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Original article

Alterations in the extracellular catabolism of nucleotides are involved in the antiproliferative effect of quercetin in human bladder cancer T24 cells

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Abstract

Bladder cancer is the most prevalent tumor in the genitourinary tract and the current treatments are not efficient to prevent recurrence and progression of tumor cases. Studies have revealed evidence of the involvement of the purinergic system in bladder tumorigenesis, particularly ecto-5′-NT/CD73, the enzyme responsible for AMP hydrolysis. Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a plant-derived flavonoid that has been shown to exert a broad range of pharmacologic properties, including potential anticancer activity. Here, we investigated the quercetin effect on the E-NTPDases and ecto-5′-nucleotidase/CD73, which catalyzes the introversion of the extracellular purine nucleotides in T24 human bladder cancer cells. The results showed that this flavonoid was able to increase ADP hydrolysis and inhibit the ecto-5′-nucleotidase/CD73 activity, with no effect on protein expression. The treatment with APCP (α,β-methyleneadenosine-5′-diphosphate), another ecto-5′-NT/CD73 inhibitor, led to a significant reduction in cell proliferation. In addition, we showed that AMP, which can be accumulating by enzyme inhibition, had an antiproliferative effect on T24 cells, which was enhanced when its hydrolysis was inhibited by APCP treatment. Otherwise, adenosine did not cause any significant effect on cell proliferation and the quercetin effects were not altered by the simultaneous presence of adenosine. Taken together, the results suggest that the antiproliferative effect of quercetin on tumor cells may occur, at least in part, via alterations in the extracellular catabolism of nucleotides, that could be by AMP accumulation, or could be due to blocked adenosine receptors by this flavonoid, supporting the potential use of quercetin in bladder cancer treatment.

Keywords: Bladder cancer; Quercetin; Ectonucleotidases; E-NTPDases; Ecto-5′-nucleotidase; Purinergic signaling

1. Introduction

Bladder cancer is the second most common malignancy of the genitourinary tract [1–3], and according to WHO statistics it is the 7th most common cancer among men in the world [4]. The majority of cases correspond to transitional cell carcinoma (TCC), a neoplasm originated from transitional urothelial cells. Tobacco is responsible for highest number of cases [5]. Moreover, other risk factors are age, occupational exposure, and habits that can induce urinary accumulation of carcinogenic factors. For advanced cases, the treatment is radical cystectomy, however, usually this kind of tumor is superficial at the time of diagnosis and it is treated with transurethral resection, which brings a greater risk of recurrence and progression [1,4,6]. In an attempt to avoid these problems, the intravesical administration of bacillus Calmette-Guerin (BCG) and chemotherapy have been used, but their efficacies are limited due to the side effects and tumor recurrence and progression during therapy that are sometimes observed [2,4].

Cancer is a multifactorial disease and, thus, added to genetic transformations that end in cellular immortality,

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there are modifications that will give physiologic support for the tumor development. Due to this fact, recent studies have focused attention on the potential involvement of the purinergic system in the genitourinary tract, especially in bladder tumors [7].

The extracellular purines and pyrimidines are signaling molecules that have many different effects in biological processes [8]. Their effects occur by induction of P1 (for adenosine) or P2 (for ATP, ADP, and UTP) receptors and are controlled by the action of the ectonucleotidases such as members of the ectonucleoside triphosphate diphosphohydrolase (E-NTPDase) family and ecto-5′-nucleotidase/CD73 (ecto-5′-NT/CD73) [9,10]. The E-NTPDases (ectonucleoside triphosphate diphosphohydrolase) hydrolyze extracellular ATP and ADP. The AMP produced by these enzymes is hydrolyzed to adenosine by the action of an ecto-5′-nucleotidase/CD73 (ecto-5′-NT/CD73). The ecto-5′-NT/CD73 is expressed in many different tissues and co-localized with the detergent-resistant and glycolipid-rich membrane subdomains, called the lipid rafts, which are important for controlling signal transduction and membrane trafficking [11]. This enzyme has been described as an important molecule in cancer progression, involved in control of cell growth, maturation, differentiation, in drug resistance, and tumor-promoting [12–15].

