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Original Research Article

Antitumoral activity of novel 1,4-naphthoquinone derivative involves L-type calcium channel activation in human colorectal cancer cell line

Juan Carlos Ramos Gonçalves [a,b,*], *Tangbadioa Herve Coulidiati* [a], *André Luís Monteiro* [a], *Laís Campos Teixeira de Carvalho-Gonçalves* [a], *Wagner de Oliveira Valença* [c], *Ronaldo Nascimento de Oliveira* [c], *Celso de Amorim Câmara* [c], *Demetrius Antônio Machado de Araújo* [a]

[a] *Department of Biotechnology, Federal University of Paraiba, João Pessoa, Brazil*
[b] *Department of Biochemistry and Pharmacology, Federal University of Piauí, Teresina, Brazil*
[c] *Department of Molecular Sciences, Rural Federal University of Pernambuco, Recife, Brazil*

ARTICLE INFO

Article history:
Received 14 January 2016
Received in revised form
9 March 2016
Accepted 23 March 2016
Available online 7 April 2016

Keywords:
Natural products
Pharmacology
Cytotoxicity
Ion channels
HT-29
Flow cytometry
Calcium imaging

ABSTRACT

Colorectal cancer (CRC) is an important public health problem estimated as the third most commonly diagnosed cancer worldwide. Naphthoquinones are compounds present in different families of plants and interesting for medicinal chemistry due to their activities as potent inhibitors of human cancer growth. In this way, our study aimed to evaluate the cytotoxicity and selectiveness of four 2,3-triazole-1,4-naphthoquinone derivatives (N1–N4) towards the CRC cell line HT-29 and normal human cells. MTT assay showed that N1, N2, N3 and N4 elicited distinct cytotoxic potency, exhibiting EC_{50} values of 40.6 ± 1.0, 100.1 ± 1.0, 241.9 ± 1.2 and 101.9 ± 1.1, respectively. Later, flow cytometry in HT-29 cells loaded with propidium iodide (5 μM), indicated the ability of N4 (0.5–50 μM) to induce cell membrane damage. Additionally, calcium imaging experiments were conducted in HT-29 cells loaded with 5 μM Fluo-3/AM to assess intracellular Ca^{2+} (iCa^{2+}). Our data demonstrated that N4 induces a fast and strong increase of iCa^{2+} in HT-29 cells, mediated by voltage-gated L-type Ca^{2+} channels activation. In conclusion, our study reported on the cytotoxicity and selectiveness of 1,2,3-triazol substituted 1,4-naphthoquinones towards the HT-29 CRC cell line. Furthermore, we have demonstrated the participation of voltage-gated L-type Ca^{2+} channels in the N4 mechanism.

* *Corresponding author at*: Department of Biochemistry and Pharmacology, Federal University of Piauí, 64049-550 Teresina, Brazil. Tel.: +55 83 3215 5631.
E-mail address: goncalvesjcr@ufpi.edu.br (J.C.R. Gonçalves).
http://dx.doi.org/10.1016/j.jab.2016.03.002

Introduction

Colorectal cancer (CRC) is an important public health problem, estimated as the third most commonly diagnosed cancer in males and the second in females worldwide, with over 1.2 million new cases and 608,700 deaths per year (Jemal et al., 2011, 2013; Pitule et al., 2013). Cancer statistics estimated that over 49,700 people in the United States would have died of CRC by the end of 2015 (Siegel et al., 2015). Even though the pharmacological remedies against cancer have improved significantly in the last twenty years, there remains the need to identify novel molecules to fight CRC.

Collective studies have shown that ion channels, the specialized membrane proteins that conduct ion fluxes, are involved in the development of many diseases including cancer (Li and Xiong, 2011). For example, the L-type calcium channel subunit, Ca_v 1.2, was found in CRC cells. Ca_v 1.2 expression increases with the differentiation of colon cells to cancer cells (Wang et al., 2000). The intracellular calcium (iCa^{2+}) ion is an important signaling factor that modulates numerous cellular processes in cancer (Clapham, 2007; Morgado et al., 2008). Ca^{2+} permeable channels, such as stored-operated calcium channels, transient receptor potential channels (TRP), and the calcium release activated channel protein 1 are involved in the iCa^{2+} homeostasis. Remodeling or deregulation of iCa^{2+} homeostasis in cancer cells causes changes in cancer progression (Parkash and Asotra, 2010). Zawadzki et al. (2008) have demonstrated that L-type calcium channels mediate calcium influx and apoptosis in human colon cancer cells that can be inhibited by verapamil a specific L-type calcium channel blocker. Thus, the identification of molecules that can activate this channel type is of great interest in CRC treatment.

