Cell Biology International 32 (2008) 888–892

Cell Biology International

www.elsevier.com/locate/cellbi

Evaluation of silibinin on the viability, migration and adhesion of the human prostate adenocarcinoma (PC-3) cell line

Mohammad Javad Mokhtari [a,b], Nasrin Motamed [b], Mohammad Ali Shokrgozar [a,*]

[a] National Cell Bank of Iran, Pasteur Institute of Iran, 69, Pasteur Avenue, P.O. Box: 1316943551, Tehran, Iran
[b] School of Biology, University College of Science, University of Tehran, Tehran, Iran

Received 15 August 2007; revised 1 March 2008; accepted 28 March 2008

Abstract

Prostate cancer (PCA) is the most common cancer diagnosed in men and the second most common cause of death due to cancers after lung cancer. Metastasis of cancer cells involves multiple processes and various cytophysiological changes, including changed adhesion capability between cells and extracellular matrix (ECM) and damaged intercellular interaction. Silibinin, a naturally occurring flavonoid antioxidant found in the milk thistle, has recently been shown to have potent antiproliferative effect against various malignant cell lines. In the present study, PC-3 cells were incubated with various concentrations of silibinin for different times; then, cell cytotoxicity, cell adhesion and cell motility were assessed using MTT assay, cell–matrix adhesion assay and cell migration assay, respectively. The results showed that silibinin exerted a dose- and time-dependent inhibitory effect on the viability, motility and adhesion of highly metastatic PC-3 cells. These observations indicate that silibinin can probably inhibit metastasis in PCA.

Keywords: Silibinin; PC-3; Migration; Adhesion; Viability

1. Introduction

Prostate cancer is the most common cancer diagnosed in men and the second most common cause of death due to cancers after lung cancer in American men. According to the American Cancer Society, 232,090 new cancer cases and 30,350 deaths were estimated to occur due to PCA in 2005. The increasing incidence and mortality due to PCA and the failure of conventional chemo- and radio-therapy against advanced invasive PCA indicate that new approaches are urgently needed to control this malignancy (Deep et al., 2006). Metastasis of cancer cells involves multiple processes and various cytophysiological changes, including changed adhesion capability between cells and ECM and damaged intercellular interaction. Degradation of ECM by cancer cells via proteases, such as serine proteinase, metalloproteinases (MMPs), cathepsins and plasminogen activators (PA), may lead to separation of intercellular matrix to promote the mobility of cancer cells and eventually lead to metastasis (Singh et al., 2002b).

Prevention and therapeutic interventions using phytochemicals are new techniques in cancer management. Administration of phytochemicals is shown to prevent initiatory, promotional and progressional events associated with carcinogenesis in different animal models, and is suggested to effectively reduce cancer mortality and morbidity. Among various groups of phytochemicals, extensive experimental data have been generated for the role of polyphenolic flavonoids in chemoprevention of various cancers including PCA (Koivisto et al., 1998; Neuhouser, 2004). Silibinin is a polyphenolic flavonoid isolated mainly from the fruits or seeds of milk thistle (*Silybum marianum*); silymarin is a flavonolignan complex, composed of silibinin and small amounts isosilybin A, isosilybin B, silychristin, isosilychristin, dehydrosilybin, silydianin and taxifolin (Sharma et al., 2003; Gazak et al., 2007). The previous studies have shown that silibinin is effective in treating a wide range of

* Corresponding author. Tel./fax: +98 2166492595.
E-mail address: mashokrgozar@pasteur.ac.ir (M.A. Shokrgozar).

1065-6995/$ - see front matter © 2008 International Federation for Cell Biology. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.cellbi.2008.03.019

liver diseases, including hepatitis and cirrhosis (Wagner et al., 1974; Saller et al., 2001). The chemopreventive role of silymarin has been extensively studied and has revealed anticancer efficacy against various cancer types, especially skin and prostate cancers (Deep and Agarwal, 2007). *In vitro* and *in vivo* studies have revealed the pleiotropic anticancer capabilities of silibinin, including significant antiproliferative effects against cancer growth and strong apoptotic death in endothelial cells (Wellington and Jarwis, 2001; Singh et al., 2002b). Furthermore, another study showed that silibinin may induce substantial growth inhibition, moderate cell cycle arrest and strong apoptotic death in both small and nonsmall human lung carcinoma cells (Sharma et al., 2003). It has been suggested that silibinin treatment causes significant growth inhibition through S-phase arrest and significant induction of apoptotic cell death in both a dose- and time-dependent manner (Singh et al., 2003). Other reports on prostate cancer cells indicate that silibinin causes a moderate increase in the expression of insulin-like growth factor-binding protein-3 (IGFBP-3) (Singh et al., 2002a; Tyagi et al., 2002; Zi et al., 2000), which may have an inhibitory effect on the mitogenic action of IGF-1. Furthermore, an increase in activated caspase 3-positive cells in silibinin-fed tumors indicates that activation of caspase cascade can be one of the molecular mechanisms in silibinin-induced apoptotic cell death in prostate cancer (Tyagi et al., 2002).

