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# SULFORAPHANE REDUCES THE ALTERATIONS INDUCED BY QUINOLINIC ACID: MODULATION OF GLUTATHIONE LEVELS

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**Abstract—Glutamate-induced excitotoxicity involves a state of acute oxidative stress, which is a crucial event during neuronal degeneration and is part of the physiopathology of neurodegenerative diseases. In this work, we evaluated the ability of sulforaphane (SULF), a natural dietary isothiocyanate, to induce the activation of transcription factor Nrf2 (a master regulator of redox state in the cell) in a model of striatal degeneration in rats infused with quinolinic acid (QUIN). Male Wistar rats received SULF (5 mg/kg, *i.p.*) 24 h and 5 min before the intrastriatal infusion of QUIN. SULF increased the reduced glutathione (GSH) levels 4 h after QUIN infusion, which was associated with its ability to increase the activity of glutathione reductase (GR), an antioxidant enzyme capable to regenerate GSH levels at 24 h. Moreover, SULF treatment increased glutathione peroxidase (GPx) activity, while no changes were observed in γ-glutamyl cysteine ligase (GCL) activity. SULF treatment also prevented QUIN-induced oxidative stress (measured by oxidized proteins levels), the histological damage and the circling behavior. These results suggest that the protective effect of SULF could be related to its ability to preserve GSH levels and increase GPx and GR activities.** 

**Key words: sulforaphane, neuroprotection, quinolinic acid, excitotoxicity, glutathione.**

## INTRODUCTION

Neurodegenerative disorders are a heterogeneous group of diseases with distinct clinical phenotypes and genetic etiologies (Jellinger, 2009). These pathologies are present sporadically and can be traced to specific genetic mutations. Although these mutations affect a wide variety of proteins, substantial evidence points to excitotoxicity as a fundamental mechanism involved in neuronal degeneration (Dong et al., 2009). Excitotoxicity usually refers to the injury and death of neurons arising from overactivation of glutamate receptors that impairs cellular $Ca^{2+}$ homeostasis, further leading to a generalized acute oxidative stress (Lau and Tymianski, 2010). Although the etiology of neurodegenerative disorders is often multifactorial, a growing body of evidence indicates crucial implications for oxidative stress in the pathogenesis of many neurodegenerative diseases (Migliore and Coppedè, 2009). Oxidative stress is generated by an imbalance in the redox state of the cell, mainly through overproduction of reactive oxygen species (ROS) (Valko et al., 2007). As mentioned above, influx of $Ca^{2+}$ activates several ion-dependent enzymes that generate highly toxic ROS such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), and hydroxyl radical ($^{\cdot}OH$). At high concentration, ROS can be important mediators of damage to cell structures, nucleic acid, lipids and proteins (Halliwell, 2006). It is well known that excessive accumulation of ROS can induce cell death by necrosis (Syntichaki and Tavernarakis, 2003).

Quinolinic acid (2,3-pyridine-dicarboxylic acid, QUIN) is an endogenous metabolite of the kynurenine pathway and it acts as an agonist of *N*-methyl-D-aspartic acid (NMDA) receptors. QUIN induces excitotoxicity by sustained receptor activation, resulting in cytotoxic

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*Abbreviations:* BSA, bovine serum albumin; DNPH, dinitrophenylhydrazine; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; GCL, γ-glutamyl cysteine ligase; GSH, glutathione reduced; GPx, glutathione peroxidase; GR, glutathione reductase; GSSG, glutathione oxidized; GST, glutathione-S-transferase; HEPES, hydroxyethyl piperazineethanesulfonic acid; HO-1, heme-oxygenase-1; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; NQO1, NADPH:quinone oxidoreductase; NMDA, *N*-methyl-D-aspartic acid; Nrf2, nuclear factor (erythroid-derived 2)-like-2; OPA, *o*-phthaldehyde; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; PMSF, phenylmethylsulfonyl fluoride; QUIN, quinolinic acid; ROS, reactive oxygen species; SULF, sulforaphane.

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intracellular $Ca^{2+}$ concentrations, ATP depletion, and massive generation of ROS (Pérez-De La Cruz et al., 2012). In addition, the ability of QUIN to produce ·OH and induce lipid peroxidation, both contributing to the pattern of toxicity elicited by QUIN, has been reported (Santamaría et al., 2001). Intrastriatal administration of QUIN causes selective loss of middle spiny neurons in the striatum and it is used as an excitotoxic/pro-oxidant model, due to its ability to reproduce behavioral, biochemical and morphological alterations observed in the brains of patients with neurological disorders (Pérez-De La Cruz et al., 2012). The high-unsaturated fatty acid content of the brain, together with its high metabolic activity rate and metal ions content – which are prone to oxidation-, makes this organ particularly susceptible to be damaged by ROS (Halliwell, 2006). In this context, it is necessary to find compounds that can stimulate antioxidant defenses for long periods of time in the same cell.

