Effect of oxidative stress on UDP-glucuronosyltransferases in rat astrocytes

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HIGHLIGHTS

► UGT1A6 and UGT1A7 expression and 1-naphthol enzymatic activity were analyzed in rat astrocytes.
► Cells were treated with xenobiotic compounds known to generate oxidative stress.
► The short term exposure inhibited the 1-naphthol glucuronidation as a result of protein oxidation.
► Oxidative stress induced deleterious changes in astrocyte morphology and decreased cell viability.
► Significantly higher glucuronidation activity and mRNA expression of detoxification enzymes in surviving impaired astrocytes.

ARTICLE INFO

ABSTRACT

The present work reports data regarding effects of an induced oxidative stress on the mainly expressed isoforms of UDP-glucuronosyltransferases (UGTs) in the brain. UGT1A6 and UGT1A7 expression and enzymatic activities toward the 1-naphthol were analyzed in rat cultured astrocytes following the exposure for 48 h to redox-cycling xenobiotic compounds such as quinones and bipyridinium ions. The expression of NADPH:cytochrome P450 reductase and NAD(P)H:quinone oxidoreductase 1 (NQO1) was also investigated. Oxidative stress induced significant deleterious changes in astrocyte morphology, decreased cell viability and inhibited catalytic function of UGTs as a result of protein oxidation. Alternatively, in the surviving impaired astrocytes, oxidative conditions induced a significant overactivity and overexpression of xenobiotic detoxification enzymes, as adaptive response. These effects were significantly prevented by the presence of melatonin, suggesting its direct antioxidant action on reactive oxygen species, reflected further on the glucuronidation activity and transcriptional regulation of both UGT1A6 and UGT1A7. Results show that both catalytic properties of UGTs and the expression of UGT1A6, UGT1A7, NQO1 and NADPH:cytochrome P450 reductase in rat astrocytes are greatly influenced by the pro-oxidative environment. In conclusion, an experimental increase in oxidative cellular status could have both immediate and long term consequences on detoxification enzymatic system activity and expression.

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1. Introduction

Reactive oxygen species (ROS) produced during the normal metabolism and under conditions of oxidative stress, have been shown to cause cell damage by attacking a great variety of biomolecules including essential enzymes and structural proteins

(Avery, 2011). The age-dependent accumulation of oxidatively modified and dysfunctional macromolecules provides the basis for the free radical theory of aging. Pro-oxidants, however, are also capable of catalyzing fully reversible modifications of proteins. It is increasingly apparent that these reactions participate in redox-dependent regulation of cell metabolism and response to stress (Humphries et al., 2006). The central nervous system (CNS) is vulnerable to oxidative stress, especially when a toxicant can modify the physiological balance between anti- and pro-oxidant mechanisms (Gonthier et al., 2004). Meanwhile, controlling oxidative stress is considered to be an important therapeutic target in neurodegenerative diseases (Park et al., 2011).

Cytochromes P450 and NADPH:cytochrome P450 reductase are phase I enzymes directly involved in the ROS production during the redox cycling xenobiotic metabolism in rat brain preparations (Ghersi-Egea et al., 1991). In contrast, phase II detoxifying

enzymes such as glutathione S-transferases, NADP(H):quinone oxidoreductases, γ-glutamyl cysteine synthethase, and UDP-glucuronosyltransferases are recently acknowledged as "indirect" antioxidants based upon their role in maintaining redox balance and thiol homeostasis (Kalthoff et al., 2010). Hence, for cellular homeostasis, an imbalance between oxidative metabolism and xenobiotic detoxification, *i.e.* by glucuronidation, could be critical. Interestingly, a growing number of studies demonstrated that ROS could initiate complex intracellular redox modulatory systems that regulate gene transcription and translation of many phase I and II drug metabolizing enzymes (Olesen et al., 2008; Vargas and Johnson, 2009; Bock, 2011).