Although it is quite clear that silibinin may inhibit the growth of various cancers by inducing cancer cells towards apoptosis, the precise involvement of silibinin in the prevention of cancer metastasis was still uncertain. Since cancer metastasis and invasion are highly related to degradation of ECM, intercellular adhesion and cellular motility, in the present study, the effects of silibinin on the viability, migration and adhesion of the PC-3 cells were investigated.

2. Materials and methods

2.1. Cell culture and silibinin treatment

PC-3, a human prostate adenocarcinoma cell line, was obtained from the National Cell Bank of Iran (NCBI), Pasteur Institute of Iran (NCBI, C427). Silibinin (Sigma, USA) was dissolved in DMSO (Sigma, USA). The DMSO in culture media never exceeded 0.1% (v/v) (Zi et al., 2000). PC-3 cells were cultured in RPMI-1640 medium supplemented with 10% heat inactivated fetal calf serum (FCS), 2 mM glutamine, penicillin (100 IU/ml) and streptomycin (100 μg/ml) (All from Gibco, Scotland) at 37 °C in an incubator containing 5% CO_2. Harvested cells with trypsin (0.25%) (Sigma, USA) were counted by neobar slide with trypan blue and then were seeded into 96-well plates (1×10^4 cells/well). The cells were incubated with different concentrations of silibinin (0, 12.5, 25, 50, 100, 200 and 400 μg/ml) at 24, 48 and 72 h. Each concentration was tested on six wells of the 96-well plates containing 1×10^4 PC-3 cells. In each experiment, six PC-3 cultured wells with no drug incubation were used as negative controls (Zali, personal communication).

2.2. Determination of cell viability

The cell viability was determined by using methyl thiazolyl tetrazolium bromide (MTT, Sigma, USA) assay (Chun et al., 2004). For the MTT assay, the dye was dissolved in PBS (phosphate buffer saline) at a concentration of 5 mg/ml and the solution was filtered through a 0.45 μm filter to sterilize and remove small amount of insoluble residues present in some batches of MTT; then it was stored at 2–8 °C for frequent use. Four hours before the end of incubation, 10 μl of MTT solution (5 mg/ml in PBS) was added to each well containing 100 μl cultured medium. Dissolved yellowish MTT is converted to an insoluble purple formazan by cleavage of the tetrazolium ring by dehydrogenase enzymes during 4 h incubation. Unlike in dead cells, active mitochondrial dehydrogenases on living cells will cause this change. The cleavage and conversion of the soluble yellow dye to the insoluble purple formazan have been used to develop an assay system alternative to other assays for measurement of cell proliferation. The produced insoluble formazan was dissolved in solution containing 100 μl isopropanol (Merck, Germany) and its optical density (OD) was read against blank reagent with a multi-well scanning spectrophotometer (ELISA reader, Organon Teknika, Netherlands) at a wavelength of 570 nm. Six PC-3 cultured wells were incubated with 100 μl DDW (deionized distilled water) for 10 min and used as positive control and six PC-3 cultured wells with no sample as negative controls. In addition, six wells containing only dye (100 μl diluted in MTT) were used as dye control (Zali et al., 2008).

The percentage of cytotoxicity was calculated according to following formulas:

$$\%\text{Cytotoxicity} = \frac{1 - \text{mean absorbance of toxicant treated cells} \times 100}{\text{mean absorbance of negative control}}$$

$$\%\text{Viability} = 100 - \%\text{Cytotoxicity}$$

2.3. LC_{50} determination

The 50% lethal concentration (LC_{50}) values of silibinin on PC-3 cells at different time intervals are shown in Fig. 2. LC_{50} was determined by probit analysis using the Pharm PCS (Pharmacologic Calculation System) statistical package (Springer-Verlag, USA).

2.4. Cell migration assay

Cell migration was determined by using a CytoSelect™ cell migration assay kit (Cell Biolabs, USA). Briefly, tumor cells (1×10^6 cells/well) were incubated with different concentrations of silibinin including 100, 150 and 200 μg/ml for 24 h, and 50, 75 and 100 μg/ml for 48 h. As LC_{50} for silibinin at 24 h was 143.4 μg/ml and at 48 h was 74.7 μg/ml, these concentrations of silibinin were selected for migration assay. After the incubation periods, cells were removed by trypsinizing, and their *in vitro* migration was tested by the CytoSelect™ cell