Butyrate activates the monocarboxylate transporter MCT4 expression in breast cancer cells and enhances the antitumor activity of 3-bromopyruvate

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Abstract Most malignant tumors exhibit the Warburg effect, which consists in increased glycolysis rates with production of lactate, even in the presence of oxygen. Monocarboxylate transporters (MCTs), maintain these glycolytic rates, by mediating the influx and/or efflux of lactate and are overexpressed in several cancer cell types. The lactate and pyruvate analogue 3-bromopyruvate (3-BP) is an inhibitor of the energy metabolism, which has been proposed as a specific antitumor agent. In the present study, we aimed at determining the effect of 3-BP in breast cancer cells and evaluated the putative role of MCTs on this effect. Our results showed that the three breast cancer cell lines used presented different sensitivities to 3-BP: ZR-75-1 ER (+)>MCF-7 ER (+)>SK-BR-3 ER (−). We also demonstrated that 3-BP reduced lactate production, induced cell morphological alterations and increased apoptosis. The effect of 3-BP appears to be cytotoxic rather than cytocstatic, as a continued decrease in cell viability was observed after removal of 3-BP. We showed that pre-incubation with butyrate enhanced significantly 3-BP cytotoxicity, especially in the most resistant breast cancer cell line, SK-BR-3. We observed that butyrate treatment induced localization of MCT1 in the plasma membrane as well as overexpression of MCT4 and its chaperone CD147. Our results thus indicate that butyrate pre-treatment potentiates the effect of 3-BP, most probably by increasing the rates of 3-BP transport through MCT1/4. This study supports the potential use of butyrate as adjuvant of 3-BP in the treatment of breast cancer resistant cells, namely ER (−).

Keywords 3-bromopyruvate · Butyrate · Monocarboxylate transporters · Warburg effect

Abbreviations

3-BP 3-bromopyruvate
ATCC American Type Culture Collection
DAB 3,3’-diamino-benzidine
DAPI 4’,6-Diamidino-2-Phenylindole, Dihydrochloride
ER Estrogen Receptor
One of the hallmarks of cancer is the “Warburg effect” or “aerobic glycolysis”, consisting in a metabolic switch in energy production, relying mostly on glycolysis with lactate production, even in the presence of O2, rather than on oxidative phosphorylation (OXPHOS), characteristic of normal tissues (Warburg 1956). Cancer cells take advantage of this metabolic switch, namely the increased access to biosynthetic precursors for anabolic reactions, provision of antioxidant defenses and higher ability to escape the immune system, to invade neighbor cells and survive in conditions of intermittent hypoxia (Pedersen 2007; Kroemer and Pouyssegur 2008). The efflux of lactate and protons, resulting from the glycolytic phenotype of cancer cells, prevents the acid-induced apoptosis and creates an extracellular acidic environment that suppresses the effect of the immune system and favors tumor invasion through the activation of metalloproteinases (Pedersen 2007; Kroemer and Pouyssegur 2008; Izumi et al. 2003; Fischer et al. 2007; Swietach et al. 2007). It is then not surprising that lactate production from cancer cells correlates positively with tumor aggressiveness and malignancy (Schwickert et al. 1995; Walenta et al. 1997; Walenta et al. 2000; Brzel et al. 2001). Exploiting the differential metabolism of cancer cells can thus be a valuable approach for the development of selective anticancer drugs, with low toxicity to normal cells.

3-Bromopyruvate (3-BP) is a potent antitumoral alkylating agent, which exerts its effect by inhibiting cancer cell energy metabolism and depleting cellular ATP (Ko et al. 2001). One major target of 3-BP is the glycolytic enzyme hexokinase II (HKII) (Ko et al. 2001; Chen et al. 2009). This hexokinase isoform is insensitive to feedback inhibition by glucose-6-phosphate and associates with mitochondria, especially in cancer cells, via Voltage Dependent Anion Channel protein (VDAC) that has privileged access to mitochondrial ATP (Mathupala et al. 2006; Bustamante and Pedersen 1977; Bustamante et al. 1981; Nakashima et al. 1986). Overexpression of HKII is associated with poor prognosis, as glycolysis is the primary energy source used by cancer cells to sustain their uncontrolled cell growth. 3-BP affects not only the energy production coming from glycolysis but also from mitochondrial respiration, inducing ATP depletion and cell death in rapidly growing tumors (Ko et al. 2001). 3-BP treatment completely eradicated advanced cancers in a rodent model without apparent toxicity to the animals, as normal cells are spared from the 3-BP effect (Ko et al. 2004). Although several 3-BP targets have been identified, in addition to HKII, its mechanism of action is not elucidated, particularly the mechanism of uptake into tumor cells.

3-BP is a synthetic derivative of pyruvate and an analogue of lactate, being likely transported by the same permeases. A family of proton-coupled monocarboxylate transporters (MCTs) was described as being involved in the transport of monocarboxylic acids (Halestrap and Price 1999; Halestrap and Meredith 2004; Halestrap and Wilson 2011; Halestrap 2011). The MCT family comprises 14 members but only four of them (MCT1-4) were functionally characterized as mediating the proton-coupled transport of monocarboxylic acids across the plasma membrane (namely lactate, pyruvate, butyrate and acetate) (Halestrap and Meredith 2004; Halestrap and Wilson 2011; Halestrap 2011; Kennedy and Dewhirst 2010). Both MCT1 and MCT4 were found in cancer cells, closely associated with CD147, also known as Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) or basigin, a chaperone needed for the correct targeting of MCT1 and MCT4 to the cell surface and for their activity (Izumi et al. 2003; Halestrap 2011; Nabeshima et al. 2006; Riethdorf et al. 2006; Hussien and Brooks 2011; Kirk et al. 2000; Wilson et al. 2005). Although these transporters are present in the plasma membrane of normal cells, there is evidence for their upregulation in cancer cells, given the increased lactic acid production and consequent efflux by the cell (Froberg et al. 2001; Fang et al. 2006; Pinheiro et al. 2008a; Pinheiro et al. 2008b; Pinheiro et al. 2010a; Pinheiro et al. 2010b). Tumor cells take up or export lactate according to the oxygen availability, lactate concentration and expression of the MCT subtype at the plasma membrane (Brooks 2000; Semenza 2008). Lactate efflux is thought to be mediated mostly by the MCT4 isoform, whereas oxidative cancer cells can take up lactate through MCT1 (Semenza 2008; Sonveaux et al. 2008; Draoui and Feron 2011). MCTs can be upregulated by different stimuli, including hormones (testosterone), exercise and also by exposure to carboxylic acids like lactic and butyric acids (Kennedy and Dewhirst 2010).