Case Research

Safety and outcome of treatment of metastatic melanoma using 3-bromopyruvate: a concise literature review and case study

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Abstract

3-Bromopyruvate (3BP) is a new, promising anticancer alkylating agent with several notable functions. In addition to inhibiting key glycolysis enzymes including hexokinase II and lactate dehydrogenase (LDH), 3BP also selectively inhibits mitochondrial oxidative phosphorylation, angiogenesis, and energy production in cancer cells. Moreover, 3BP induces hydrogen peroxide generation in cancer cells (oxidative stress effect) and competes with the LDH substrates pyruvate and lactate. There is only one published human clinical study showing that 3BP was effective in treating fibrolamellar hepatocellular carcinoma. LDH is a good measure for tumor evaluation and predicts the outcome of treatment better than the presence of a residual tumor mass. According to the Warburg effect, LDH is responsible for lactate synthesis, which facilitates cancer cell survival, progression, aggressiveness, metastasis, and angiogenesis. Lactate produced through LDH activity fuels aerobic cell populations inside tumors via metabolic symbiosis. In melanoma, the most deadly skin cancer, 3BP induced necrotic cell death in sensitive cells, whereas high glutathione (GSH) content made other melanoma cells resistant to 3BP. Concurrent use of a GSH depletor with 3BP killed resistant melanoma cells. Survival of melanoma patients was inversely associated with high serum LDH levels, which was reported to be highly predictive of melanoma treatment in randomized clinical trials. Here, we report a 28-year-old man presented with stage IV metastatic melanoma affecting the back, left pleura, and lung. The disease caused total destruction of the left lung and a high serum LDH level (4,283 U/L). After ethics committee approval and written patient consent, the patient received 3BP intravenous infusions (1–2.2 mg/kg), but the anticancer effect was minimal as indicated by a high serum LDH level. This may have been due to high tumor GSH content. On combining oral paracetamol, which depletes tumor GSH, with 3BP treatment, serum LDH level dropped maximally. Although a slow intravenous infusion of 3BP appeared to have minimal cytotoxicity, its anticancer efficacy via this delivery method was low. This was possibly due to high tumor GSH content, which was increased after concurrent use of the GSH depletor paracetamol. If the anticancer effectiveness of 3BP is less than expected, the combination with paracetamol may be needed to sensitize cancer cells to 3BP-induced effects.

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doi: 10.5732/cjc.013.10111

Metastatic melanoma, the most deadly skin cancer, is resistant to current treatment modalities. Melanoma energy supply is derived from glucose oxidation (glycolysis) in the tumor center and oxidative phosphorylation in the tumor periphery[1,2]. However, inhibiting glycolysis is sufficient to limit energy delivered to melanoma through

both pathways, as glucose oxidation produces enormous amounts of lactate per the Warburg effect[3]. The resultant lactate is used to fuel oxidative phosphorylation in the tumor periphery in a phenomenon called metabolic symbiosis[1,2] (**Figure 1A**). Melanoma cells exhibit the Warburg effect, as they use more glucose and produce more lactate than normal melanocytes[4]. Lactate induces acidosis of the cancer cell microenvironment and creates a toxic microenvironment for surrounding normal and susceptible cancer cells. On the other hand, cancer cells that can survive this unfavorable microenvironment thrive[5], facilitating progressive malignancy and metastasis (**Figure 1A**).

Interestingly, lactate mediates inflammatory reactions[6], enhances angiogenesis[7], and is associated with decreased patient survival, chemoresistance, radioresistance, and decreased immunity against cancer[8] (**Figure 1A**). "Lactate is the mirror and motor of malignancy"[9], facilitating cancer cell survival, progression, and distant metastasis[8]. Lactate formation is the last step in glycolysis in cancer cells and is catalyzed by lactate dehydrogenase (LDH). LDH has been reported as the most significant marker for melanoma progression and is included in the American Joint Committee on Cancer (AJCC) melanoma staging system: patients with high LDH are diagnosed with stage IV M1c melanoma[10]. Serum LDH level was reported to be highly predictive of melanoma treatment in randomized clinical trials[11].

3-Bromopyruvate (3BP) is a pyruvate and lactate analog (**Figure 1B**) that has shown antitumor activity against a number of cancers. 3BP was reported to induce necrotic cell death in sensitive melanoma cells[12] and to decrease the viability of glucocorticoid-resistant childhood acute lymphoblastic leukemia (ALL) cells[13]. Recently, 3BP was reported to treat aggressive neuroblastoma[14], as well as glioma and glioblastoma[15]. El Sayed et al.[15] reported that 3BP exerted potent anti-glioma effects by depleting glioma cell energy sources and inducing oxidative stress. There is only one published clinical study

showing the effectiveness of 3BP as a potent anticancer agent, and in that study, it was used to treat human fibrolamellar hepatocellular carcinoma (HCC)[16]. 3BP was administered via transcatheter arterial chemoembolization and induced necrotic cell death in tumor tissue as evidenced by positron emission tomography-computed tomography (PET-CT)[16]. Indeed, 3BP was reported to eradicate HCC[17]. Selectivity of 3BP towards cancer tissue has been noted in several studies[15,17,18]. 3BP was also reported to be less toxic to normal cells both *in vitro* and *in vivo*[15,17,19].

