J Bioenerg Biomembr (2012) 44:7–15
DOI 10.1007/s10863-012-9419-2

MINI-REVIEW

3-bromopyruvate: Targets and outcomes

Maria C. Shoshan

Published online: 2 February 2012
© Springer Science+Business Media, LLC 2012

Abstract The pyruvate mimetic 3-bromopyruvate (3-BP) is generally presented as an inhibitor of glycolysis and has shown remarkable efficacy in not only preventing tumor growth, but even eradicating existant tumors in animal studies. We here review reported molecular targets of 3-BP and suggest that the very range of possible targets, which pertain to the altered energy metabolism of tumor cells, contributes both to the efficacy and the tumor specificity of the drug. Its in vivo efficacy is suggested to be due to a combination of glycolytic and mitochondrial targets, as well as to secondary effects affecting the tumor microenvironment. The cytotoxicity of 3-BP is less due to pyruvate mimicry than to alkylation of, e.g., key thiols. Alkylation of DNA/RNA has not been reported. More research is warranted to better understand the pharmacokinetics of 3-BP, and its potential toxic effects to normal cells, in particular those that are highly ATP-/mitochondrion-dependent.

Keywords 3-bromopyruvate · Cancer · Energy metabolism · Chemotherapy · toxicity

Introduction

At first glance, 3-bromopyruvate (3-BP) seems unlikely as a novel candidate anti-cancer drug. In contrast to many other novel drugs, e.g., receptor tyrosine kinase inhibitors which are non-polar, non-reactive and tailored to target a specific molecule or class of molecule, 3-BP is a highly reactive electrophilic alkylator, and can be expected to unspecifically affect a number of macromolecules. Indeed, its reactivity towards protein thiols/cysteines has made it useful for affinity labeling of protein thiols (Apfel et al. 1984; Banas et al. 1988). In line with this property, the literature reports many targets of 3-BP, some of which will be discussed below. Briefly, these targets include, but are not restricted to, hexokinase-II (HK-II) (Mathupala et al. 2009) and GAPDH (Dell'Antone 2009; Ganapathy-Kanniappan et al. 2010a; da Silva AP et al. 2009) in the glycolytic pathway, and mitochondrial succinate dehydrogenase (da Silva AP et al. 2009), the endoplasmic reticulum (Ganapathy-Kanniappan et al. 2010a) and the lysosomes (Dell'Antone 2006). Most of the known targets are thus involved in energy metabolism, and the anti-cancer effect of 3-BP is accordingly proposed to be due to the high dependence of tumor cells on glycolysis (Mathupala et al. 2009).

With this range of key metabolic targets, and the combination of effects on both glycolysis and mitochondria/mitochondrial respiration (Ko et al. 2001; Geschwind et al. 2002), it is not surprising that 3-BP efficiently kills proliferating tumor cells with a high demand for ATP as well as anabolic building-blocks. However, such a wide range of targets would also be expected to lead to serious side toxicity in normal tissues, especially in those which are highly dependent on functional glucose metabolism and on mitochondrial oxidative phosphorylation, e.g., in heart, brain and kidney. One may compare 3-BP with the clinically indispensable chemotherapeutic drugs cisplatin and doxorubicin—they are both electrophilic alkylators with multiple intracellular targets ranging from nuclear DNA to mitochondria, and have the disadvantage of being neuro-, nephro-and/or cardiotoxic; in particular cisplatin is also extremely emetic (induces vomiting). By contrast, 3-BP has been reported to elicit a massive anti-tumoral effect but no or low-level concomitant toxic effects in in vivo models of implanted hepatocarcinomas (Geschwind et al. 2002; Ko et al. 2004).

M. C. Shoshan (✉)
Department of Oncology-Pathology, Cancer Center Karolinska, Karolinska Institute,
S-171 76 Stockholm, Sweden
e-mail: mimmi.shoshan@ki.se

This review presents an overview of molecular targets and/or mechanisms of 3-BP, and a discussion of possible reasons for its tumor specificity and relative lack of toxicity.

Targets and mechanisms

Glycolysis

Hexokinase and its molecular partners

Glycolysis as a target pathway and HK-II as a target molecule of 3-BP have been extensively reviewed (Mathupala et al. 2009; Mathupala et al. 2006). Importantly, many tumors show greatly increased glucose uptake and glycolysis, and preferential overexpression of HK-II, whose association with mitochondria via the voltage-dependent anion channel (VDAC, or porin) is essential for the tumor cell metabolic phenotype and also contributes to chemoresistance (Mathupala et al. 2009; Mathupala et al. 2010; Shoshan-Barmatz et al. 2010). The vital role of the mitochondrial complex of HK-II, VDAC and the adenine nucleotide transporter (ANT) in particular in tumor cells has also been extensively reviewed (Mathupala et al. 2009; Shoshan-Barmatz et al. 2010; Ramsay et al. 2011; Pastorino & Hoek 2003); briefly, this complex regulates life and death by coordinating energy metabolism, metabolite transport between cytosol and mitochondria and also the opening of the mitochondrial permeability transition pore involved in cell death (Mathupala et al. 2009; Shoshan-Barmatz et al. 2010; Ramsay et al. 2011; Pastorino & Hoek 2003). Altogether, glycolysis, HK-II and VDAC are now established as potential therapeutic targets (Mathupala et al. 2010; Pelicano et al. 2006; Hanahan & Weinberg 2011; Tennant et al. 2010; Shoshan-Barmatz et al. 2011).

In vitro inhibition of HK-II activity by 3-BP has been demonstrated (Ko et al. 2001). In a study on cellular HK activity it was found that in HepG2 cells, treatment with 150–5,000 μM 3-BP for up to 30 min had only minor inhibitory effects, while as little as 100 μM led to substantial loss of cell viability (da Silva AP et al. 2009). It is thus unlikely that 3-BP-induced cell death is due only to inhibition of HK-II activity per se. Instead, disruption by 3-BP of the vital HK-VDAC complex may be crucial. In support of this model, it was recently reported that 3-BP (100 μM, 3–6 h) caused covalent modification of HK-II, likely at cysteines, leading to its dissociation from mitochondria and to release of the mitochondrial apoptosis-inducing factor (AIF), to which it was otherwise bound (Chen et al. 2009). Secondly, using N-HK-II (2–30 μM), an HK-II derived dissociating peptide, dissociation of HK-II from mitochondria (in fact, from VDAC) was shown to be sufficient to induce cell death, but unlike 3-BP it did so without induction of reactive oxygen species (ROS) or loss of mitochondrial membrane potential (Chen et al. 2009). Another suggestion to explain the observed dissociation of HK-II (da Silva AP et al. 2009) is based on inhibition of GAPDH and phosphoglycerate kinase further downstream in the glycolytic pathway leads to accumulation of glucose-6-phosphate which is known to lead to feed-back dissociation of HK-II.

