

Antioxidant activity of *N*-acetylcysteine, flavonoids and α -tocopherol on endometrial cells in culture

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Abstract

An appropriate local environment is necessary for successful implantation. Oxidative stress is implicated in the pathogenesis of several pathologies, and may contribute to early pregnancy failure. Antioxidant therapies have been studied in infertility. In this study, we have assessed the antioxidant activity of *N*-acetylcysteine (NAC), flavonoids (quercetin, catechin) and α -tocopherol in an oxidative model of endometrial cells (RL95). Endometrial cells were incubated at several hydrogen peroxide concentrations. Antioxidant effects of NAC (15 mM), quercetin (150 μ M), catechin (150 μ M) and α -tocopherol included in liposomes (1.6 μ g) were assessed by measuring cell viability by the MTT assay. α -Tocopherol-liposomes taken up by endometrial cells were assessed by HPLC. All liposomes used were able to introduce α -tocopherol into cells. The antioxidant effect of NAC and quercetin improved the viability of oxidised cells, and this effect was observed when the oxidant and antioxidant were coincubated. No viability change occurred when the antioxidant was added before or after the oxidant. The antioxidant effect of NAC was better than that of quercetin. When catechin or α -tocopherol were used in the same conditions, no antioxidant effect was detected in cells in culture. These results demonstrate that NAC and quercetin are good H_2O_2 scavengers.

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1. Introduction

Oxidative stress is generated by an imbalance between oxidants and antioxidants in favour of oxidants, which may cause tissue damage. Oxidative stress associated with chronic inflammatory conditions can have detrimental effects mediated by the ability of reactive oxygen species (ROS) to induce cell death in a number

of different cell types. (Agarwal et al., 2005; Iborra et al., 2005).

ROS include several molecules and radicals, such as the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and others. Under physiological conditions, ROS are constantly generated within cells by several intracellular oxidase enzymes and by mitochondrial respiration. H_2O_2 , which easily diffuses inside and outside cells, is able to modulate multiple cellular processes: cell proliferation, signal transduction pathways, gene expression, DNA damage, apoptosis and necrosis (Agarwal et al., 2005; Stone and Yang, 2006). Although H_2O_2 is not a radical *per se*, it is the primary precursor for the generation of hydroxyl radicals by

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Fenton reaction, and this is highly reactive with cellular macromolecules such as DNA, lipids and proteins.

Oxidative stress is involved in the pathogenesis of several diseases and, in the last decade, more diseases have been related to oxidation. For instance, some studies on several neurodegenerative diseases (Gilgun et al., 2001), cardiovascular disease (Black and Garbutt, 2002), cancer (Senthil et al., 2004) and, recently, some male and female infertility diseases (Agarwal et al., 2003) describe the relationship between these pathologies and oxidative stress. Recently, the presence of ROS in reproductive tissue, in peritoneal fluid of patients with endometriosis and in follicular fluid of infertile patients undergoing *in vitro* fertilization has been demonstrated (Agarwal et al., 2003, 2005). The embryotoxicity of ROS has also been described (Bedaiwy et al., 2002). Moreover, oxidative stress in endometrial cells could have a role in the low implantation rates described in some pathologies. For these reasons, antioxidant therapy turns out to be important in the treatment of many pathologies (Abudu et al., 2004; Marchand, 2002; Zafarullah et al., 2003).

Compounds with antioxidant properties have been widely studied, among them *N*-acetylcysteine (NAC), a sulfhydryl-containing antioxidant, which has been used in antioxidant therapy because of its role in reducing endothelial dysfunction, the inflammatory process, fibrosis or cartilage erosion (Buhimschi et al., 2002; Zafarullah et al., 2003). Another important group of compounds with antioxidant activity is flavonoids, a polyphenolic molecule present in fruits, vegetables and seeds. Flavonoids have many biological and pharmacological activities with antioxidative, antiallergic, antiviral, antiinflammatory and antitumoral effects (Nijveldt et al., 2001). Flavonoids are divided into six groups based on their molecular structure: flavanol, flavone, flavonol, flavanone, isoflavone and anthocyanidin (Rice-Evans et al., 1996). Quercetin and catechin belong to flavonol and flavanol, respectively, and they have been widely studied because of their high free radical-scavenging activity.

Tocopherols are a group of vegetable molecules with lipophilic characteristics and phenol antioxidant properties. The principal tocopherol in mammalian tissue is α -tocopherol, although significant quantities of γ -tocopherol are also present. Although many studies have shown that α -tocopherol reduces lipid peroxidation (John et al., 2001) and protects cells from oxidative stress (Kalender et al., 2004), its applicability in human diseases is disputed (Morris and Carson, 2003; Vivekananthan et al., 2003).

Oxidation and inflammation have an enormous influence in the pathogenic process of several diseases and

it is of interest to research the antioxidant ability of some antioxidants in the prevention and development of these pathologies. Using optimal doses of antioxidant agents, we could inhibit both oxidative biochemical modifications and tissue inflammation. In this study, the antioxidant capacity of NAC, quercetin, catechin and α -tocopherol has been evaluated to prevent H_2O_2 -induced cell cytotoxicity in the *in vitro* oxidative model with endometrial cells.

2. Materials and methods

2.1. Cell culture and oxidative treatment

The human endometrial carcinoma cell line (RL95-2) was obtained from American Type Culture Collection (ATCC). Cells were grown until confluence at 37 °C in a humidified atmosphere of 10% CO_2 in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies) supplemented with several growth factors [bovine insulin (0.05 μ g/ml), transferrin (0.05 μ g/ml), glutathione (0.1 μ g/ml), hydrocortisone (0.02 μ g/ml)], antibiotics (1% of a mixture of penicillin, streptomycin and fungizone) (Sigma) and 10% fetal calf serum (FCS) (Life Technologies).

Cell culture was trypsinised using 0.05% trypsin with 0.53 mM EDTA in Hanks' Balanced Salt Solution (Gibco). Cells were cultured in 96-well plates (BD Falcon) at 2.5×10^4 cells/well and allowed to attach overnight in culture conditions. Before starting experiments, cell morphology aspect was assessed by optical microscopy.

For oxidative experiments, the cell culture in 96-well plates was rinsed twice with sterile phosphate-buffered saline (PBS), pH 7.2, and incubated with increasing concentrations of H_2O_2 (10^{-6} to 10^2 mM) in DMEM for 3 h. Then, H_2O_2 cytotoxicity was analysed just after oxidative conditions and after 3 h oxidation + 24 h in DMEM-supplemented medium.

2.2. Assessment of cell viability by succinate dehydrogenase activity (MTT assay)

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay assesses the reduction of MTT to formazan catalysed by mitochondrial dehydrogenase. Before analysis, the culture was washed once. Then, 100 μ l/well of 0.5 mg/ml MTT (Sigma) in supplemented DMEM medium was added to the culture and incubated for 3 h. Preliminary experiments showed that the maximum reduction of MTT was obtained at 2 h and maintained until 4 h total time (data not shown). The

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