3BP has several anticancer mechanisms. It is a powerful inhibitor of angiogenesis[20] and ATP-binding cassette transporters, which efflux chemotherapeutic drugs and cause chemoresistance[19]. Furthermore, 3BP inhibits key enzymes involved in glycolysis, including hexokinase II[17], glyceraldehyde-3-phosphate dehydrogenase[21], and LDH[22]. El Sayed et al.[23] reported that 3BP antagonized the effects of lactate and pyruvate. In addition, 3BP was reported to inhibit oxidative phosphorylation[16] and induce cancer cell death by generating hydrogen peroxide and causing oxidative stress[15]. Further research is needed to explore the potential of 3BP as an anticancer agent that exploits the Warburg effect and targets critical energy pathways in cancer cells. The information gained from such work may be useful for future development of 3BP as a therapeutic option for cancer patients.

Treatments based on better understanding of the biology of melanoma may be promising. We report a 28-year-old man with stage IV metastatic melanoma who received intravenous infusions of 3BP with limited effect. However, combining 3BP with an oral glutathione (GSH) inhibitor produced a better response. Combining 3BP with paracetamol may be necessary when the initial response to 3BP is not satisfactory. Here, we provide our experience with this case, concisely review the related literature, and discuss important future directions.
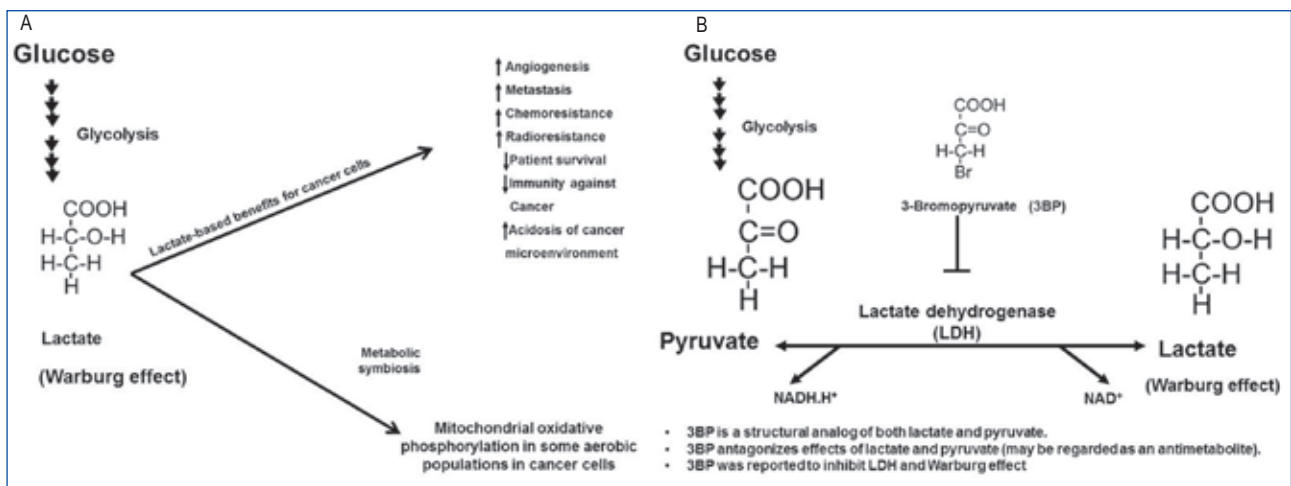
Figure 1. Glycolysis and metabolic symbiosis in cancer and melanoma metastasis. A, 3-bromopyruvate (3BP) targets critical steps in cancer cell biology. Glycolysis is the major source of energy in cancer cells. Lactate production that occurs through this process provides many benefits to cancer cells. 3BP is a structural analog of pyruvate and lactate and can be regarded as an antimetabolite. B, lactate-based benefits in cancer cells. Lactate can help oxidative phosphorylation in some aerobic portions of cancer cells and exerts important benefits to cancer cells.

Case report

A 28-year-old man weighing 60 kg presented for the first time with a hard, painless mass in his left forearm in January 2012 and was admitted to Sohag Cancer Institute. A sample was collected from the mass for biopsy, and histopathology indicated melanoma (**Figure 2A**). The mass was surgically excised, and the patient was discharged 2 weeks after admission. Regular follow-up was done monthly.