Naphthoquinones are compounds present in different families of plants and considered privileged structures in medicinal chemistry due to their biological activities and structural properties (Castro et al., 2011; Padhye et al., 2012). In fact, the 1,4-naphthoquinone nucleus has become attractive as a potent inhibitor of human cancer growth and has been suggested for use in cancer therapy (Kayashima et al., 2009; Wellington, 2015). On the other hand, 1,2,3-triazoles have occupied an important role in medicinal chemistry since their facilitated synthesis by click chemistry and its attractive features (Kolb and Sharpless, 2009). They also have numerous biological activities: antifungal, antibacterial (Wang et al., 2010), anti-inflammatory (Simone et al., 2011) and anti-HIV (Giffin et al., 2008).

Inspired by the biological importance of 1,2,3-triazoles and 1,4-naphthoquinones and our recent results on the field of anticancer agents (da Cruz et al., 2014; Coulidiati et al., 2015), in this study, we relate the cytotoxicity effect of novel 1,2,3-triazole-1,4-naphthoquinone derivatives towards the human CRC cell line. In addition, we evaluate the involvement of voltage-gated calcium channels in the cytotoxic mechanisms.

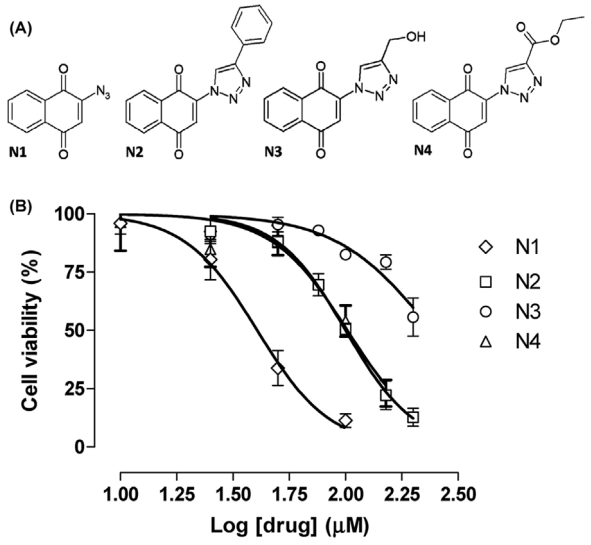

Fig. 1 – Cytotoxicity of 1,4-naphtoquinone derivatives (N1–N4) in HT-29 cells. (A) Chemical structure of the N1, N2, N3 and N4 derivatives. (B) Concentration–response curves of HT-29 colon adenocarcinoma cells incubated for 24 h with the 1,4-naphtoquinone derivatives (0.1–200 μM). Cell viability and oxidoreductase profile were determined by the MTT assay and data are displayed as mean ± SEM, obtained from at least three independent experiments in triplicate.

Materials and methods

Drugs

The 1,4-naphthoquinone derivatives ((N1, N2, N3 or N4), Fig. 1A) were synthesized at the Laboratory for Synthesis of Bioactive Compounds (UFRPE, Brazil) as previously reported (Nascimento et al., 2011).

Chemicals

RPMI-1640, propidium iodide (PI), Fluo-3 A/M, streptomycin, penicillin, etoposide were from Sigma-Aldrich (USA); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), dimethylsulfoxide (DMSO), ethylenediamine tetraacetic acid (EDTA) were from Amresco (USA); foetal bovine serum (FBS) was purchased from Cripion (Brazil); ficoll-hypaque was purchased from GE Healthcare (Sweden).

Cell culture

Human colon adenocarcinoma cell line (HT-29) was acquired from Banco de Células do Rio de Janeiro (BCRJ, Brazil) and cultured in a DMEM (Himedia, India) medium supplemented with 10% FBS, 100 IU/mL penicillin, 100 mg/mL streptomycin and placed in humidified air at 37 °C with 5% CO_2 atmosphere. Normal peripheral blood mononuclear cells (PBMC) were isolated from healthy donors using Ficoll-Hypaque (Coulidiati et al., 2015), in agreement with the Committee of Ethics in Research with Humans (CEP/HULW/UFPB, Brazil), protocol #655/10-318119.