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Original article

C-phycocyanin to overcome the multidrug resistance phenotype in human erythroleukemias with or without interaction with ABC transporters

E. Fernandes e Silva[a,b,*], F.S. Figueira[c], A.D. Cañedo[f], K.S. Machado[d], M.T.S.F. Salgado[a,b], T.K. Silva[a,c], E.F. Wagner[a,c], F.H. Mattozo[a,b], É.A. Lima[e], J.M. Sales-Neto[e], V.U. Ferreira[f], A.A. Comitre[a,c], S.R. Mascarenhas[e], S.J. Kalil[c], A.P.S. Votto[a,b]

[a] Laboratório de Cultura Celular, ICB, FURG, RS, Brazil
[b] Programa de Pós-Graduação em Ciências Fisiológicas, ICB, FURG, RS, Brazil
[c] Escola de Química e Alimentos, EQA, FURG, RS, Brazil
[d] Centro de Ciências Computacionais, FURG, RS, Brazil
[e] Centro de Biotecnologia, Universidade Federal da Paraíba, PB, Brazil
[f] Universidade Federal do Pampa, Campus São Gabriel Brazil

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ABSTRACT

The phenotype of multidrug resistance (MDR) is one of the main causes of chemotherapy failure. Our study investigated the effect of C-phycocyanin (C-PC) in three human erythroleukemia cell lines with or without the MDR phenotype: K562 (non-MDR; no overexpression of drug efflux proteins), K562-Lucena (MDR; overexpression of ATP-binding cassette, sub-family B/ABCB1), and FEPS (MDR; overexpression of ABCB1 and ATP-binding cassette, sub-family C/ABCC1). Using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, we showed that 20 and 200 µg/mL C-PC decreased K562 viable cells after 24 h and 200 µg/mL C-PC decreased K562-Lucena cell proliferation after 48 h. C-PC did not decrease viable cells of FEPS cells. On the other hand, the MTT assay showed that exposure of 2, 20, and 200 µg/mL C-PC for 24 or 48 h was not cytotoxic to peritoneal macrophages. At 72 h, the trypan blue exclusion assay showed that 20 µg/mL C-PC decreased K562 and K562-Lucena cell proliferation and in FEPS cells, only 200 µg/mL C-PC decreased proliferation. In addition, protein–protein docking showed differences in energy and binding sites of ABCB1 and ABCC1 for C-PC, and these results were confirmed by the efflux protein activity assay. Only ABCC1 activity was altered in the presence of C-PC and FEPS cells showed lower C-PC accumulation, suggesting C-PC extrusion by ABCC1, conferring C-PC resistance. In combination with chemotherapy (vincristine [VCR] and daunorubicin [DNR]), the sensitivity of K562-Lucena cells for C-PC + VCR did not increase, whereas FEPS cell sensitivity for C-PC + DNR was increased. In molecular docking experiments, the estimated free energies of binding for C-PC associated with chemotherapy were similar (VCR: -6.9 kcal/mol and DNR: -7.2 kcal/mol) and these drugs were located within the C-PC cavity. However, C-PC exhibited specificity for tumor cells and K562 cells were more sensitive than K562-Lucena cells, followed by FEPS cells. Thus, C-PC is a possible chemotherapeutic agent for cells with the MDR phenotype, both alone in K562-Lucena cells (resistance due to ABCB1), or in combination with other drugs for cells similar to FEPS (resistance due to ABCC1). Moreover, C-PC did not damage healthy cells (peritoneal macrophages of *Mus musculus*).

1. Introduction

The number of new cases of cancer has increased largely because of growth and aging of the world's population [1]. In 2030, it is expected that cancer will result in approximately 5.76 million deaths worldwide [2,3]. Cancer treatment usually involves chemotherapeutic agents to reduce mortality/morbidity and increase quality of life [4]. However, several chemotherapeutic agents have a low therapeutic index, generating serious problems, such as a multidrug resistance (MDR) phenotype [5]. The MDR phenotype is the most significant reason for cancer chemotherapeutic failures and plays a central role in cancer metastasis and recovery [6].