In July 2012, there was a local recurrence, where a progressively hard nodule was felt in the left forearm at the site of the excised mass (**Figure 2B**). The patient also presented with pain in the back and left chest wall. Chest X-ray and computed tomography (CT) scan revealed a progressively growing left pleural mass that caused destruction of a wide portion of the left lung, resulting in its collapse (**Figures 2C-F**) and shifting of the heart to the right side (dextrocardia). The patient was treated with bisphosphonates. Pain in the chest wall and back was intolerable, and the patient was treated with non-steroidal, anti-inflammatory drugs (ibuprofen and diclofenac), which were insufficient for pain management. As the

mass at the left chest wall continued to grow, a hard metastatic mass (5 cm × 3 cm) bulged outside of the chest wall (**Figure 2C**). Local radiotherapy, which entailed a 25-Gy total dose administered in 5-Gy fractions 5 days per week using a linear accelerator, was applied to the bulging mass.

The patient sought medical advice at the Department of Medical Oncology and Nuclear Medicine in Sohag Faculty of Medicine at Sohag University (Egypt) and was admitted September 13, 2012. Immediately after admission, initial evaluation revealed that he had dyspnea, orthopnea, hypotension (BP, 90/60 mmHg), anorexia, generalized anasarca (mostly nutritional edema), rightward shift in cardiac apical beat (dextrocardia), bulging metastatic mass through the left chest wall, and painful regions over his left chest wall and back. Laboratory evaluation confirmed hypoalbuminemia, hypoproteinemia, and anemia, along with normal renal and liver functions. The patient's whole left lung was destroyed by lung metastasis, per CT scan (**Figures 2C-F**), with no air entry on the left side. Thus, the patient was maintained on oxygen by mask when necessary. Radiologic evaluation revealed collapsed left lung, pleural
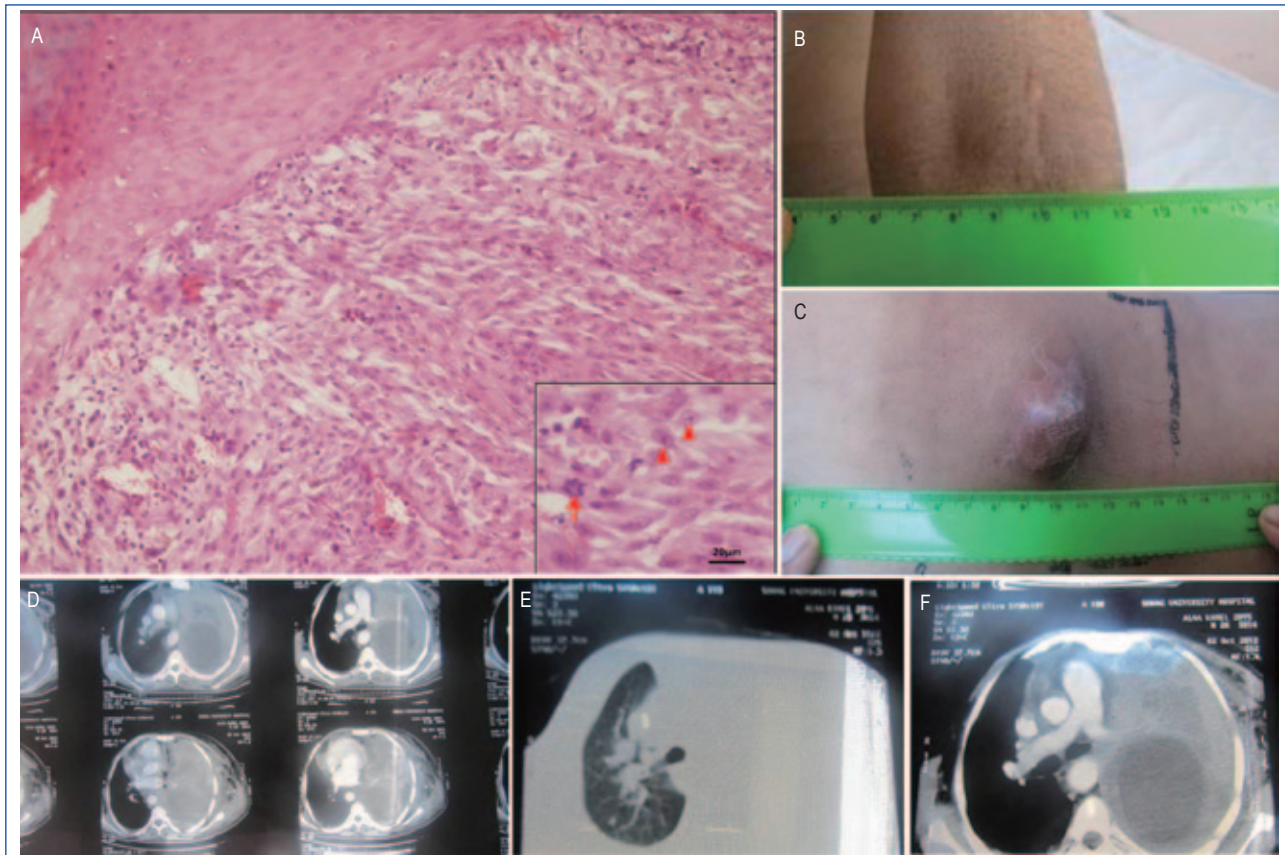
Figure 2. Melanoma metastatic to the lung and chest wall. A, histopathology of primary melanoma. Hematoxylin and eosin stained section shows infiltration of the subcutaneous tissue by sheets of atypical melanocytes with prominent nucleoli (arrow head) and abnormal mitosis (arrow). Magnifications are 100× for the main slide and 400× for the inset. **B**, scar left after excision of primary melanoma. Primary melanoma tumor presents as a hard mass (1 cm × 1 cm) at the upper part of frontal aspect of the left forearm. The mass was hard in consistency, dark reddish in color (with color variation), and shows asymmetry with irregular borders. **C–F**, metastatic melanoma to the left chest wall. **C**, bulging metastatic mass through the left chest wall for which local radiotherapy was given. **D**, computed tomography (CT) shows total destruction of the left lung caused by metastatic melanoma. **E**, chest X-ray shows that the right lung is spared, whereas the left lung is totally destroyed. **F**, CT scan shows that huge circular metastatic tumor mass occupies a major part of the left lung.