We have previously shown a clear distinct pattern of expression of ectonucleotidases between 2 different cell lines, where a more malignant bladder cancer cell line (T24) present a low ATP- and ADP-hydrolysis activity and a significantly higher ecto-5′-NT/CD73 activity, while a less malignant bladder tumor cell line (RT4) presents higher ATP- and ADP- hydrolysis activity than AMP-degrading activity [16]. These results led us to propose the participation of E-NTPDases and ecto-5′-NT/CD73 in tumorigenesis of the urinary bladder, as it was seen in other tumors [13,17].

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a plant-derived flavonoid widely distributed in the plant kingdom; it is present in many fruits and vegetables such as apple, garlic, red wine, and black tea. Quercetin is often glycosylated and there is controversial information about the absorption and bioavailability of quercetin glycosides in relation to pure aglycones [18–21].

This compound has been shown to exert a broad range of pharmacologic properties, including potential antioxidative, cardioprotective, and anticancer activity. Among its antitumor effects, there are the ability to prevent cancer by inhibiting cell proliferation, promoting cell cycle arrest or cell death by inhibiting different enzyme systems (including ecto-5′-NT/CD73) [17,21–23]. Quercetin has already demonstrated growth inhibitory effects through decrease of cell viability, inhibition of colony formation, induction of apoptosis, and cell cycle arrest in G0/G1 in T24, EJ, and J82 bladder cancer cell lines [4].

In the present study, we evaluated the effect of quercetin on extracellular hydrolysis of ATP, ADP, and AMP in T24 human bladder cancer cell line and the possible relationship between the inhibition of ecto-5′-NT/CD73 and the antiproliferative effect of quercetin on this tumor cell line.

2. Materials and methods

2.1. Materials

RPMI1640, penicillin/streptomycin, trypsin/EDTA solution, and fungizone were purchased from Gibco BRL, Grand Island, NY. Fetal bovine serum (FBS) was purchased from Cultilab, Campinas, SP, Brazil. Quercetin, DMSO (dimethyl sulfoxide), nucleotides, adenosine and (α,β-methyleneadenosine-5′-diphosphate (APCP) were purchased from Sigma Chemical Company, St Louis, MO). All other chemicals and solvents used were of analytical grade.

2.2. Cell culture and treatments

The T24 human bladder cancer cell line was obtained from the American Type Culture Collection (Rockville, MD). Cells were grown in culture flasks in RPMI culture medium supplemented with 10% fetal bovine serum (FBS) and containing 0.5 U/ml penicillin/streptomycin antibiotics. Cells were maintained in 5%CO_2/95% air at 37°C and seeded in 24 multiwell plates at densities of 1×10^4 cells/well in 500 μl medium per well.

To evaluate the effect of quercetin on ATP, ADP, and AMP hydrolysis, the cells were treated with 10, 30, 50, and 100 μM quercetin (dissolved in DMSO) or 0.5% DMSO (control) for 24, 48, and 72 hours. To measure the direct effect of quercetin on ecto-5′-NT/CD73 activity, the cells were pre-incubated in incubation medium with the same concentrations of quercetin for 10 minutes and then AMP hydrolysis assay was performed as described below.

For the cell counting experiments, the T24 cell cultures were treated with 10, 30, 50, and 100 μM quercetin for 24, 48 and 72 hours, and 100 and 1000 μM AMP, 100 μM adenosine, or 1 μM APCP (another ecto-5′-NT/CD73 inhibitor) for 48 hours. Cells were also treated simultaneously with APCP (10 μM) and AMP (1 mM) for 48 hours, and with quercetin (50 and 100 μM) plus adenosine (0.5 and 1.0 μM), for 48 hours. For IC50 determination, cells were treated with 10, 30, 50, 75, 100, and 200 μM of quercetin for 48 hours.

2.3. Enzymatic assay

The T24 multiwell plates were washed 3 times with phosphate-free incubation medium. The reaction was started by the addition of 200 μl of incubation medium containing 2 mM $MgCl_2$, 120mM NaCl, 5 mM KCl, 10mM glucose, 20 mM Hepes, pH 7.4, and 2 mM of AMP or 2.5 mM of ATP or ADP. After 30 minutes of incubation, the reaction was stopped by taking an aliquot of the reaction medium and transferring to Eppendorf tubes containing