In the brain, among metabolizing enzymes which could be the subject of oxidative stress-induced modifications at transcriptional and posttranslational levels, UDP-glucuronosyltransferases (UGTs, EC:2.4.1.17) may represent a privileged target. Indeed, these enzymes catalyze the transfer of the glucuronic acid moiety from the high energy donor uridine diphosphate glucuronic acid (UDPGA) to a wide range of exogenous and endogenous substrates, being actively involved in phase II biotransformation process (Bock and Köhle, 2005). Until now, the mainly expressed isoform of UGTs in the brain appeared to be UGT1A6, which conjugates a variety of planar phenolic xenobiotic compounds such as 1-naphthol, 4-nitrophenol, 4-methylumbelliferone, paracetamol, resveratrol, and endogenous substrates such as serotonin (Suleman et al., 1993, 1998; Shelby et al., 2003; Sabolovic et al., 2007). The enzymatic activity and the expression of the constitutive UGT1A6 present in rat brain and in primary cerebral cultured cells have already been characterized. Thus, the glucuronidation process, displays a regional and cellular heterogeneity, with high levels in the cerebral structures forming either blood–brain- and cerebrospinal fluid–brain interfaces, as well as in the olfactory tissues (Minn et al., 2000, 2002; Gradinaru et al., 2009; Heydel et al., 2001, 2010). Also, previous studies evidenced an increase in rat brain conjugation activities of 1-naphthol during aging (Gradinaru et al., 1999). At cellular level, the 1-naphthol UGT1A6 specific activity was 10-fold higher in astrocytes, as compared with neurons and endothelial cerebrovascular cells, suggesting an important role in the protection against neurotoxic actions of drugs or environmental pollutants (Suleman et al., 1998). Another isoform – UGT1A7, which is widely active toward benzo(*a*)pyrene metabolites, particularly the phenols, exhibits a similar pattern of constitutive expression with UGT1A6 (Grove et al., 1997; Webb et al., 2005). In spite of that this enzyme was identified in many rat and human extrahepatic tissues (Strassburg et al., 1997; Daidoji et al., 2005), no study was carried out to check for its presence in astrocytes. Besides, it has been shown that astrocytes have an important role in protecting neurons against the deleterious effect of ROS, through their high levels of antioxidant systems: glutathione and enzymes involved in glutathione metabolism, ascorbate, vitamin E, catalase, superoxide dismutase (Makar et al., 1994; Aschner et al., 2002; Liddell et al., 2010). ROS production has been demonstrated in rat astrocytes following the reductive metabolism of some redox-cycling xenobiotic compounds such as quinones and bipyridinium ions (Bayol-Denizot et al., 2000; Heurtaux et al., 2004).

This work continues our previous studies concerning the free-radicals mediated toxicity/detoxification cerebral processes and the age-related modifications in the rat cerebral glucuronidation, with the aim to approach interrelated mechanisms of aging, oxidative stress and detoxification. Therefore, the present study was designed to evaluate the effects of an induced oxidative stress on the brain UGT1A6 and UGT1A7 isoforms. The mRNA expression and enzymatic activities were analyzed in rat astrocytes treated with xenobiotic compounds known to generate oxidative stress. Furthermore, the expression of other two essential phase I and II drug metabolizing enzymes, namely NADPH:cytochrome P450

reductase and NAD(P)H:quinone oxidoreductase 1, was investigated in the astrocytes subjected to this oxidative environment.

2. Materials and methods

2.1. Chemicals

Menadione (2-methyl-1,4-naphthoquinone), benzyl viologen (1,1′-dibenzyl-4,4′-bipyridinium dichloride), paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride), 1-naphthol (1-NP), 1-[^{14}C] naphthol, UDP-glucuronic acid (UDP-GlcA), melatonin (N-acetyl-5-methoxytryptamine), cycloheximide (3-[2-(3,5-dimethyl-2-oxocyclohexyl)-2-hydroxyethyl]glutarimide), were purchased from Sigma (Saint-Quentin Fallavier, France). Cell culture products were provided by Gibco (Cergy-Pontoise, France). All reagents for reverse transcription – polymerase chain reaction (RT-PCR) were obtained from Eurobio (Les Ulis, France). All other routine reagents were of the highest purity commercially available.