That 3-BP induces release of AIF and its translocation to the nuclei has been shown also by others (Kim et al. 2008). In this scenario, loss of glycolytically produced ATP might be regarded as a secondary effect which in turn could further stimulate cell death and/or induce a necrotic rather than apoptotic, ATP-requiring death. It would therefore be interesting to assess the effects of 3-BP in AIF-deficient cells.

Despite its name and its ability to induce chromatinolysis in the nucleus, AIF function is not restricted to apoptosis. On the contrary, it is becoming clear that AIF is involved in maintaining oxidative phosphorylation, and at least in part by regulating complex I content (Vahsen et al. 2004; Porter & Urbano 2006; Joza et al. 2009; Sevrioukova 2011). It is thus possible that in addition to inhibiting HK-II activity and/or dissociating it from mitochondria, 3-BP supports further dysregulation of energy metabolism by forcing AIF to abandon its role(s) in mitochondrial metabolism. It would thus be interesting to compare cell death events induced by 3-BP, N-HK-II and other agents shown to dissociate HK-II from mitochondria, e.g., clotrimazole (Penso & Beitner 1998; Majewski et al. 2004) and methyl jasmonate (Goldin et al. 2008).

Due to the pro-survival role of the HK-II/VDAC complex, dissociation of HK-II from mitochondria would furthermore sensitize cells to pro-apoptotic Bcl-2 family signaling, e.g., via Bax (Pastorino & Hoek 2003; Pastorino et al. 2002). Accordingly, N-HK-II and also clotrimazole, have been found to potentiate apoptosis induced in HCT116 colon cancer cells by cisplatin (30 μM) (Shulga et al. 2009). Although we did not assess displacement of HK-II, we have reported that while 3–5 μM cisplatin did not induce cell death in HCT116 cells (Berndtsson et al. 2007; Ihrlund et al. 2008), cell death (necrosis in particular) was greatly enhanced by combinations of low-dose cisplatin and 3-BP at 25 μM (Ihrlund et al. 2008). We also compared cellular effects of 3-BP and 2-deoxyglucose (2-DG), a more specific inhibitor of glycolysis as it is phosphorylated by HK but not further metabolized, and concluded that at low doses (<100 μM) 3-BP acts less as an inhibitor of glycolysis than as a double-edged sword with effects also on mitochondria, e.g., mitochondrial depolarization within 3 h that was not seen with 2-DG (Ihrlund et al. 2008).

Might 3-BP target VDAC or ANT in such a manner as to disrupt the HK/VDAC complex? Cysteines are the major alkylating targets of 3-BP, but the two cysteines of VDAC were recently shown to not be required for VDAC channel

activity, oligomerization and apoptosis (Aram et al. 2010). Furthermore, the interaction of VDAC with HK occurs within two cytosolic loops that do not contain the cysteines (Abu-Hamad et al. 2008). ANT contains three cysteine residues that are differentially critical to the opening of the MPT pore; modification of one of these, Cys-56, leads to permeabilization that cannot be blocked by anti-apoptotic Bcl-2 proteins (Halestrap et al. 2002; Costantini et al. 2000). There is, however, as yet no report of 3-BP alkylation of ANT.

Glycolysis downstream of hexokinase

Several reports show alkylation and inhibition of GAPDH by 3-BP (Dell'Antone 2009; da Silva AP et al. 2009; Ganapathy-Kanniappan et al. 2009). Cytosolic fractions from rat liver were treated with 3-BP and among the six glycolytic enzymes tested, including HK, GAPDH was by far the most affected (70% inhibition) by 150 μM 3-BP for 30 min (da Silva AP et al. 2009). In another report, HepG2 cells were first treated with 3-BP and GAPDH activity was then assessed; the K_i for this inhibition was 25 μM (Dell'Antone 2009). Boar spermatozoal GAPDH was found to be inhibited by 3-BP at 500 μM (Jones et al. 1996). Obviously, GAPDH inhibition leads to loss of both of the ATP-producing steps downstream of this enzyme. However, new non-glycolytic roles of GAPDH are emerging, e.g., as regulator of gene expression and apoptosis via the nucleus (Sirover 2005). Although these functions are regulated mainly via nitrosative modification (Sirover 2005; Hara et al. 2006), they may turn out to be of interest with respect to 3-BP. Of note, GAPDH is nitrosylated on the active site Cys-150 (Hara et al. 2006), but it remains to be seen whether this is also the site of alkylation by 3-BP (Ganapathy-Kanniappan et al. 2009).

In vitro treatment of yeast and human pyruvate kinase with 3-BP has been reported to result in alkylation of active site cysteines (Yun & Suelter 1979; Acan & Ozer 2001). Presumably, this would inactivate the kinase, but in the above mentioned study on cytosolic fractions, pyruvate kinase activity was almost doubled by 3-BP treatment (Dell'Antone 2009). There is, to our knowledge, no cellular study on 3-BP effects on pyruvate kinase.

Post-glycolytic targets

Pyruvate dehydrogenase and lactate dehydrogenase

Pyruvate is the end-product of glycolysis, and is normally then converted by the pyruvate dehydrogenase complex (PDC) into acetyl-CoA for further metabolization in the Krebs' cycle. A major metabolic difference between normal and cancer cells is that in the latter, PDC activity is often inhibited by pyruvate dehydrogenase kinase (PDK), and pyruvate is then instead converted by lactate dehydrogenase (LDH) into lactate. Normal oxidative metabolism is thus suppressed (Ralph et al. 2010). Restoration of oxidative metabolism by inhibition of PDK, and/or stimulation of PDC activity, has been suggested as a possible therapeutic strategy; the pyruvate mimetic dichloroacetate which inhibits PDK1-4 is likely the best example of this (Bonnet et al. 2007; Pan et al. 2007; Papandreou et al. 2011). Although 3-BP is a pyruvate analog, it does not appear to have a similar effect as dichloroacetate; in a cell-free system, 3-BP (6 μM) inhibited pyruvate dehydrogenase (PDH), the first catalytic component of the PDC (Korotchkina 1999). 3-BP has also been used as an inhibitor of PDH in intact hamster spermatozoa (Kumar et al. 2008). It is unclear whether this inhibition is due to alkylation or to competitive titration of endogenous pyruvate. Either way, inhibition of PDH does not explain the anticancer efficacy of 3-BP since it would support rather than counteract the tumor cell metabolic phenotype. To our knowledge, there are no reports on 3-BP and PDK.

