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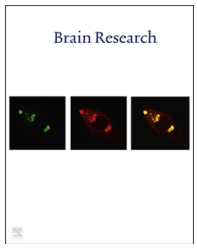

Research Report

Attenuation of rotenone toxicity in SY5Y cells by taurine and N-acetyl cysteine alone or in combination

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ABSTRACT

There is accumulating evidence that supports the involvement of reactive oxygen species (ROS), mitochondrial dysfunction and inflammation in the pathogenesis of neurodegenerative diseases. Thus, it is plausible that a multi-targeted therapeutic approach may be a more effective strategy to retard or even potentially halt the progression of the disease. Taurine is an organic acid that has a role in the regulation of oxidative stress and promoting mitochondrial normal functions, and N-Acetyl cysteine (NAC) is a well-known anti-oxidant and glutathione precursor. The main purpose of this study was to examine the cytoprotective effects of taurine alone or in combination with NAC against rotenone-induced toxicity in the SH-SY5Y neuroblastoma cell line. Taurine treatment produced a concentration-dependent reduction in rotenone-induced cell death. From this, we tested sub-effective concentrations of taurine in combination with low, sub-effective concentrations of NAC against rotenone toxicity, and found the combined treatment afforded greater cytoprotection than either treatment alone. The combined taurine/NAC treatment also attenuated rotenone-induced reductions in aconitase activity suggesting the cytoprotection afforded by the combined treatment may be associated with anti-oxidative mechanisms. Together, our data suggest that a multi-targeted approach may yield new avenues of research exploring the utility of combining therapeutic agents with different mechanisms of actions at concentrations lower than previously tested and shown to be cytoprotective.

1. Introduction

A substantial body of evidence demonstrates that mitochondrial dysfunction and oxidative damage play critical roles in the etiology of cell death associated with several neurodegenerative diseases (Albers and Beal, 2000; Uttara et al., 2009). A large number of studies exploring the therapeutic utility of agents with metabolic and/or anti-oxidative properties have been shown to be effective in providing beneficial effects in both in vitro and in vivo models of neurological diseases. However, fewer studies have explored combining agents to assess whether greater therapeutic utility could be achieved. Given

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the difficulty of achieving appropriate brain levels of such agents to achieve desired biological effects without incurring systemic toxicity makes development of new therapeutic strategies for neurological indications all the more challenging.

Emerging data suggests that taurine may be involved in a wide range of biological processes exerting its beneficial effects through a variety of mechanisms. For example, taurine has been shown to possess anti-oxidative effects by decreasing the levels of reactive oxygen species (ROS) and enhancing electron transport chain activity (Jong et al., 2012). Taurine has also been demonstrated to decrease intracellular calcium levels following an excitotoxic insult (Wu et al., 2005). Anti-inflammatory effects have been demonstrated by showing taurine interacting with myeloperoxidase–halide system of leukocytes to produce taurine halo amines resulting in inflammation reduction, or by down-regulation for of ADP ribose polymerase (PARP) and nuclear factor-kappaB (NF-κB) (Sun et al., 2012; Marcinkiewicz and Kontny, 2014). Finally, taurine's beneficial effects have been linked to activating specific signaling pathways involved with cellular osmotic processes (Schaffer et al., 2000), or that are essential to stimulating glucose utilization and/or insulin secretion (Carneiro et al., 2009; Nandhini and Thirunavukkarasu Anuradha).

Based on these putative mechanisms, we tested taurine's ability to provide cytoprotection against the toxicity caused by rotenone, a pesticide that inhibits complex I activity of the mitochondrial electron transport chain (Orth and Tabrizi, 2003). Previous reports have shown rotenone exposure to result in both metabolic and oxidative stress resulting from ATP depletion and oxidative damage to cellular macromolecules (Fiskum et al., Chinopoulos). Several compounds and mechanisms have been demonstrated to reduce the toxic effects of rotenone, many of which counter the metabolic abnormalities and/or free radical production resulting from the pesticide. In particular, a recent study by Han and colleagues (2013) showed substrates of ATP-generating biochemical pathways rescued rat retinal cells form rotenone-induced toxicity. The authors concluded that substrates, such as glucose, were able to overcome rotenone-induced decreases in ATP, which in turn, reduced oxidative damage.

