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Cancer cell sensitization and improved treatment efficacy by combined sodium butyrate and paclitaxel formulations is cancer-type specific

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ABSTRACT

We queried whether cancer treatment by combinations of paclitaxel and butyrate – free or formulated in drug delivery systems – can improve therapeutic responses compared to each drug alone. Combination treatments were conducted with HT-29 and HeLa cells, as representatives of differentiation-induced and cell-death-induced cancer lines, respectively. Pre-treatment of the HT-29 cells with butyrate (at doses inducing differentiation), followed by butyrate + paclitaxel generated changes in cell cycle profile, increased the level of dead cells beyond that of each drug alone, and allowed reduction in paclitaxel doses. A similar combination treatment of HeLa cells was detrimental, indicating that whether the combination is beneficial or not is cancer-type specific. We hypothesize that while butyrate-treated HT-29 cells became sensitive to paclitaxel-induced Fas-mediated apoptosis, butyrate-adapted HeLa cells became apoptosis-resistant. We next tested the same drug combination on HT-29 cells, but each drug in a specific tumor-targeted carrier. The combination of drug carriers outperformed an equidose combination of the free drugs, showing potential to achieve high therapeutic responses (even in drug-resistant cells) at significantly lower and detergent-free paclitaxel doses, which should allow for reduction in adverse effects and risks of toxicity.

1. Introduction

The prevailing approach to cancer treatment is acute therapy aiming at cancer cell kill. The well-known tumor heterogeneity is addressed by applying drug cocktails made of therapeutic agents that may operate in different mechanisms and at different cellular loci (Cao and Heng, 2005; Carrick et al., 2009; Goetz and Grothey, 2004). An alternative approach is to turn cancer from an acute into a chronic disease applying differentiation agents with the hope of using drug doses well below toxic thresholds, thus overcoming a major problem frequently encountered in the acute treatment mode (Cao and Heng, 2005). In the present study we questioned whether a different type of drug cocktail, combining a chemotherapeutic drug driving cell kill with a drug that may drive differentiation or apoptosis, can provide a therapeutic benefit over treatment with each drug alone. Our test combination consisted of: (i) paclitaxel (PTX), a well-established acute-treatment chemotherapy known to drive cell kill through apoptosis (Panchagnula, 1998) and (ii) sodium butyrate (SB), reported to act as a differentiation agent in erythroleukemia, embryonic carcinoma and colon carcinoma and to induce apoptosis in cervix adenocarcinoma and colonic adenomas and carcinomas (Boren et al., 2003; Orchel et al., 2005).

To assess SB's cellular effects on human cancer cell lines, we combined three independent measures: (1) cell viability, determined by total cell protein (Papazisis et al., 1997); (2) changes in the activity of mitochondrial succinate-coenzyme Q reductase (SQR), the rational based on the Warburg effect that mitochondrial activity may serve as a general marker for the differentiation process in cancer cells (Bensaid et al., 2000; Gatenby and Gillies, 2004; Kim and Dang, 2006; Scudiero et al., 1988; Warburg, 1956) and (3) changes in the activity of alkaline phosphatase (ALP) – an enzyme well-known as a specific differentiation marker in intestinal cancer cells (Deng et al., 1992; Popovtzer et al., 2008). The cell lines tested were from human breast, colon and cervical cancer, and were a mix of drug-sensitive and drug-resistant cells.

Based on the results with SB alone, we opted to study the effects of the PTX + SB combination treatment on two cell lines: colon cancer (HT-29) cells in which SB acts as a differentiation agent, and cervical cancer (HeLa) cells in which SB induces apoptosis (Boren et al., 2003; Chen et al., 2006).