Sulforaphane (4-methylsulfinylbutyl isothiocyanate, SULF) is an abundant naturally occurring isothiocyanate contained in cruciferous vegetables (also called *Brassica*) such as broccoli, brussels sprouts and cabbage. This compound is obtained from glucoraphanin (4-metylsulfoniylbutyl glucosinolate) by the enzymatic action of myrosinase (Fimognari and Hrelia, 2007). SULF has gained attention as a cytoprotective and chemopreventive compound (Juge et al., 2007; Negrette-Guzmán et al., 2013a,b). The cytoprotective effect exerted by SULF is mediated by its property as an inducer of transcription nuclear factor (erythroid-derived 2)-like-2 (Nrf2). In turn, Nrf2 plays a crucial role in the cellular protection against oxidative stress regulating the cellular antioxidant response since it modulates the expression of several genes encoding phase II and antioxidant enzymes, including heme-oxygenase-1 (HO-1), NADPH:quinone oxidoreductase (NQO1), glutathione-S-transferase (GST), glutathione peroxidase (GPx), glutathione reductase (GR) and γ-glutamyl cysteine ligase (GCL), among others (Lee et al., 2003; Guerrero-Beltrán et al., 2010, 2012). Several of these enzymes need optimal levels of glutathione (GSH) to exert its proper function. Thus, Nrf2 plays an important role in detoxification of ROS and electrophilic species.

GSH is a major non-enzymatic antioxidant in the brain and its synthesis is regulated by the Nrf2-dependent expression of GCL, the rate-limiting enzyme in GSH synthesis (Aoyama et al., 2008). GSH plays a critical role in protecting cells from oxidative stress and xenobiotics, also maintaining the thiol redox state, most notably in the CNS (Dringen, 2000). Disturbances in GSH homeostasis are implicated in the etiology and/or progression of a number of human diseases, including cancer, aging and inflammatory and neurodegenerative diseases (Ballatori et al., 2009).

Considering the crucial role that GSH plays in the antioxidant defense of the brain, the aim of the present study was to investigate the *in vivo* effect of SULF on GSH levels elicited by the induction of Nrf2 in a toxic model induced by QUIN in the rat striatum.

## EXPERIMENTAL PROCEDURES

### Chemicals

SULF was obtained from LKT Laboratories (St. Paul, MN, USA). QUIN, apomorphine, 2,4-dinitrophenylhydrazine (DNPH), *o*-phthaldehyde (OPA), hydrogen peroxide ($H_2O_2$), dithiothreitol (DTT), phenylmethylsulfonyl fluoride (PMSF), NADPH, GR, oxidized (GSSG) and reduced glutathione (GSH), 2,3-naphthalendicarboxyaldehyde (NAD), L-cysteine, sulfosalicylic acid, ATP, boric acid, L-serine, streptomycin sulfate, sodium azide, guanidine hydrochloride, bovine serum albumin (BSA), and trichloroacetic acid were obtained from Sigma (St. Louis, MO, USA). Glutamic acid was from JT Baker (Center Valley, PA, USA). Primary antibodies anti-Nrf2 (H-300) and anti-proliferating cell nuclear antigen (PCNA) were obtained from Santa Cruz Biotechnology Inc. (Dallas, TX, USA) and Abcam (Cambridge, MA, USA), respectively. Donkey-anti rabbit IgG horseradish peroxidase-conjugate (secondary antibody) used for Western blot was from Jackson Immunoresearch Laboratories Inc. (West Grove, PA, USA). All other reagents were obtained from other known commercial sources. Deionized water from a Milli-Q system (Millipore, Bedford, MA, USA) was used for preparation of solutions.

### Animals

Male Wistar bred in-house rats (280–320 g) were used throughout the study. For all experimental purposes, animals were housed five per cage in acrylic box cages and provided with a standard commercial rat chow diet (Laboratory rodent diet 5001; PMI Feeds Inc., Richmond, IN, USA) and water *ad libitum*. Housing room was maintained under constant conditions of temperature (25 ± 3 °C), humidity (50 ± 10%), and lighting (12-h light/dark cycles). All procedures with animals were carried out strictly according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Local Guidelines on the Ethical Use of Animals from the Health Ministry of Mexico. During the experiments, all efforts were made to minimize animal suffering.

### Experimental design

The animals were randomly divided into four groups ($n = 6$), as follows: (1) control group (SHAM) treated with saline; (2) SULF group treated with SULF plus saline; (3) QUIN group treated with saline plus QUIN; and (4) SULF + QUIN group treated with SULF plus QUIN. Animals from SULF and SULF + QUIN groups received SULF 5 mg/kg, i.p., twice at 24 h and 5 min before the intrastriatal injection of saline or QUIN. SHAM and QUIN groups received only saline twice at the same times. QUIN and SULF + QUIN were intrastriatally infused with 1 µl of QUIN (240 nmol) in the right striatum, according to the following stereotaxic coordinates: +0.5 mm anterior to bregma, −2.6 lateral to bregma, and +4.5 mm ventral to the dura (Paxinos and