In line with being a pyruvate mimetic, 3-BP was found to be a substrate for LDH (Dell'Antone 2009). Based on the low affinity and the fact that NAD^+ is produced, the same study argues that the production of 3-bromolactate is not critically involved in 3-BP antiproliferative effects (Dell'Antone 2009). However, it might be speculated that this effect could have a role in vivo for at least some tumors, since an ensuing decrease in lactate export would affect neighboring tumor cells dependent on lactate for energy (Sonveaux et al. 2008).

Other mitochondrial targets

Isolated mitochondria treated with 1.2 mM 3-BP showed rapid inhibition of ADP-driven oxygen consumption in the presence of succinate, as assessed in a Clark electrode (Ko et al. 2001). In line with this, inhibition of succinate dehydrogenase (SDH), i.e., complex II of the electron transport chain but also part of the citric acid cycle, and of succinate-driven ATP production has been reported as important for 3-BP-induced loss of ATP and induction of cytotoxicity (Dell'Antone 2009; da Silva AP et al. 2009). Because inhibition of SDH in the citric acid cycle would also impair glutaminolysis—a major source of energy and building-blocks in tumor cells—this effect may be important for the tumor specificity of 3-BP (Dell'Antone 2009).

Likely depending on molecular mechanism, inhibition of SDH/Complex II may lead to induction of reactive oxygen species (ROS), more specifically superoxide, and to tumor cell death, as exemplified by α-tocopheryl succinate (Neuzil et al. 2007). It is not known if 3-BP, similar to this agent, acts by displacing ubiquinone binding in SDH, but ROS induction by 3-BP has indeed been reported (Ganapathy-Kanniappan et al. 2010a; Kim et al. 2008; Qin et al. 2010).

There are no reports on 3-BP acting on complex III, which is generally seen as a major source of ROS.

Within 15 min of treatment with 100 μM 3-BP, two hepatoma cell lines showed ROS induction, as determined by CM-H_2DCFDA staining and flow cytometry, and within 1–6 h necrosis ensued (Kim et al. 2008). Although the cell line with lower expression of HK-II was, as expected, more resistant to 50–200 μM 3-BP over 72 h, ROS induction was higher in these cells (Kim et al. 2008). This might be explained by HK-II displacement being a key effect of 3-BP but per se not leading to ROS induction, as shown using the N-HK-II peptide (Chen et al. 2009).

Another study reports the opposite result; after treatment with 200 μM 3-BP for 0.5–6 h, two resistant melanoma cell lines showed no induction of superoxide, as assessed by dihydroethidium staining, and a 20% decrease in ATP, while sensitive cells showed 2–3 fold superoxide induction after 3 h (Qin et al. 2010). However, these cells were nearly completely depleted of ATP within 1 h, i.e., before superoxide could be detected (Qin et al. 2010), suggesting that inhibition of complex II was not a primary cause of superoxide formation. Although HK-II levels were not determined, the same study also showed that glutathione levels were critical determinants for the effect of 3-BP, and indeed that 3-BP depleted the cells of this important thiol (Qin et al. 2010).

The affinity of 3-BP to thiols should be kept in mind when agents such as NAC (N-acetylcysteine) and DTT (dithiothreitol) are used to assess the role of ROS in 3-BP-induced effects. NAC was found to prevent cell death induced by 25–200 μM BP in different cancer cell lines (Ihrlund et al. 2008; Qin et al. 2010); similarly, also DTT (Dell'Antone 2006), but this may to a significant degree be due to mere titration of 3-BP. To obtain unequivocal data on the role of ROS in cell death induction by 3-BP therefore requires further investigation.

Degradation of cytochrome c, possibly as a consequence of oxidative damage, was observed in glioblastoma cells treated with 3-BP (60–80 μM, 18 h). The outcome was autophagic rather than apoptotic or necrotic cell death (Macchioni et al. 2011). Complex V, or the β-ATP synthetase/ATPase which either phosphorylates ADP to ATP, or consumes ATP when required to pump out protons from the mitochondrial matrix, could be another putative target of 3-BP. However, it was reported that in submitochondrial particles, proton pumping was not affected by 3-BP (500 μM), and the ATPsynthase function was suggested not to be affected either (Dell'Antone 2009).

Other targets and mechanisms

Uptake of the dye acridine orange into acidic organelles, and acidification of lysosomes, were inhibited by 3-BP (20–500 μM), indicating inhibition of vacuolar H+ATPase, likely via modification of key cysteines (Dell'Antone 2006). The resulting dissipation of pH gradients was found to precede ATP depletion and could be important as a determinant of more general toxicity (Dell'Antone 2006).

While massive depletion of ATP leads to necrosis, a moderate decrease leads to induction of cellular autophagy, a common cancer cell strategy to overcome situations of energetic or metabolic stress (White & DiPaola 2009). Autophagy induction by 3-BP has been reported, but its role in the overall cytotoxicity is not clear (Ganapathy-Kanniappan et al. 2010b). Given the ability of 3-BP to interfere with thiols and to induce ROS, oxidative damage and loss of ATP, it is perhaps not surprising that it in addition induces endoplasmic reticulum stress (unfolded protein response) (Ganapathy-Kanniappan et al. 2010b).

Although induction of ROS is known to cause DNA damage, treatment with 3-BP (100–500 μM; 4 h) did not induce phosphorylation of H2AX (γ-H2AX), nor activation of ATM, two well-known markers of DNA strand breaks. On the contrary, 3-BP decreased endogenous γ-H2AX and ATM activation, and interestingly, dichloroacetate had the opposite effect (Zhao et al. 2007). To explain this, the authors propose that reduced aerobic metabolism, i.e., some effect on mitochondrial respiration, as induced by 3-BP protects against oxidative damage to DNA (Zhao et al. 2007). This needs further investigation to be confirmed, however, especially in view of demonstrated induction of ROS (Kim et al. 2008).

Given its reactivity as alkylator, 3-BP would be expected to alkylate not only proteins but also DNA and RNA, similar to, e.g., cisplatin. No such DNA damage or DNA/RNA alkylation have been reported, however.

