renal Nrf2 nuclear translocation that may be involved in the protection of this compound against I/R injury in rats.