Pharmacological Reports
2012, 64, 991–996
ISSN 1734-1140

Short communication

Verapamil-induced autophagy-like process in colon adenocarcinoma COLO 205 cells; the ultrastructural studies

Beata Pająk[1], Elżbieta Kania[1], Barbara Gajkowska[1], Arkadiusz Orzechowski[1,2]

[1]Electron Microscopy Platform, Mossakowski Medical Research Centre, Polish Academy of Sciences, Pawińskiego 5, PL 02-105, Warszawa, Poland

[2]Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences (SGGW), Nowoursynowska 159, PL 02-776 Warszawa, Poland

Correspondence: Beata Pająk, e-mail: bepaj@wp.pl

Abstract:
Background: Verapamil (Ver) is a well known, worldwide used drug to correct cardiac arrhythmias. The main Ver target is the L-type calcium channel. Modulation of calcium homeostasis vaulted Ver into use in medical applications.
Methods: To examine COLO 205 cells morphology after Ver treatment, an electron microscopy technique was used.
Results: This study shows ultrastructural evidence that Ver initiates autophagy-like process in human colon adenocarcinoma COLO 205 cells. TEM photographs revealed the presence of differently developed autophagic vacuoles in response to Ver administration. Furthermore, extensive ultrastructural cell alterations confirmed that cancer cells died *via* necrosis or apoptosis, as demonstrated by ruptured plasma membrane or condensed chromatin, respectively.
Conclusions: It is the evidence that apoptosis resistant COLO 205 cells are overruled by autophagy-like process. Autophagy-like cell death could be a promising venue to delete cancer cells. Ver appears to be a new potentially effective anticancer compound.

Key words:
verapamil, autophagy, transmission electron microscopy, colon cancer, cell death

Introduction

COLO 205 cells represent colon adenocarcinomas, the most common cancer type in human population. These cells are characterized by an extreme resistance to cell death induction *via* natural death receptor ligands (TNF-α, FasL, TRAIL), as well as chemotherapeutic drugs [10]. It was shown that one of the mechanisms important for resistance to apoptosis relies on changes in calcium signaling in neoplastic cells [2]. Encouraged by our previous observations [11], concerning the proapoptotic effects of ethylene glycol tetraacetic acid (EGTA) and ethylenediaminetetraacetic acid (EDTA) (calcium ions chelators), we investigated the possible mechanisms of calcium signaling in COLO 205 cells. Among several tested compounds was verapamil, a well-known L-type calcium channel blocker. L-type calcium channels are expressed in skeletal and heart muscle tissue. Wang et al. [16] reported that in HT-29, Caco-2 and T84 colon cancer cells, as well as colon cancer biopsies, the

L-type calcium channel is upregulated. According to Loza et al. [6], increased mRNA of the calcium channel may correlate with enhanced proliferation induced by epidermal growth factor (EGF). Apart from L-type channel blockage, Ver has been reported to exert other cellular responses, such as inhibition of MDR1/P-gp activity [15] and increased reactive oxygen species (ROS) production leading to oxidative stress [17]. Oxidative stress results in both autophagy activation and mitochondrial dysfunction [17]. Furthermore, mitochondrial ROS can further increase calcium release from the endoplasmic reticulum (ER), thereby causing protein misfolding. ROS production and protein misfolding together activate calcium-dependent kinases such as JNK eventually leading to cell death [7]. On the other hand, changes in calcium homeostasis may foster ER stress triggering autophagy by the ER-activated autophagy pathway. The latter could be mediated by limited unfolded protein response (UPR) signals involving PERK and/or IRE1 as well as UPR-independent mechanism such as calcium leakage (JNK-AKT/mTOR signaling) [4]. Meister et al. [8] demonstrated that Ver enhanced IRE1- and PERK-mediated pathways, further activated the UPR and might trigger an autophagic, caspase-independent cell death. According to latest studies published by Kuo et al. [5], the Ver-induced ER stress is determined by increase in GRP78 chaperone protein expression and by upregulation of a heat shock protein 70 kDa family member that prevents the unfolded protein from further transit and secretion [3].

Autophagy, an intracellular degradation system of cytoplasmic contents and organelles, is required for normal turnover of cellular components during starvation in eukaryotic cells and is characterized by distorted mitochondria. The autophagosomes, with two or more membranes enclosed vesicles, engulf various cellular constituents that fuse with lysosomes for degradation and recycling [12]. Besides promoting cell survival, autophagy can trigger caspase-independent cell death [12]. Furthermore, various observations show that autophagy contributes to cell death induction in apoptosis competent cells, while it becomes the major cell death-inducing pathway in apoptosis deficient cells [9].

We present the first ultrastructural evidence that in colon adenocarcinoma COLO 205 cells, Ver treatment induces massive autophagy-like process leading to necrotic cells death.

Materials and Methods

Cell culture

Human colon adenocarcinoma cell line COLO 205 was purchased from American Type Culture Collection (ATCC). Cells were maintained in the exponential phase of growth in growth medium [GM, 100 ml/l Fetal Bovine Serum (FBS)/Dulbecco's modified Eagle medium (DMEM) with Glutamax and antibiotic-antimycotic mixture (penicillin G sodium salt 50 IU/ml, streptomycin sulfate 50 μg/ml, gentamycin sulfate 20 μg/ml, fungizone – amphotericin B 1 μg/ml)]. The cells were grown at 37ºC, in a controlled, humidified 50 ml/l CO_2 atmosphere, on 96-well flat-bottomed or tissue culture Petri dishes (100 mm diameter, BD Biosciences Pharmingen, San Jose, CA USA).

Experimental procedure

During propagation, the medium was changed every other day until cultures reached 100% confluence. One-day (24 h) prior to the experiment, confluent cells (cells of the same cell density fully covering the surface of the dish) were switched to post-mitotic status to induce quiescence (withdrawal from cell cycle) by replacing GM with 20 g/l BSA/DMEM designated as a control medium (CTRL). In the above-mentioned conditions divisions of COLO 205 cell have been completed. Next, cells were treated with Ver (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) [50, 100 or 200 μM] for 24 or 48 h.

Ultrastructural studies

Cells were fixed in 2.5% paraformaldehyde and 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 2 h at 4ºC. Cells were washed with the same buffer and post-fixed with 1% OsO_4 in 0.1 M sodium cacodylate buffer for 1 h. Cells were dehydrated in a graded ethanol series, and embedded in Epon 812. Ultrathin sections were mounted on copper grids, air-dried, and stained for 10 min with 4.7% uranyl acetate and for 2 min with lead citrate. The sections were examined and photographed with a JEOL JEM 1011 electron microscope (Jeol, Tokyo, Japan).