Each of the drugs is known to have drawbacks when applied in free form. Treatment with free PTX is fraught with adverse effects due to its chemotherapeutic nature and to the presence of

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detergent in its clinical formulation (needed to overcome the drug's poor solubility) (Fetterly and Straubinger, 2003; Gelderblom et al., 2001; Gianni et al., 1995). PTX is a known substrate of multidrug (MDR) resistance pumps, in particular ABCB1 (P-glycoprotein) (Argov et al., 2009). Treatment with free SB requires mM doses within the cancer cell, it undergoes fast metabolism and has to be supplied continuously to maintain the (reversible) differentiation state (Augenlicht et al., 2003; Janssen and Schink, 1995; Orchel et al., 2005; Serpe et al., 2004).

To address these free-drug deficiencies, we formulated each drug in a suitable tumor-targeted carrier. In both cases, the targeter was hyaluronan (HA), its ability to target a carrier (hence its drug load) to tumors evolving from the overexpression of hyaluronan receptors (the CD44 family and CD168) on the membranes of many tumor types (Peer et al., 2007; Peer and Margalit, 2004a,b; Platt and Szoka, 2008). For the poorly soluble PTX we applied our previously developed carriers named gagomers, in which hyaluronan is the carrier's surface and in which PTX is formulated without the need for detergent (Rivkin et al., 2010). For the soluble SB we applied our targeted liposomes, in which hyaluronan is covalently anchored to the liposomal surface. We have previously shown these targeted liposomes to be particularly suitable for small soluble anticancer drugs (Peer and Margalit, 2004a,b) and as will be shown here, this also holds for SB. The combination treatments thus included two types of dosage forms: free SB with free PTX, carrier-loaded SB with carrier-loaded PTX.

2. Materials and methods

2.1. Materials

Phospholipon 90G (high purity Soybean phosphatidylcholine (SPC)) was a kind gift from Nattermann Phospholipid GmbH (Cologne, Germany). Dipalmitoylphosphatidylethanolamine (DPPE), ethyl-dimethyl-aminopropyl-carbodiimide (EDC), sodium butyrate (SB), sulfarhodamine B (SRB), trichloroacetic acid (TCA), Trizma base, alkaline phosphatase yellow liquid substrate (pNPP), pepsin, propidium iodide, mitoxanthrone and etoposide were from Sigma Chemical Co. (St. Louis, USA). Hyaluronan (HA) 1.5 MDa used for the liposomes was a kind gift from Genzyme (Cambridge, MA, USA) and HA 0.6–1.2 $\times 10^6$ Da used for the gagomers was obtained from Calbiochem® (Nottingham, UK). 1,2-Dilauroyl-sn-glycero-3-phosphoethanolamine (DLPE) and 1,2-dilauroyl-sn-glycero-3-glycerol (DLPG) was from Avanti Polar Lipids Inc. (Alabaster, AL, USA). ^{14}C-sodium butyrate was from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA). Liquid scintillation cocktail, Ultima Gold™, was from PerkinElmer Life and Analytical Sciences Inc. (USA). Complete™ solution for protease inhibition was from Roche Molecular Biochemicals (Mannheim, Germany). Precision Plus Protein™ Standards were from Bio-Rad Laboratories (Hercules, USA). Enhanced chemiluminescence (ECL) reagents were from Amersham Pharmacia Biotech (Buckinghamshire, UK). Films were from Kodak. Anti-human MRP clone, MRPm6 clone, was from Chemicon Intl. Inc. (Temecula, USA). Anti-human BCRP clone BXP-21 was from Sigma–Aldrich (St. Louis, USA). Secondary goat anti-mouse horseradish-peroxidase conjugated antibody was from Jackson ImmunoResearch Laboratories, Inc., (PA, USA). Tissue culture flasks and 96-well plates were from Corning Inc. (Corning, NY). Dialysis tubing (molecular weight cutoff of 12,000–14,000) was from Spectrum Medical Industries (Los Angeles, CA). Fetal bovine serum (FBS), McCoy's 5a medium, MEM-Eagle medium, RPMI 1640 medium, MEM-Eagle non-essential amino acids (×100), L-glutamine, penicillin (10,000 U/ml) + streptomycin (10 mg/ml) + nystatin (1250 U/ml) solution, sodium bicarbonate, sodium pyruvate, 0.25% trypsin–EDTA solution and XTT kit were from Biological Industries (BeitHaemek, Israel). All other reagents were of analytical grade. Ultracentrifugation was performed with a Sorval Discovery M120 SE micro ultracentrifuge (TN, USA). Lyophilization was performed with a HETO Drywinner 3 (Alleraod, Denmark).