effusion on the left side, and metastatic mass on the left chest wall causing shift of the mediastinum to the right. Ultrasonography-guided aspiration failed to remove fluid, and little of the hemorrhagic effusion was aspirated. Intercostal tube was not used. The patient did not receive melanoma-related treatment but did receive supportive treatment in the form of salt-free human albumin infusion, diuretics, tonics, non-steroidal analgesics, hemostatic agents (e.g., capron), and one unit of fresh blood via transfusion.

The patient asked for treatment with new lines of chemotherapy for his current status and was accordingly informed about 3BP, including its mechanism of action and possible side effects. After receiving approval from the Ethics Committee of Sohag Faculty of Medicine and written consent from the patient according to the Declaration of Helsinki, treatment using 3BP was planned at safe, low therapeutic doses, based on previous reports and published studies of 3BP[15,16,18,24,25]. The therapeutic plan was devised to safely benefit the patient starting at lowest possible dose, which would be administered through intravenous drip infusion. This novel route for 3BP administration fractionates the calculated dose. This may be safer than direct intra-arterial injection of a bolus dose, which was reported to effectively, with minimal toxicity, treat liver tumors implanted in rabbits[25]. Further dose modification was considered in light of treatment safety and tolerability.

The patient's general condition was fair except for mild to moderate anasarca, which was partially relieved with salt-free albumin and diuretics. The patient also maintained a good urine output. With treatment, the patient had normal renal and liver functions with no orthopnea or dyspnea. Serum LDH level was tested before and during treatment with 3BP using Beckman Coulter AU analyzer through the automated clinical pathology laboratory in Sohag University Hospital (Egypt). Before treatment, serum LDH level was high, which reflects high glycolysis rate and energy metabolism in tumor and metastatic tissue[26]. Follow-up of serum LDH level indicated response to planned treatment with 3BP.

The first infusion of 3BP was administered on September 18,

2012. Based on the reference 3BP dosage range in humans (2–3.5 mg/kg body weight) reported recently by Ko *et al*.[16], the patient received 3BP at a dose of 1 mg/kg, added to 500 mL of glucose (5%), by slow-drip intravenous infusion over 2 h (**Figure 3A**). Treatment was tolerated, with no anaphylaxis or unexpected adverse events. Members of the treatment team attended beside the patient during 3BP infusion, and measures for emergency treatment were available. The only adverse event was a mild to moderate burning sensation at the infusion site, which decreased upon slowing the infusion rate. No phlebitis, local inflammatory reactions, or allergic reactions were encountered. At the end of infusion, the patient was in a good general condition, lying comfortably in bed and being able to sit, stand, and walk. He went to the toilet and his appetite improved.

The next day, results were promising, as LDH decreased moderately from 4,283 U/L to 3,126 U/L (**Figure 3B**). Both renal and liver functions were normal (**Figure 4**). No metabolic abnormalities were recorded with regard to arterial blood gases, serum glucose, or serum uric acid. Blood cellular count was within normal indices, with no evidence of hemolytic anemia (**Figure 5**). Bowel habits were normal after treatment using 3BP. Pain at the left chest wall and back was controlled with duragesic (fentanyl) trans-dermal skin patch.

Four days later, serum LDH level started to rise again and reached 4,353 U/L. The patient received a second dose of 3BP—this time, 1 mg/kg added to 500 mL normal saline (0.9%) was administered via intravenous drip infusion over 3 h. Treatment was tolerated, and the patient had little burning sensation at the infusion site compared to the first time, as the infusion rate was lower. There was no phlebitis or anaphylaxis.