MDR is a well known phenomenon that results in the resistance of cancer cells to one chemotherapeutic drug accompanied by resistance

* Corresponding author at: Universidade Federal do Rio Grande - FURG, Instituto de Ciências Biológicas, Av. Itália, Km 8, 96201-900, Rio Grande, RS, Brazil.
E-mail addresses: star.fs@hotmail.com, fernandesesilvae@furg.br (E. Fernandes e Silva).

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to other chemotherapeutic drugs that may have different structures and/or mechanisms of action [7]. The mechanisms that cancer cells use and/or develop to evade chemotherapy treatment are complex and not fully understood. However, one of the most prominent mechanisms is the overexpression of ATP-binding cassette (ABC) transporters [8]. Resistant cancer cells exhibit high levels of expression of several ABC transporter pumps, resulting in an increase in drug efflux [9]. ABC transporters include the well known P-glycoprotein (P-gp, encoded by the *ABCB1* gene), MDR-associated protein 1 (MRP1, encoded by the *ABCC1* gene), and ABC subfamily G member 2, also known as breast cancer resistance protein, BCRP, which is encoded by the *ABCG2* gene [10].

Although the overexpression of drug efflux proteins is often associated with the MDR phenotype, it consists of several other mechanisms as well. Thus, substances with different mechanisms of action would be an alternative to overcome the MDR phenotype. In this context, C-phycocyanin (C-PC) appears promising, with several cellular targets and mechanisms of action [11].

C-PC is a water-soluble protein, which was first reported by Lemberg in 1928 [12] as the main phycobiliprotein found in cyanobacteria/microalgae of the genus *Nostoc*, *Spirulina*, *Aphanizomenon*, and others. According to Vonshak [13], the protein fraction of *Spirulina platensis* cyanobacterium may contain up to 20% C-PC [12,14–17]. C-PC is widely used in food and pharmaceutical industries and has other applications, such as its use as a fluorophore in clinical and immunological analyses [18–20]. C-PC has been reported to exhibit anticancer properties by inducing cytotoxicity and inhibiting cell proliferation [17,21–25].

The potential of C-PC to inhibit the growth of cancer-resistant cells with the MDR phenotype has remained poorly explored. There is a study which shown that C-PC increase the intracellular accumulation of doxorubicin in HepG2 cells, which are resistant to chemotherapy, and increase the anti-cancer effects of doxorubicin, indicating the ability of C-PC to act on cells with the MDR phenotype [25]. Here, to understand the effects of C-PC on the MDR phenotype, three human erythroleukemia cell lines were used: one non-MDR, K562, that was isolated and first cultured by Lozzio and Lozzio [26], and two MDR cell lines, K562-Lucena and FEPS.

The K562-Lucena cell line was established from the K562 parental cell line by Rumjanek et al. [27,28], using vincristine (VCR), an anticancer drug that targets components of the cytoskeleton [29–31]. K562-Lucena cells are resistant to non-related drugs [32,33] and overexpress P-gp [34].

The FEPS cell line was selected from K562 with daunorubicin (DNR), an anti-cancer drug that inhibits DNA synthesis [35,36], which has characteristics that distinguish it from the K562-Lucena cell line, such as lower expression of the cellular death receptor CD95 and overexpression of P-gp and MRP1 [37]. The aim of this study was to compare the anti-cancer properties of C-PC in one non-MDR (K562) and two MDR (K562-Lucena and FEPS) cell lines, as well as to verify the ability of C-PC to increase the action of traditional chemotherapeutic agents (VCR and DNR).

2. Materials and methods

2.1. Human erythroleukemic cells culture

K562, K562-Lucena, and FEPS cell lines were obtained from Laboratório de Imunologia Tumoral of Instituto de Bioquímica Médica Leopoldo de Meis (Universidade Federal do Rio de Janeiro, Brazil). K562 parental cells were grown in RPMI 1640 medium (Sigma), supplemented with sodium bicarbonate (0.2 g/l) (Synth), L-glutamine (0.3 g/l) (Vetec), with 10% fetal bovine serum (FBS) (Gibco) and 1% antibiotic and antimycotic (penicillin [100 U/mL], streptomycin [100 µg/mL], and amphotericin B [0.25 µg/mL]) (Gibco), in disposable plastic flasks at 37 °C and 5% of CO_2. K562-Lucena and FEPS cells were grown under the same conditions above, but with concentrations of chemotherapeutic agents: 60 nM vincristine (VCR) [27,28] or 532 nM daunorubicin (DNR) [37], respectively, in order to preserving MDR phenotype. In the experiments VCR or DNR was not added to K562-Lucena and FEPS cells only in the assays of combination chemotherapeutic plus C-PC.