In our current study, we assessed the cytoprotective potential of taurine, a semi-essential amino acid that has been shown previously to enhance glucose utilization, alone or in combination with N-acetyl cysteine (NAC), a well-known free radical scavenger, against the cellular toxicity induced by rotenone. Specifically, lactate dehydrogenase (LDH) activity was measured to assess cellular toxicity while aconitase activity was measured as marker of oxidative stress (Lobner, 2000). Aconitase is an iron-sulfur containing enzyme catalyzing the isomerization of citrate within the tricarboxylic acid cycle. The activity of aconitase is particularly sensitive to oxidative damage and has been used previously as an indirect marker for oxidative stress (Gardner et al., 1995).

2. Results

2.1. Assessment of rotenone cytotoxicity

A concentration-response toxicity profile of rotenone in SH-SY5Y cell line is shown in Fig. 1. We observed a statistically significant increase in LDH activity in cells treated with rotenone at 3 (403±55), 6 (611±38), and 9 (674±49) uM as compared to control (232±38). Based on this data, we selected 6 μM for all subsequent studies, which provided a large enough toxicity window relative to control to capture any potential cytoprotective effects of taurine alone or in combination with NAC.

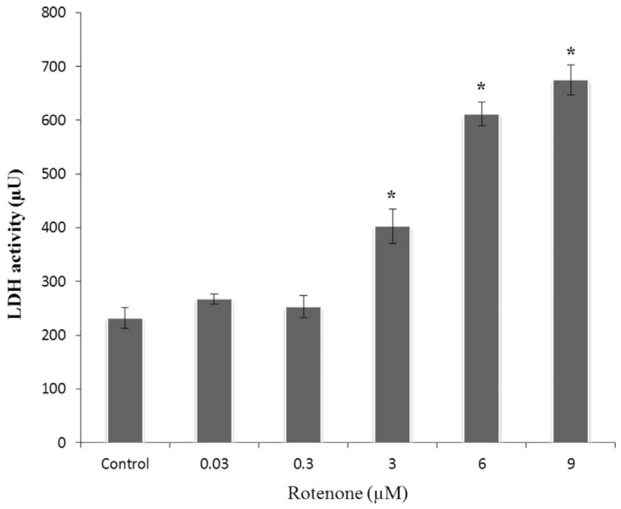

Fig. 1 – Rotenone cellular toxicity in SH-SY5Y cells as determined by LDH release and activity: LDH assay was performed to evaluate the induction of cell death in normal SH-SY5Y cell line using different concentrations of rotenone (0.03 μM, 0.3 μM, 3.0 μM, 6.0 μM and 9.0 μM) after 48 h exposure. Data are presented as mean±SEM, $n=4$. $^{*}p<0.05$ compared to control group.

2.2. Cytoprotective effect of taurine and NAC

We observed a concentration-dependent reduction in rotenone-induced toxicity by increasing concentrations of taurine (Fig. 2a). Cells concurrently treated with rotenone and taurine at 5 or 10 mM, produced statistically significant decreases in LDH activity as compared to cells treated with rotenone alone, while taurine at 2.5 mM did not have any effect on modulating rotenone toxicity. All concentrations of taurine tested by itself did not produce any cell damage as measured by changes in LDH activity (data not shown).

As expected, NAC produced concentration-dependent decreases in rotenone-induced toxicity (Fig. 2b), which is consistent with previous reports (Sun et al., 2012; Molina-Jimenez et al., 2003). NAC, at either 2.5 or 5 mM, produced significant differences as compared to rotenone alone whereas NAC at 0.5 mM produced no cytoprotective effect. Similar to taurine, all concentrations of NAC tested by itself did not produce any cell damage as measured by changes in LDH activity (data not shown).

Based on these data sets, we combined the two sub-effective concentrations of taurine (2.5 mM) and NAC (0.5 mM) and tested this mixture against rotenone-induced cytotoxicity (Fig. 3). We observed a statistically significant decrease in rotenone-induced toxicity by the combination treatment as compared to cells treated with rotenone alone. By itself, the combined