Over the next 10 days, the patient received 6 doses of 3BP (1.5-2.2 mg/kg added to 500 mL normal saline, given by intravenous drip infusion) (**Figure 3A**). All laboratory evaluations revealed normal liver and renal functions (**Figure 4**), with no hematologic impairments such as neutopenia or hemolytic anemia (**Figure 5**). The patient's general condition was stable on 3BP treatment. Serum LDH level was around half the initial level at presentation but did not fall to normal range
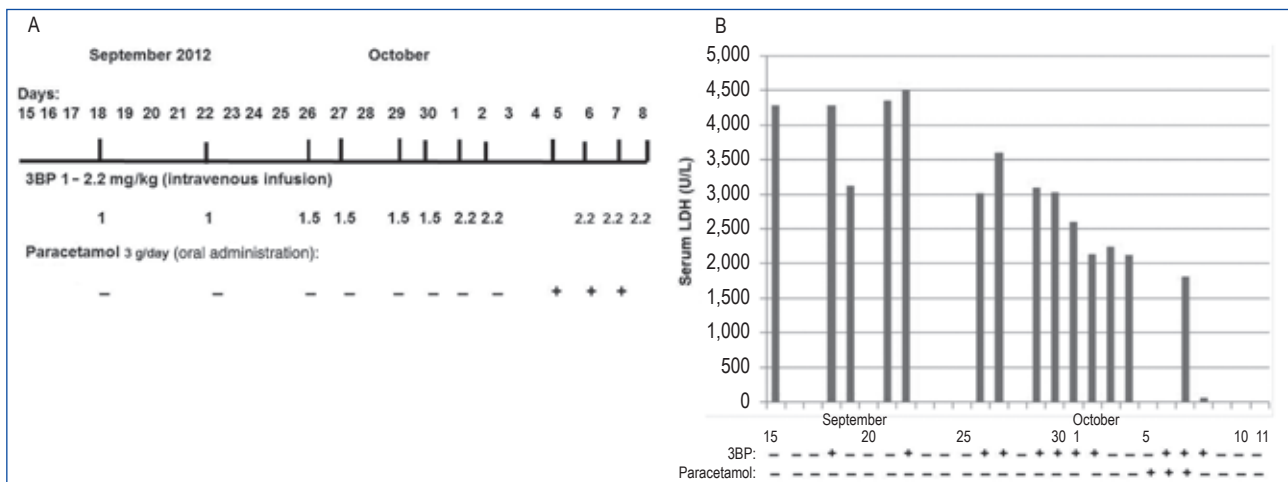
Figure 3. **Treatment using 3BP (intravenous infusion). A, treatment doses given to the patient. B, 3BP treatment caused a moderate decrease in serum lactate dehydrogenase (LDH) level, a metabolic predictor of cancer cell energy. Serum LDH level decreased maximally on combining paracetamol and 3BP.**
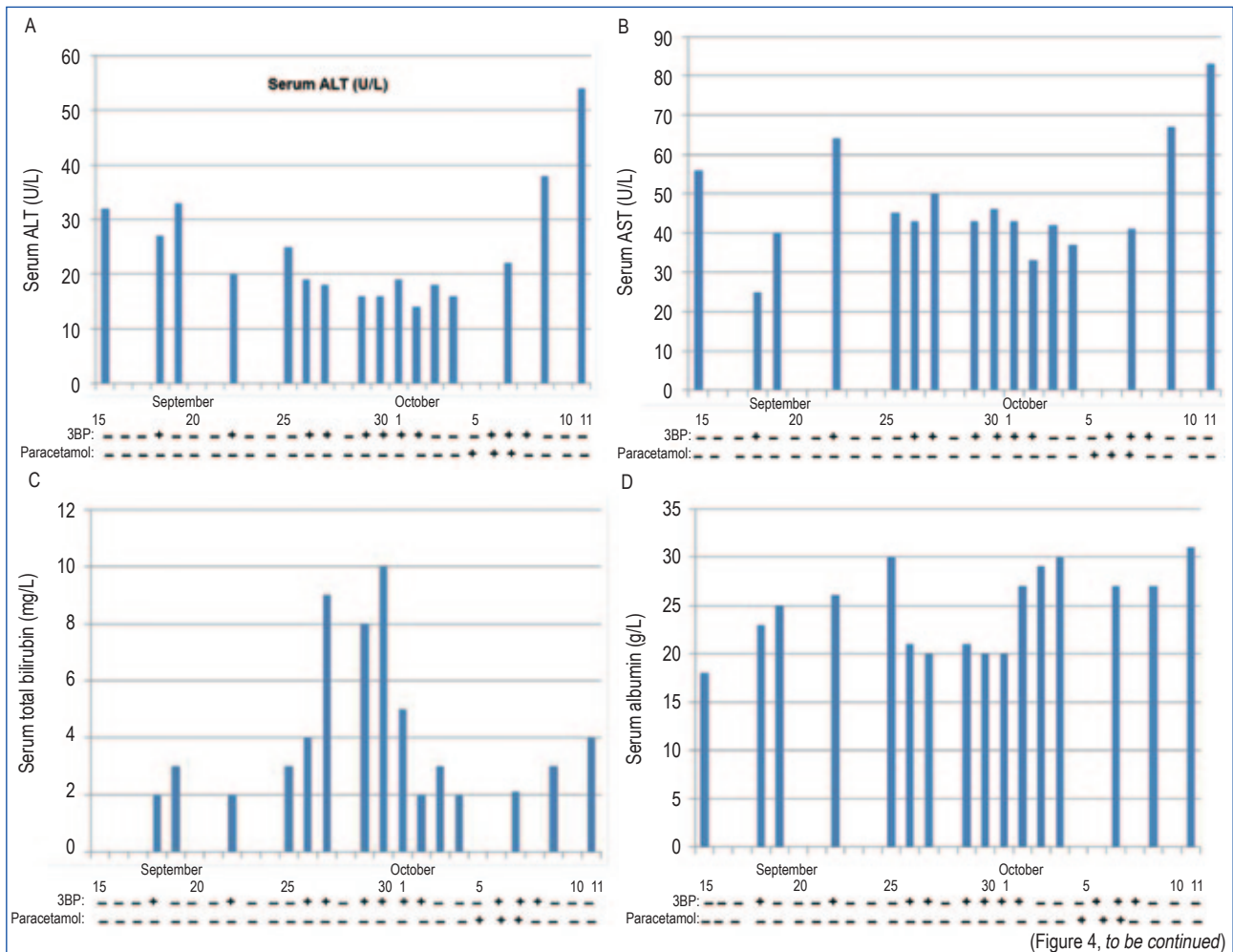
(Figure 3B).