2.2. C-phycocyanin extraction and purification

Spirulina platensis LEB 52 was cultivated [38] and supplied by Laboratório de Engenharia Bioquímica (Universidade Federal do Rio Grande – FURG, Brazil).

C-phycocyanin extraction process occurred according to Moraes et al. [39]. Briefly, crude broth containing cells was centrifuged (1890*g*, 20 min) and pH was adjusted at 6.5. Purification process was by ultrafiltration performed in dead-end ultrafiltration cell with 50 kDa polyethersulfone membrane (UH050 P, Nadir, Germany) at 25 °C and 1.0 kgf/cm^2 in diafiltration/ultrafiltration mode [40]. Purity of C-PC extract was calculated according to Abalde et al., [41], using absorbance ratio OD_{620}/OD_{280}. Treatments were constructed using purified extract with purity degree of 1.00, considered as food grade [42]. C-PC concentration (mg/mL) was calculated according to Bennet and Bogorad [43] by following equation, with changes in the wavelength:

$$\text{C-PC} = \frac{(OD620 - 4.74 \times (OD652))}{5.34}$$

2.3. MTT assay with human erythroleukemic cells

Cells were centrifuged (197*g*; 2 min), suspended (2.10^4 cells/mL) in RPMI 1640 medium (Sigma), plated in 96-well culture plates, and treated with different concentrations of C-PC (2.0, 20.0 and 200.0 µg/mL of C-PC). In addition, a control group (without C-PC), receiving the same volume of sterile water was used. Cells were incubated at 37 °C.

MTT assay was performed after 24 and 48 h of C-PC exposure. Briefly, after incubation with C-PC the cells were centrifuged (524*g*, 5 min) for removal of the culture medium and washed with phosphate buffer saline (PBS). Subsequently, the cells were centrifuged again (524*g*, 5 min), the PBS was removed and added 200 µl of RPMI 1640 medium and 20 µl of MTT (5 mg/mL) in each well. Cells were incubated for 3 h at 37 °C. Supernatant was removed after centrifugation (524*g*, 5 min) and formazan crystals were dissolved in 200 µl of dimethylsulfoxide (DMSO, Sigma), shaking gentle. Absorbance values at 490 nm were determined on multiwell plate reader (ELX 800 Universal Microplate Reader, Bio-TEK).

2.4. MTT assay with peritoneal macrophages

Animals: Five female Swiss albino mice (6–8 weeks) were obtained from Thomas George animal house of Instituto de Pesquisa em Fármacos e Medicamentos (Universidade Federal da Paraíba). Animals were kept under standard laboratory conditions on a constant 12 h light/dark cycle with controlled temperature (21 ± 1 °C). Food and water were given *ad libitum*. After manipulation, euthanasia was employed by cervical dislocation. All procedures adopted in this study were approved by Institutional Ethics Committee of Biotechnology Center/UFPB (protocol number: 111/2016).

Peritoneal macrophage elicitation: Peritoneal inflammation was induced by injection of 4 mL of 4% thioglycollate (Sigma Aldrich). Four days after the i.p. thioglycolate injection, animals were euthanized by cervical dislocation and the peritoneal cavity was washed with 8 mL of PBS (phosphate buffer saline), supplemented with 3% fetal bovine serum (FBS) (Gibco). Cell suspension obtained from peritoneal lavage was centrifuged (390*g*; 5 min; 4 °C). After that, supernatant was discarded and the pellet was resuspended in 1 mL of complete RPMI medium. Viable cells were counted with a Neubauer chamber using