Combinations with chemotherapeutic drugs

Metabolism-targeted drugs may turn out to be especially efficient in combination with standard chemotherapy. A chemopotentiating potential of 3-BP has been shown in combination with platinum drugs (Berndtsson et al. 2007; Ihrlund et al. 2008) and with 5-fluorouracil (Sanchez-Arago & Cuezva 2011). The mechanism(s) of potentiation are not known, but loss of ATP would affect a number of processes, e.g., efficient DNA repair or the function of efflux pumps of the ABC transporter type, a major factor of chemoresistance. Accordingly, 3-BP (50 μM) led to a decrease in ATP that was proposed to promote intracellular retention of daunorubicin and mitoxantrone by inhibiting the transporters (Nakano et al. 2011). This would then contribute to 3-BP-mediated potentiation of doxorubicin in a xenograft model (Nakano et al. 2011). A recent study supports these findings by demonstrating the role of higher absolute levels of intracellular ATP for chemoresistance, and that 3-BP reverted such resistance (Zhou et al. 2011).

Mice expressing the RIP1-Tag2 transgene developed multistage pancreatic carcinoma, and were treated intraperitoneally with 3-BP and in combination with the Hsp90 inhibitor geldanamycin. While 3-BP alone had no effect at all on tumor volume, the combination elicited 90% inhibition of tumor growth, and also of metastasis. This was attributed to reduced levels of VEGF and AKT in response to geldanamycin combined with the energy starvation and loss of anti-survival pathways elicited by 3-BP (Cao et al. 2008).

We have shown that in HCT116 colon cancer cells, 3-BP at 25 μM potentiated cell death induced by 5 μM cisplatin or 5 μM oxaliplatin, doses which otherwise have only little effect in these cells (Ihrlund et al. 2008). As 3-BP had a greater decreasing effect on ATP levels than did the glycolysis inhibitor 2-deoxyglucose, it was perhaps not surprising that ATP-dependent apoptosis was not the main outcome, but rather massive necrosis evident at 48 h of treatment (Ihrlund et al. 2008). Loss of p53 function is known to contribute to chemoresistance, in particular to DNA-damaging agents such as platinum drugs, wherefore it is of interest that potentiation was observed also in HCT116 cells lacking the p53 gene (Ihrlund et al. 2008).

Furthermore, we have recently created cisplatin-resistant subclones of ovarian cancer cell lines by long-term treatment schedules with increasing doses of cisplatin. One of these cell lines, SKOV-3, lacks p53 expression due to an N-terminal stop codon, and is per se fairly resistant to cisplatin (IC_{50} over 72 h: 5 μM). In our cisplatin-resistant subclone, the IC_{50} had increased to 25 μM (manuscript in progress), and we therefore wished to test whether 3-BP could reverse this resistance. Since successful chemotherapeutic treatment is defined by absence of tumor regrowth, cisplatin-resistant SKOV-3R cells were treated with cisplatin and 3-BP for 48 h, and were then given the chance to recuperate in fresh, drug-free medium during 2–3 days post-treatment. The results show that, similar to low-dose cisplatin (2.5–5 μM), 3-BP at 10 μM had little effect per se on the cisplatin-resistant cells, both at 48 h and after an additional 48 h in drug-free medium. By contrast, the combination of 10 μM 3-BP with low-dose cisplatin led to massive cell death and completely prevented re-growth of any survivors (Fig. 1).

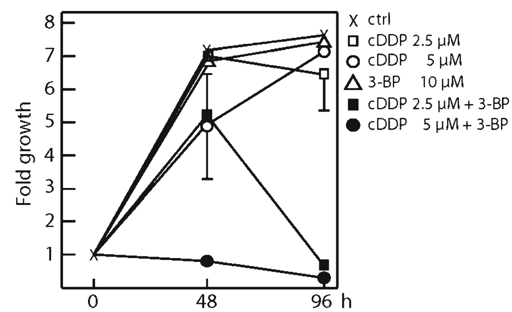

Fig. 1 3-BP prevents tumor cell regrowth in combination with low-dose Cisplatin. SKOV-3 ovarian cancer cells resistant to cisplatin (cDDP; IC_{50} 25 μM) were treated with low-dose cisplatin alone (2.5 and 5 μM) or in combination with 3-BP (10 μM) for 48 h, after which time all media were exchanged for fresh, drug-free medium. Cells were then allowed to recuperate for another 48 h. Growth was assessed as fold increase in total cellular protein at 48 h and 96 h compared to the level at 0 h. Cellular protein was quantified using the sulphorhodamine B assay (Sigma-Aldrich). Error bars represent S.E.M. If absent, they were smaller than the symbol

Tumor specificity, in vivo studies and toxicity

The tumor specificity of 3-BP and the lack of systemic toxicity in animal models is generally attributed to tumor-specific upregulation of, and dependence on, HK-II and glycolysis (Mathupala et al. 2009). Accordingly, compared to hepatocarcinoma cells, normal hepatocytes did not show reduced ATP levels in response to treatment with 3-BP (Ko et al. 2004). Other explanations include tumor-specific uptake of 3-BP via pyruvate-lactate transporters (Dell'Antone 2009; Ko et al. 2001). Of these, MCT1 and 4 are often upregulated in cancer cells, where they are of key importance for regulating intracellular pH (Pinheiro et al. 2009; Pinheiro et al. 2010; Chiche et al. 2009). MCT1 expression is promoted by loss of p53 (Le Floch et al. 2011; Boidot et al. 2011). Altogether, these findings thus support the idea that the tumor specificity of 3-BP is intimately linked to at least two types of tumor-specific protein.

Mutations in KRAS and BRAF are other common oncogenic alterations in human cancer; 3-BP (110 μM, 3 days in vitro) was found to prevent colony formation preferentially in cells with these mutations, and to significantly decrease tumor growth of such cells in a xenograft model (Yun et al. 2009). It would be interesting to examine in particular MCT in these models, but also HK-II, and also in the other in vivo models outlined below.

The rabbit VX2 model of implanted liver cancer was first used to demonstrate the in vivo anti-tumor effect of 3-BP (Ko et al. 2001). In this study, 3-BP was injected at 0.5 mM (25 mL) into the hepatic artery, resulting in massive loss of tumor burden and with low or no toxic side effects (Ko et al. 2001). The model has been used in several studies, e.g., to assess 3-BP effects on glucose uptake, and thus in effect on glycolysis, using FDG-PET (Liapi et al. 2011). This study showed a rapid inhibitory effect on uptake (within hours) of intraarterially administered 3-BP (1.75 mM; 25 mL) and substantial tumor necrosis after 1 week; there was, however, no reduction in tumor volume over the same period (Liapi et al. 2011). Longer times may be required, since with the same tumor model and 3-BP dosage, treated animals showed considerably better survival than controls over 2 months; death was most commonly due to lung metastases

(Vali et al. 2008). When intravenous and intraarterial administration were compared (3-BP at 1.75 mM; 25 mL), the latter route of delivery resulted in a better biodistribution profile (Vali et al. 2008). In addition, intraarterial administration reduces the risk of systemic toxicity. Nevertheless, higher intraarterial doses (5–25 mM, ×25 mL) were lethal to rabbits, with toxicity to the gastrointestinal tract (Chang et al. 2007).