On October 5, 2012, the patient started treatment with paracetamol, a safe GSH depletor[27-32], in the form of oral, 500-mg tablets taken twice every 8 h for 3 consecutive days. By the next day, the patient received 3BP treatment (2.2 mg/kg added to 500 mL of normal saline, administered by slow-drip intravenous infusion over 3 h for 3 consecutive days), which was tolerated with no anaphylaxis or unexpected adverse events. Clinical follow-up revealed good response to treatment as evidenced by a decrease in pain with duragesic dermal patch and moderate improvement in appetite. Mild lower limb edema persisted and was controlled with diuretics. Renal and liver functions were within normal range. There was a sharp decrease in serum LDH level to 1,809 U/L (October 7), 58 U/L (October 8), and 12 U/L (October 9) (Figure 3B). The treatment was stopped, and the patient was in a fair condition apart from an intercurrent chest infection and mild to moderate lower limb edema.

The chest infection manifested with fever, cough, and respiratory distress (dyspnea). Chest examination revealed that there was no air entry on the left side of the chest due to the previously noted destruction of the lung. Complete blood count revealed moderate leukocytosis (Figure 5D) and neutrophilia,

consistent with the infection. Bacterial pneumonia was considered in light of immunocompromise due to malignancy and borderline hypoproteinemia, and the patient received intravenous injection of appropriate broad-spectrum antibiotics. The patient's blood pressure was 90/60 mmHg, and renal and liver functions were normal (Figure 4). Hypoalbuminemia and hypoproteinemia were persistent, mostly due to anorexia and nutritional deficiency.

The patient still had pain in the back region and left chest wall at the metastatic points, and this was controlled with duragesic transdermal patch. Edema in both lower limbs was moderate under treatment with diuretics (with good urine output). The next day, fever, cough, respiratory distress, and decreased air entry on the right side was noted, and a further decrease in blood pressure occurred. Lower limb edema persisted and urine output decreased. Because of the fluid restriction, the patient received dopamine infusion at the intensive care unit, where blood pressure increased to 100/60 mmHg. Urine output increased, and fresh urine was voided in a urine collection bag. Liver and renal functions were normal. Echocardiographic evaluation revealed a metastatic mass in the wall of the left ventricle (2.5 cm × 2.5 cm) together with moderate pericardial effusion. The diagnosis was impending cardiac