Cell lines: HeLa (human cervical cancer) and two colon cancer lines HCT-15 andHT-29 (human colon carcinomas) were from ATCC; MCF-7 (human breast adenocarcinoma), the parent cell line and two drug-resistant sublines MCF-7/MX and MCF-7/VP16 were a kind gift from the M. Moscovitch Laboratory in the Weizmann Institute (Rehovot, Israel).

2.2. Methods

2.2.1. Cell culture growth and maintenance

Cultures were maintained at 37 °C in 5% CO_2. For all experiments cells were harvested from sub confluent cultures using 0.25% trypsin–EDTA solution and were resuspended in fresh full serum-supplemented growth medium before plating. HT-29 cells (human colon carcinoma) were grown in McCoy's 5A medium containing 1.5 mM L-glutamine, 1% penicillin–streptomycin–nystatin (PSN), 10% fetal bovine serum (FBS) and 2.2 g/l sodium bicarbonate. HCT-15 cells (human colon carcinoma) were grown in RPMI 1640 medium containing 2 mM L-glutamine, 1% penicillin–streptomycin–nystatin (PSN), 10% fetal bovine serum (FBS) and 0.2 mM Hepes buffer. HeLa cells (human cervical cancer) were grown in MEM-Eagle medium containing 1.5 mM L-glutamine, 1% PSN, 10% FBS, 0.1 mM NEAA, 1.5 g/l sodium bicarbonate and 1.0 mM sodium pyruvate. Three lines of MCF-7 cells (human breast adenocarcinoma) – MCF-7, MCF-7/MX (mitoxanthrone-resistant) and MCF-7/VP16 (etoposide-resistant) – were grown in RPMI 1640 medium containing 2 mM L-glutamine, 10% FBS and 1% PSN; for MCF-7/MX the medium also included 250 nM mitoxanthrone and for MCF-7/VP16, 4 μM etoposide.

2.2.2. Cell plating and treatments

Plating, growing and treatment procedures were similar for all cell lines. In most experiments, exposure time for SB was 72 h, except the HT-29 cells, that were also exposed for 144 h ("long" treatment). On the first day (time 0), the cells were seeded onto 96-well plates, 100 μl/well, at the following densities: HT-29 – 120,000 cells/ml for the "short" 72 h treatment and 35,000 cells/ml for the "long" 144 h treatment; HCT-15 – 110,000 cells/ml; HeLa and the three MCF-7 lines – 30,000 cells/ml. Twenty four hours after seeding, treatments with free or liposomal SB were initiated by means of refreshing the medium in the wells with medium containing the selected drug formulation. The range of SB concentrations was 0–40 mM. The cells were incubated at 37 °C and 5% CO_2 for 72 h. For the "long" HT-29 plating, there were two treatment-time-points: on the second day (24 h after seeding of cells) and on the fourth day (96 h after seeding); and the cells were incubated at the same conditions for additional 72 h. Mitoxanthrone-resistant or etoposide-resistance MCF-7 cell lines were obtained by the following procedure: after three passages in cell growth medium without the drugs, the cells were grown in flasks for one week (two passages) in medium containing 250 nM of MX or 4 μM of etoposide; then the drugs were removed from the medium and three days later the plating experiment was performed. For treatment with MX or etoposide, cell seeding was at 10,000 cells/well. Treatment was similar to that described for SB, except termination was 24 h post-treatment, and drug concentration range (for each drug) was 0–300 μM.

2.2.3. Growth inhibition assay

Quantitating cell viability was performed by the trypan blue method using a homocytometer and by the sulfarhodamine B (SRB)