A rat model of hepatocellular carcinoma either as an ascitic or a solid cancer has also been used to demonstrate the effects of 3-BP (Geschwind et al. 2002; Ko et al. 2004). The animals were treated intraperitoneally (ascitic model) or locally (solid subcutaneous tumors) with 2 mM 3-BP (1 mL) repeatedly over several days, again with complete eradication of tumors and no side toxicity (Geschwind et al. 2002; Ko et al. 2004). It may be noted in this context of hepatic tumors that the liver isoform of hexokinase, glucokinase, was reported to be more sensitive to 3-BP than the more tumor-specific HK-II that is usually regarded as the prime target of 3-BP (da Silva AP et al. 2009).

A rat model of breast cancer was used for a dose-escalation study (Buijs et al. 2009). Here, the rats were injected intravenously with 2.5 mL 3-BP at 5–50 mM. With 30–50 mM, all animals died within 15 min, whereas no deaths occurred with doses under 20 mM. Therefore, 15 mM was chosen for antitumor studies, which showed greatly decreased FDG uptake in the tumors of treated animals, but not in healthy tissue, thus supporting the tumor specificity of 3-BP action. However, increased, rather than decreased, FDG uptake was observed in liver and kidney tissue; this was proposed to be due to energy-demanding involvement of these tissues in the clearance of 3-BP (Buijs et al. 2009). This implies that 3-BP affected mitochondrial production of ATP in these normal cells which then responded with a temporary upregulation of glucose uptake to fuel glycolysis.

Other models include a mouse model of spontaneous pancreatic carcinoma, in which the animals were treated intraperitoneally twice weekly with 5 mg/kg 3-BP, with no reported toxicity (Cao et al. 2008). As mentioned, this treatment did not affect tumor volume at all (Cao et al. 2008). In a recent study, SCID mice with highly aggressive implanted lymphoma were also treated intraperitoneally with 3-BP (10 mg/kg), resulting in significant reduction in tumor growth in animals treated daily for 7 days, with approx 20% weight loss compared to controls as the only reported side effect (Schaefer et al. 2012).

Toxic effects of 3-BP have been reported for some types of non-tumor cells in vitro. The effects on HK, GAPDH, SDH and vacuolar H^+ ATPase mentioned above were observed in rat liver mitochondria and in normal rat thymocytes (Dell'Antone 2009); it was indeed proposed that above 20–30 μM, 3-BP may lead to severe toxic side effects (Dell'Antone 2006). Direct effects on highly ATP-dependent spermatozoa have been reported (Jones et al. 1996; Kumar et al. 2008), and on similarly ATP-dependent kidney proximal tubule cells (Jones et al. 1996); these findings are in line with effects on mitochondria and with increased uptake of FDG in liver and kidney (Vali et al. 2008; Buijs et al. 2009). Clearly, more investigation of 3-BP effects on normal cells is warranted.

Concluding remarks

Many direct and indirect targets of 3-BP have thus been reported. Although some of them have been investigated only in normal cells, and others only in tumor cells, most, if not all, of them are involved in energy metabolism. However, their respective role(s) in the anticancer effect and the toxic effects of 3-BP need to be further investigated. The many targets may also allow 3-BP to as it were lay siege to cancer cells: since these can adapt to various environmental and energy-related challenges, e.g., can switch between glycolytic, glutaminolytic and oxidative metabolism (Smolkova et al. 2010) and can use autophagy to overcome metabolic stress (White & DiPaola 2009), an efficient drug should target each of these escape routes.

Furthermore, the total antitumoral effect of 3-BP in vivo may include as yet unexamined secondary effects on the complex tumoral environment, e.g., via normalization of extracellular pH. Increased extracellular pH can have several effects, e.g., it supports the activity of chemotherapeutic drugs that are mildly basic and reduces metastasis (McCarty & Whitaker 2010). Another secondary effect of 3-BP could be to decrease extracellular lactate levels leading to starvation of neighboring lactate-dependent tumor or stromal cells. Indeed, the in vivo anticancer effect of inhibition of lactate uptake combined with inhibition of glycolysis may be considerable (Sonveaux et al. 2008).

As suggested above, the antitumor efficacy of 3-BP as single agent in some reports might in part be due to an extreme sensitivity of hepatical tumors to the drug. If other tumor types turn out to be less sensitive, this does not preclude the possible use of 3-BP in combinations with, e.g., standard chemotherapeutic drugs. As we have discussed earlier (Berndtsson et al. 2007; Ihrlund et al. 2008), any potentiating or synergistic effect of 3-BP with such drugs could potentially both decrease the necessary dose of chemotherapeutic drug and increase the tumor specificity of the treatment.

Although 3-BP is often presented as a pyruvate mimetic, its effects appear to be mediated mainly by its alkylating properties, notably towards thiols. In addition to the metabolism-modulating effects of the drug, 3-BP titrates glutathione (Qin et al. 2010), the major cellular thiol; this

may be of major importance for some of its chemopotentiating effects. However, considering the importance of thiols in many enzymes, the affinity for thiols increases the risk for toxic side effects. Thus, the pharmacokinetics and other in vivo properties of 3-BP need to be further investigated. Possible toxicities must also be further examined both in cell culture and in vivo. Optimal means of administration must be determined; if low side toxicity turns out to depend on low drug uptake from blood vessels during systemic treatment, perhaps future nanoparticle technology may be able to overcome this problem.

It is in the context of toxicity versus efficacy interesting to compare 3-BP with cisplatin—a clinically very important small-molecule drug (MW 301.1) that creates DNA adducts but whose total cytotoxic effects involve its high reactivity towards protein thiols, effects on mitochondria and induction of ROS (Goodisman et al. 2006; Custodio et al. 2009; Rybak et al. 2007) and which via some such mechanism is toxic to normal, non-dividing inner ear cells and kidney tubule cells (Custodio et al. 2009; Rybak 2007; Kruidering et al. 1997; Santos et al. 2007). The latter cells have been reported to be sensitive to 3-BP, and 3-BP was also found to induce diuresis (Jones et al. 1996). It may thus be important to assess both ototoxicity and nephrotoxicity of 3-BP.