2.2. Animals

Pregnant Sprague-Dawley rats were obtained from Iffa Credo (Saint-Germain-sur-l'Arbresle, France). The animals were maintained under standard laboratory conditions in 12:12 h light/dark cycle with unrestricted access to standard food and tap water. The study was approved by the local Animal Ethic Committee, and all procedures were carried out according to the Directive 86/609 EEC guidelines for the care and use of laboratory animals.

2.3. Primary cultures of astrocytes

Astrocytes were prepared from the cerebral cortex of newborn Sprague-Dawley rats, according to the method described previously (Heurtaux et al., 2004). Cell culture purity was characterized by immunocytochemical detection of a specific astrocyte marker, the glial fibrillary acidic protein (GFAP). Cells were fixed with paraformaldehyde (4%, w/v) and marked with a primary antibody anti-GFAP (1:100, polyclonal rabbit anti-GFAP; Sigma, France), and a fluorescein isothiocyanate (FITC) – labeled secondary antibody (1:100, goat anti-rabbit IgG (H + L); Zymed, France). Cells were co-stained with 4 μM propidium iodide, a nuclear marker. Fluorescent labeled cells were observed using an epifluorescence microscope (AX 70 Provis, Olympus, France) equipped with a mercury vapor lamp, a FITC filter (excitation between 450 and 490 nm/emission at 505 nm) and a propidium iodide filter (excitation between 540 and 580/emission at 590 nm). Pictures were then recorded using a 40× oil immersion objective.

The protein content of cell homogenates was determined according to the method of Lowry et al. (1951) using bovine serum albumin as a standard.

2.4. ROS generation in rat astrocyte cultures

The redox-cycling xenobiotic compounds used as $O_2^{•-}$ generators were menadione and bipyridinium compounds benzyl viologen and paraquat. Menadione (2-methyl-1,4-naphthoquinone) is a synthetic quinoid molecule that has been extensively used for studying the mechanisms underlying oxidative injury in the brain (Bayol-Denizot et al., 2000; Heurtaux et al., 2004). Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride) and benzyl viologen (1,1′-dibenzyl-4,4′-bipyridinium dichloride) are bipyridyl herbicides that generate active oxygen species by redox cycling and have been shown to be neurotoxins *in vivo* and *in vitro* (Bonneh-Barkay et al., 2005). An additional pro-oxidant system used was the lipophilic iron donor ferric (Fe^{3+}) complexed to 8-hydroxyquinoline (FeHQ), in which the oxidative injury results from the generation of hydrogen peroxide (H$_2$O$_2$) and its intracellular conversion to HO$^•$ through an iron-dependent mechanism (Demougeot et al., 2003). It is noteworthy that all these ROS generators are widely used as standard oxidative stress inducing compounds.

2.4.1. In vitro studies

Before *in vitro* ROS generation assay cells were rinsed twice with 0.9 g% NaCl, then harvested by scraping in 0.1 M Tris–HCl buffer (pH 7.4) and homogenized by sonication. Sonicated astrocytes suspensions (cell lysates, 250 μg of protein), were incubated in 0.1 M Tris–HCl buffer (pH 7.4), at 37 °C for 20 min with one of the following ROS generator xenobiotic compounds: menadione (10 μM and 100 μM), benzyl viologen (60 μM) or paraquat (400 μM), used as superoxide radical ($O_2^{•-}$) producers, and the mixture FeCl$_3$/8-hydroxyquinoline (FeHQ) in a molar ratio of 1:2, used as hydroxyl radical (HO$^•$) producer system. FeHQ was calculated for an iron content of 2 μM. Menadione was dissolved in dimethyl sulfoxide (DMSO). Benzyl viologen, paraquat and FeHQ were dissolved in 0.1 M Tris–HCl buffer (pH 7.4). Control samples were incubated with 0.1 M Tris–HCl buffer (pH 7.4) containing the vehicle (0.1% DMSO, v/v). Cell lysates were subsequently used for the assessment of glucuronidation enzymatic activity.

2.5. Cell cultures treatments

All treatments were applied to confluent cultures. Cultured astrocytes allowed to grow for 14 days, were exposed over 48 h to ROS generators xenobiotic