(Figure 4, *to be continued*)
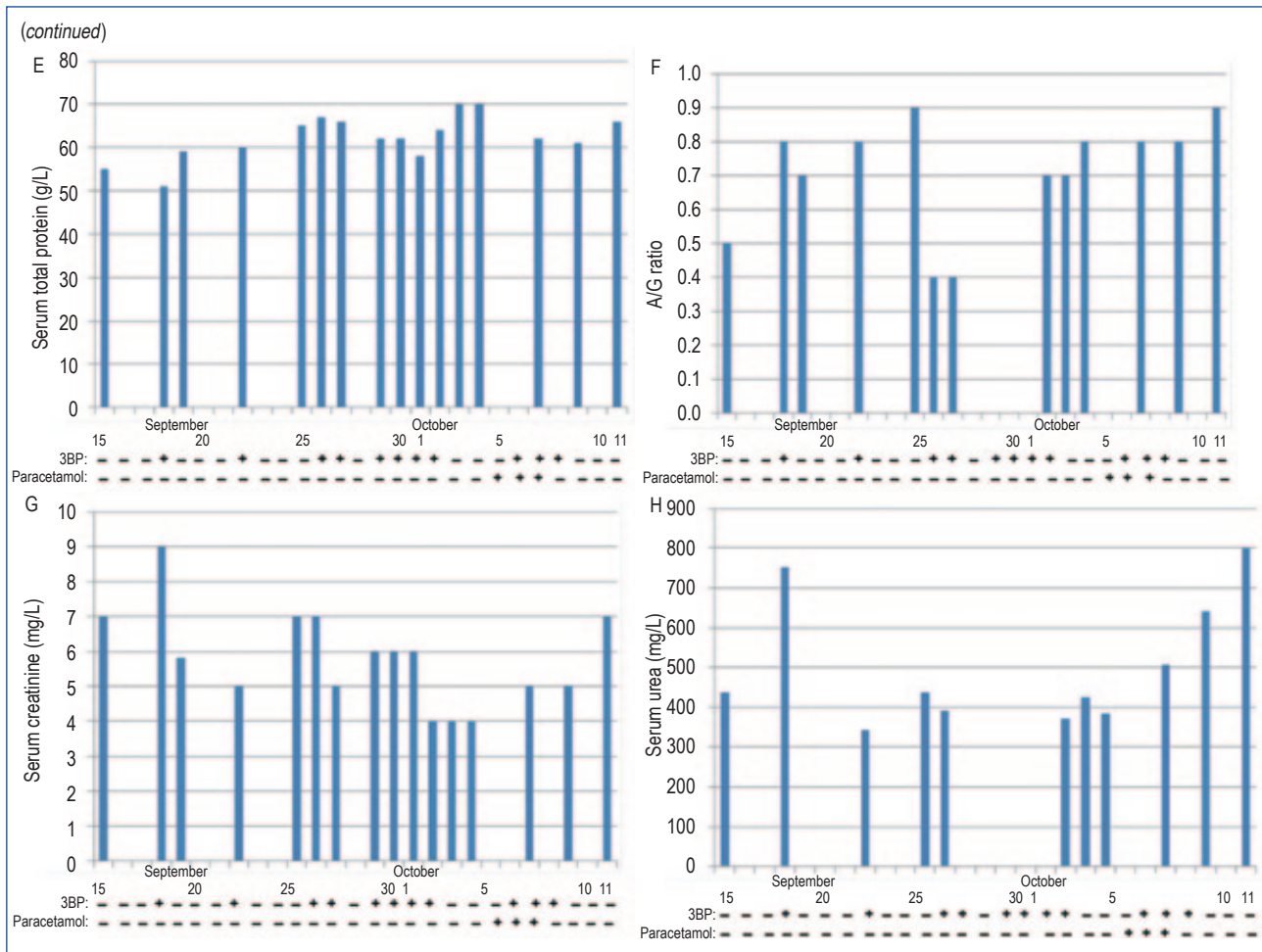
Figure 4. **3BP is not toxic to liver or renal functions.** A, serum alanine transaminase (ALT) is within normal range with 3BP treatment (mild elevation may occur). B, serum aspartate transaminase (AST) shows moderate elevations with 3BP treatment. C, serum bilirubin is within normal range with 3BP treatment. D, serum albumin did not decrease (compared to pretreatment level) with 3BP treatment. E, serum protein is within normal range with 3BP treatment. F, serum albumin/globulin (A/G) ratio did not decrease (compared to pretreatment level) with 3BP treatment. G, serum creatinine is within normal range with 3BP treatment. H, serum urea is within normal range with 3BP treatment (moderate elevation may occur).

tamponade, which may have been secondary to metastasis that shifted the heart to the right, as well as nutritional hypoproteinemia. Overall cardiac movement was normal. Patient was maintained on treatment with antibiotics, dopamine, dobutamine, and diuretics in the intensive care unit. Edema in the lower limbs gradually decreased and blood pressure was stable at 100/60 mmHg.

Leukocytosis (**Figure 5D**) and absolute neutrophilia increased despite broad-spectrum antibiotics. Liver function tests, including serum alanine transaminase (ALT) (**Figure 4A**) was within the normal range with a slight elevation in serum aspartate transaminase (AST) (**Figure 4B**). In addition, serum bilirubin (**Figure 4C**) was within the normal range. Renal function tests such as serum creatinine (**Figure 4G**) were within the normal range, with a moderate elevation of serum urea (**Figure 4H**). The patient was in respiratory distress and hypoxemia was evident. The patient died because of hypoxemia on October 12, 2012.

Discussion

Serum LDH, a good parameter for the evaluation of tumors such as melanoma, is superior to the presence of a residual tumor mass for predicting treatment outcome. Targeting glycolysis and the Warburg effect with agents like 3BP deprives melanoma cells of the energy necessary for survival, proliferation, and metastasis.