In summary, the hitherto impressive tumor-specific efficacy of 3-BP, its promise as a chemopotentiating agent and its as yet unclear pharmacokinetics and possible side effects together give reason to be both optimistic and cautious about the future of 3-BP.

Acknowledgements The author is grateful to My Björklund, Lina Löfstedt and Srikanth Sathu for discussions and technical assistance, and to the Swedish Cancer Society, the Radiumhemmet Research Funds and the foundations Goljes Minne and Olle Engkvist Byggmästare for financial support.

References

Apfel MA, Ikeda BH, Speckhard DC, Frey PA (1984) Escherichia coli pyruvate dehydrogenase complex. Thiamin pyrophosphate-dependent inactivation by 3-bromopyruvate. J Biol Chem 259:2905–2909

Banas T, Gontero B, Drews VL, Johnson SL, Marcus F, Kemp RG (1988) Reactivity of the thiol groups of Escherichia coli phosphofructo-1-kinase. Biochim Biophys Acta 957:178–184

Mathupala SP, Ko YH, Pedersen PL (2009) Hexokinase-2 bound to mitochondria: cancer's stygian link to the "Warburg Effect" and a pivotal target for effective therapy. Semin Cancer Biol 19:17–24

Dell'Antone P (2009) Targets of 3-bromopyruvate, a new, energy depleting, anticancer agent. Med Chem 5:491–496

Ganapathy-Kanniappan S, Vali M, Kunjithapatham R, Buijs M, Syed LH, Rao PP, Ota S, Kwak BK, Loffroy R, Geschwind JF (2010a) 3-bromopyruvate: a new targeted antiglycolytic agent and a promise for cancer therapy. Curr Pharm Biotechnol 11:510–517

da Silva AP Pereira, El-Bacha T, Kyaw N, dos Santos RS, da-Silva WS, Almeida FC, Da Poian AT, Galina A (2009) Inhibition of energy-producing pathways of HepG2 cells by 3-bromopyruvate. Biochem J 417:717–726

Dell'Antone P (2006) Inactivation of H+-vacuolar ATPase by the energy blocker 3-bromopyruvate, a new antitumour agent. Life Sci 79:2049–2055

Geschwind JF, Ko YH, Torbenson MS, Magee C, Pedersen PL (2002) Novel therapy for liver cancer: direct intraarterial injection of a potent inhibitor of ATP production. Cancer Res 62:3909–3913

Ko YH, Pedersen PL, Geschwind JF (2001) Glucose catabolism in the rabbit VX2 tumor model for liver cancer: characterization and targeting hexokinase. Cancer Lett 173:83–91

Ko YH, Smith BL, Wang Y, Pomper MG, Rini DA, Torbenson MS, Hullihen J, Pedersen PL (2004) Advanced cancers: eradication in all cases using 3-bromopyruvate therapy to deplete ATP. Biochem Biophys Res Commun 324:269–275

Mathupala SP, Ko YH, Pedersen PL (2006) Hexokinase II: cancer's double-edged sword acting as both facilitator and gatekeeper of malignancy when bound to mitochondria. Oncogene 25:4777–4786

Mathupala SP, Ko YH, Pedersen PL (2010) The pivotal roles of mitochondria in cancer: Warburg and beyond and encouraging prospects for effective therapies. Biochim Biophys Acta 1797:1225–1230

Shoshan-Barmatz V, De Pinto V, Zweckstetter M, Raviv Z, Keinan N, Arbel N (2010) VDAC, a multi-functional mitochondrial protein regulating cell life and death. Mol Aspects Med 31:227–285

Ramsay EE, Hogg PJ, Dilda PJ (2011) Mitochondrial metabolism inhibitors for cancer therapy. Pharm Res 28:2731–2744

Pastorino JG, Hoek JB (2003) Hexokinase II: the integration of energy metabolism and control of apoptosis. Curr Med Chem 10:1535–1551

Pelicano H, Martin DS, Xu RH, Huang P (2006) Glycolysis inhibition for anticancer treatment. Oncogene 25:4633–4646

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. Cell 144:646–674

Tennant DA, Duran RV, Gottlieb E (2010) Targeting metabolic transformation for cancer therapy. Nat Rev Cancer 10:267–277

Shoshan-Barmatz V, Ben-Hail D: VDAC, a multi-functional mitochondrial protein as a pharmacological target, Mitochondrion 2011,

Chen Z, Zhang H, Lu W, Huang P (2009) Role of mitochondria-associated hexokinase II in cancer cell death induced by 3-bromopyruvate. Biochim Biophys Acta 1787:553–560

Kim JS, Ahn KJ, Kim JA, Kim HM, Lee JD, Lee JM, Kim SJ, Park JH (2008) Role of reactive oxygen species-mediated mitochondrial dysregulation in 3-bromopyruvate induced cell death in hepatoma cells: ROS-mediated cell death by 3-BrPA. J Bioenerg Biomembr 40:607–618

Vahsen N, Cande C, Briere JJ, Benit P, Joza N, Larochette N, Mastroberardino PG, Pequignot MO, Casares N, Lazar V, Feraud O, Debili N, Wissing S, Engelhardt S, Madeo F, Piacentini M, Penninger JM, Schagger H, Rustin P, Kroemer G (2004) AIF deficiency compromises oxidative phosphorylation. EMBO J 23:4679–4689

Porter AG, Urbano AG (2006) Does apoptosis-inducing factor (AIF) have both life and death functions in cells? Bioessays 28:834–843

Joza N, Pospisilik JA, Hangen E, Hanada T, Modjtahedi N, Penninger JM, Kroemer G (2009) AIF: not just an apoptosis-inducing factor. Ann N Y Acad Sci 1171:2–11

Sevrioukova IF (2011) Apoptosis-inducing factor: structure, function, and redox regulation. Antioxid Redox Signal 14:2545–2579

Penso J, Beitner R (1998) Clotrimazole and bifonazole detach hexokinase from mitochondria of melanoma cells. Eur J Pharmacol 342:113–117

Majewski N, Nogueira V, Bhaskar P, Coy PE, Skeen JE, Gottlob K, Chandel NS, Thompson CB, Robey RB, Hay N (2004) Hexokinase-mitochondria interaction mediated by Akt is required

to inhibit apoptosis in the presence or absence of Bax and Bak. Mol Cell 16:819–830

Goldin N, Arzoine L, Heyfets A, Israelson A, Zaslavsky Z, Bravman T, Bronner V, Notcovich A, Shoshan-Barmatz V, Flescher E (2008) Methyl jasmonate binds to and detaches mitochondria-bound hexokinase. Oncogene 27:4636–4643

Pastorino JG, Shulga N, Hoek JB (2002) Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome c release and apoptosis. J Biol Chem 277:7610–7618

Shulga N, Wilson-Smith R, Pastorino JG (2009) Hexokinase II detachment from the mitochondria potentiates cisplatin induced cytotoxicity through a caspase-2 dependent mechanism. Cell Cycle 8:3355–3364

Berndtsson M, Hagg M, Panaretakis T, Havelka AM, Shoshan MC, Linder S (2007) Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA. Int J Cancer 120:175–180

Ihrlund LS, Hernlund E, Khan O, Shoshan MC (2008) 3-bromopyruvate as inhibitor of tumour cell energy metabolism and chemopotentiator of platinum drugs. Molecular Oncology 2:94–101

Aram L, Geula S, Arbel N, Shoshan-Barmatz V (2010) VDAC1 cysteine residues: topology and function in channel activity and apoptosis. Biochem J 427:445–454

Abu-Hamad S, Zaid H, Israelson A, Nahon E, Shoshan-Barmatz V (2008) Hexokinase-I protection against apoptotic cell death is mediated via interaction with the voltage-dependent anion channel-1: mapping the site of binding. J Biol Chem 283:13482–13490

Halestrap AP, McStay GP, Clarke SJ (2002) The permeability transition pore complex: another view. Biochimie 84:153–166

Costantini P, Belzacq AS, Vieira HL, Larochette N, de Pablo MA, Zamzami N, Susin SA, Brenner C, Kroemer G (2000) Oxidation of a critical thiol residue of the adenine nucleotide translocator enforces Bcl-2-independent permeability transition pore opening and apoptosis. Oncogene 19:307–314

Ganapathy-Kanniappan S, Geschwind JF, Kunjithapatham R, Buijs M, Vossen JA, Tchernyshyov I, Cole RN, Syed LH, Rao PP, Ota S, Vali M (2009) Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is pyruvylated during 3-bromopyruvate mediated cancer cell death. Anticancer Res 29:4909–4918

Jones AR, Porter KE, Dobbie MS (1996) Renal and spermatozoal toxicity of alpha-bromohydrin, 3-bromolactate and 3-bromopyruvate. J Appl Toxicol 16:57–63

Sirover MA (2005) New nuclear functions of the glycolytic protein, glyceraldehyde-3-phosphate dehydrogenase, in mammalian cells. J Cell Biochem 95:45–52

Hara MR, Cascio MB, Sawa A (2006) GAPDH as a sensor of NO stress. Biochim Biophys Acta 1762:502–509

Yun SL, Suelter CH (1979) Modification of yeast pyruvate kinase by an active site-directed reagent, bromopyruvate. J Biol Chem 254:1811–1815

Acan NL, Ozer N (2001) Modification of human erythrocyte pyruvate kinase by an active site-directed reagent: bromopyruvate. J Enzyme Inhib 16:457–464

Ralph SJ, Rodriguez-Enriquez S, Neuzil J, Moreno-Sanchez R (2010) Bioenergetic pathways in tumor mitochondria as targets for cancer therapy and the importance of the ROS-induced apoptotic trigger. Mol Aspects Med 31:29–59

Bonnet S, Archer SL, Allalunis-Turner J, Haromy A, Beaulieu C, Thompson R, Lee CT, Lopaschuk GD, Puttagunta L, Harry G, Hashimoto K, Porter CJ, Andrade MA, Thebaud B, Michelakis ED (2007) A mitochondria-K+channel axis is suppressed in cancer and its normalization promotes apoptosis and inhibits cancer growth. Cancer Cell 11:37–51

Pan JG, Mak TW: Metabolic targeting as an anticancer strategy: dawn of a new era?, Sci STKE 2007, 2007:pe14

Papandreou I, Goliasova T, Denko NC (2011) Anticancer drugs that target metabolism: Is dichloroacetate the new paradigm? Int J Cancer 128:1001–1008

Korotchkina LG (1999) Showkat Ali M, Patel MS: Involvement of alpha-cysteine-62 and beta-tryptophan-135 in human pyruvate dehydrogenase catalysis. Arch Biochem Biophys 369:277–287

Kumar V, Kota V, Shivaji S (2008) Hamster sperm capacitation: role of pyruvate dehydrogenase A and dihydrolipoamide dehydrogenase. Biol Reprod 79:190–199

Sonveaux P, Vegran F, Schroeder T, Wergin MC, Verrax J, Rabbani ZN, De Saedeleer CJ, Kennedy KM, Diepart C, Jordan BF, Kelley MJ, Gallez B, Wahl ML, Feron O, Dewhirst MW (2008) Targeting lactate-fueled respiration selectively kills hypoxic tumor cells in mice. J Clin Invest 118:3930–3942

Neuzil J, Dyason JC, Freeman R, Dong LF, Prochazka L, Wang XF, Scheffler I, Ralph SJ (2007) Mitocans as anti-cancer agents targeting mitochondria: lessons from studies with vitamin E analogues, inhibitors of complex II. J Bioenerg Biomembr 39:65–72

Qin JZ, Xin H, Nickoloff BJ (2010) 3-Bromopyruvate induces necrotic cell death in sensitive melanoma cell lines. Biochem Biophys Res Commun 396:495–500

Macchioni L, Davidescu M, Sciaccaluga M, Marchetti C, Migliorati G, Coaccioli S, Roberti R, Corazzi L, Castigli E (2011) Mitochondrial dysfunction and effect of antiglycolytic bromopyruvic acid in GL15 glioblastoma cells. J Bioenerg Biomembr 43:507–518

White E, DiPaola RS (2009) The double-edged sword of autophagy modulation in cancer. Clin Cancer Res 15:5308–5316

Ganapathy-Kanniappan S, Geschwind JF, Kunjithapatham R, Buijs M, Syed LH, Rao PP, Ota S, Kwak BK, Loffroy R, Vali M (2010b) 3-Bromopyruvate induces endoplasmic reticulum stress, overcomes autophagy and causes apoptosis in human HCC cell lines. Anticancer Res 30:923–935

Zhao H, Tanaka T, Halicka HD, Traganos F, Zarebski M, Dobrucki J, Darzynkiewicz Z (2007) Cytometric assessment of DNA damage by exogenous and endogenous oxidants reports aging-related processes. Cytometry A 71:905–914

Sanchez-Arago M, Cuezva JM (2011) The bioenergetic signature of isogenic colon cancer cells predicts the cell death response to treatment with 3-bromopyruvate, iodoacetate or 5-fluorouracil. J Transl Med 9:19

Nakano A, Tsuji D, Miki H, Cui Q, Sayed SM, Ikegame A, Oda A, Amou H, Nakamura S, Harada T, Fujii S, Kagawa K, Takeuchi K, Sakai A, Ozaki S, Okano K, Nakamura T, Itoh K, Matsumoto T, Abe M (2011) Glycolysis Inhibition Inactivates ABC Transporters to Restore Drug Sensitivity in Malignant Cells. PLoS One 6:e27222

Zhou Y, Tozzi F, Chen J, Fan F, Xia L, Wang J, Gao G, Zhang A, Xia X, Brasher H, Widger W, Ellis LM, Weihua Z: Intracellular ATP levels are a pivotal determinant of chemoresistance in colon cancer cells, Cancer Res 2011

Cao X, Jia G, Zhang T, Yang M, Wang B, Wassenaar PA, Cheng H, Knopp MV, Sun D (2008) Non-invasive MRI tumor imaging and synergistic anticancer effect of HSP90 inhibitor and glycolysis inhibitor in RIP1-Tag2 transgenic pancreatic tumor model. Cancer Chemother Pharmacol 62:985–994

Pinheiro C, Longatto-Filho A, Pereira SM, Etlinger D, Moreira MA, Jube LF, Queiroz GS, Schmitt F, Baltazar F (2009) Monocarboxylate transporters 1 and 4 are associated with CD147 in cervical carcinoma. Dis Markers 26:97–103

Pinheiro C, Reis RM, Ricardo S, Longatto-Filho A, Schmitt F, Baltazar F (2010) Expression of monocarboxylate transporters 1, 2, and 4 in human tumours and their association with CD147 and CD44. J Biomed Biotechnol 2010:427694

Chiche J, Brahimi-Horn MC, Pouyssegur J: Tumor hypoxia induces a metabolic shift causing acidosis: a common feature in cancer, J Cell Mol Med 2009

Le Floch R, Chiche J, Marchiq I, Naiken T, Ilk K, Murray CM, Critchlow SE, Roux D, Simon MP, Pouyssegur J (2011) CD147 subunit of lactate/H+symporters MCT1 and hypoxia-inducible MCT4 is critical for energetics and growth of glycolytic tumors. Proc Natl Acad Sci U S A 108:16663–16668

Boidot R, Vegran F, Meulle A, Lebreton A, Dessy C, Sonveaux P, Lizard-Nacol S, Feron O: Regulation of monocarboxylate transporter MCT1 expression by p53 mediates inward and outward lactate fluxes in tumors, Cancer Res 2011

Yun J, Rago C, Cheong I, Pagliarini R, Angenendt P, Rajagopalan H, Schmidt K, Willson JK, Markowitz S, Zhou S, Diaz LA Jr, Velculescu VE, Lengauer C, Kinzler KW, Vogelstein B, Papadopoulos N (2009) Glucose deprivation contributes to the development of KRAS pathway mutations in tumor cells. Science 325:1555–1559

Liapi E, Geschwind JF, Vali M, Khwaja AA, Prieto-Ventura V, Buijs M, Vossen JA, Ganapathy S, Wahl RL (2011) Assessment of tumoricidal efficacy and response to treatment with 18 F-FDG PET/CT after intraarterial infusion with the antiglycolytic agent 3-bromopyruvate in the VX2 model of liver tumor. J Nucl Med 52:225–230

Vali M, Vossen JA, Buijs M, Engles JM, Liapi E, Ventura VP, Khwaja A, Acha-Ngwodo O, Shanmugasundaram G, Syed L, Wahl RL, Geschwind JF (2008) Targeting of VX2 rabbit liver tumor by selective delivery of 3-bromopyruvate: a biodistribution and survival study. J Pharmacol Exp Ther 327:32–37

Chang JM, Chung JW, Jae HJ, Eh H, Son KR, Lee KC, Park JH (2007) Local toxicity of hepatic arterial infusion of hexokinase II inhibitor, 3-bromopyruvate: In vivo investigation in normal rabbit model. Acad Radiol 14:85–92

Buijs M, Vossen JA, Geschwind JF, Ishimori T, Engles JM, Acha-Ngwodo O, Wahl RL, Vali M (2009) Specificity of the anti-glycolytic activity of 3-bromopyruvate confirmed by FDG uptake in a rat model of breast cancer. Invest New Drugs 27:120–123

Schaefer NG, Geschwind JF, Engles J, Buchanan JW, Wahl RL (2012) Systemic administration of 3-bromopyruvate in treating disseminated aggressive lymphoma. Transl Res 159:51–57

Smolkova K, Plecita-Hlavata L, Bellance N, Benard G, Rossignol R, Jezek P: Waves of gene regulation suppress and then restore oxidative phosphorylation in cancer cells, Int J Biochem Cell Biol 2010

McCarty MF, Whitaker J (2010) Manipulating tumor acidification as a cancer treatment strategy. Altern Med Rev 15:264–272

Goodisman J, Hagrman D, Tacka KA, Souid AK (2006) Analysis of cytotoxicities of platinum compounds. Cancer Chemother Pharmacol 57:257–267

Custodio JB, Cardoso CM, Santos MS, Almeida LM, Vicente JA, Fernandes MA (2009) Cisplatin impairs rat liver mitochondrial functions by inducing changes on membrane ion permeability: prevention by thiol group protecting agents. Toxicology 259:18–24

Rybak LP, Whitworth CA, Mukherjea D, Ramkumar V (2007) Mechanisms of cisplatin-induced ototoxicity and prevention. Hear Res 226:157–167

Rybak LP (2007) Mechanisms of cisplatin ototoxicity and progress in otoprotection. Curr Opin Otolaryngol Head Neck Surg 15:364–369

Kruidering M, Van de Water B, de Heer E, Mulder GJ, Nagelkerke JF (1997) Cisplatin-induced nephrotoxicity in porcine proximal tubular cells: mitochondrial dysfunction by inhibition of complexes I to IV of the respiratory chain. J Pharmacol Exp Ther 280:638–649

Santos NA, Catao CS, Martins NM, Curti C, Bianchi ML, Santos AC (2007) Cisplatin-induced nephrotoxicity is associated with oxidative stress, redox state unbalance, impairment of energetic metabolism and apoptosis in rat kidney mitochondria. Arch Toxicol 81:495–504