Interestingly, serum LDH level reflects metabolic energy activity of cancer cells inside tumor mass, which may be a more sensitive indicator of tumor activity than tumor size. Early measurement of serum LDH level was reported to be useful in identifying response to chemotherapy. For example, in pediatric leukemia, higher LDH levels in ALL were associated with high counts of leukocytes and blast cells. In pediatric solid tumors, high LDH levels were associated with the extent of tumor mass and stage of the disease[33]. Moreover, LDH-A contributes to development of resistance of cancer cells to chemotherapy[34]. In melanoma, LDH is a metabolic marker to detect
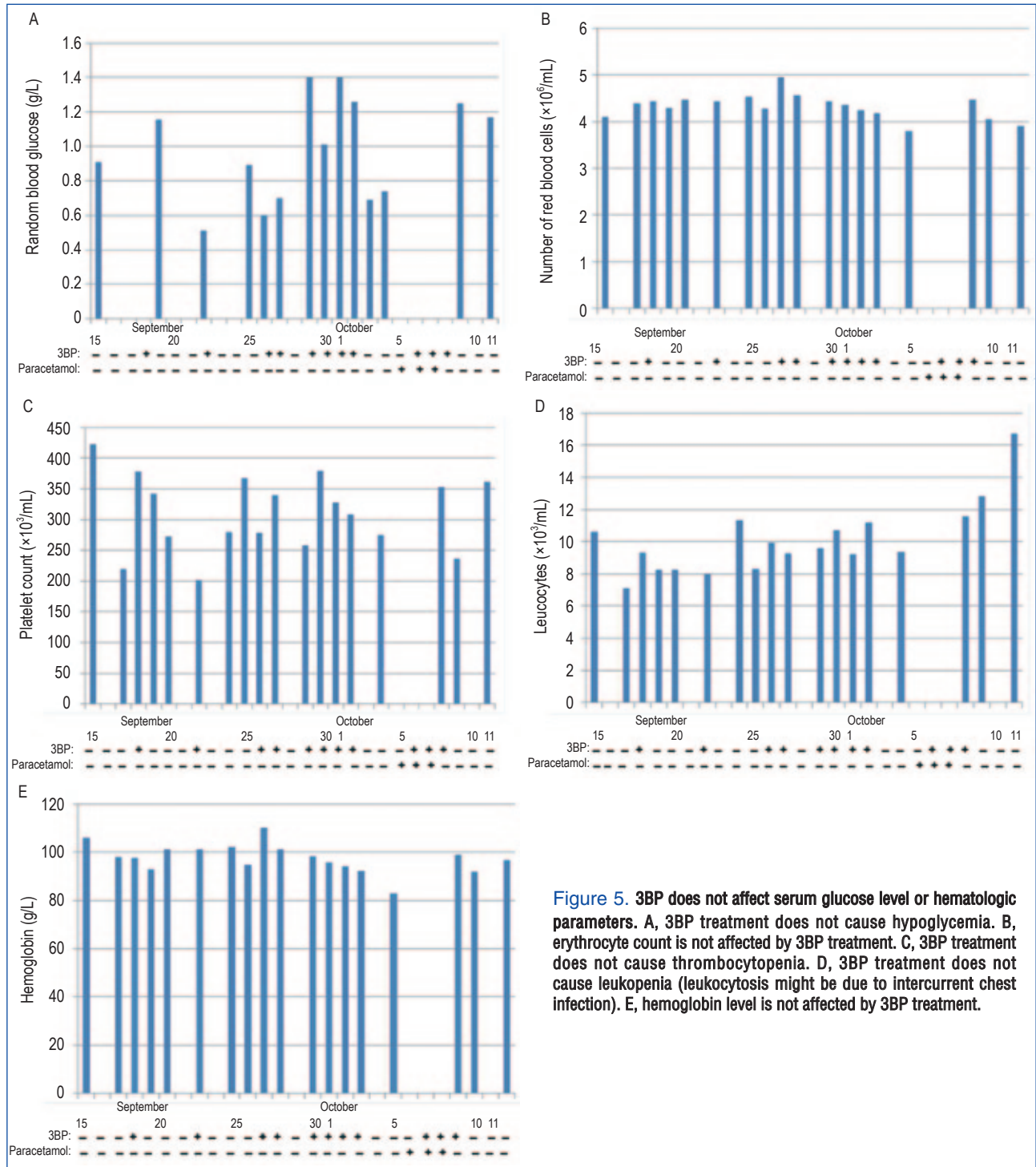
Figure 5. **3BP does not affect serum glucose level or hematologic parameters. A, 3BP treatment does not cause hypoglycemia. B, erythrocyte count is not affected by 3BP treatment. C, 3BP treatment does not cause thrombocytopenia. D, 3BP treatment does not cause leukopenia (leukocytosis might be due to intercurrent chest infection). E, hemoglobin level is not affected by 3BP treatment.**

progression and predict prognosis in stage IV of the disease[35]. When discussing anticancer effects of 3BP, serum LDH level estimation as a response to treatment is critical, as 3BP is a structural analog of both lactate and pyruvate. Lactate produced through activity of LDH fuels aerobic populations inside tumors via metabolic symbiosis (**Figure 1A**)[1].

Combining 3BP with lactate or pyruvate, substrates of LDH, protected cancer cell viability, suggesting that 3BP is an antagonist to lactate and pyruvate. 3BP was reported to compete with pyruvate for LDH[36]. Furthermore, it may be transported to the inside of cancer cells through the pyruvate-lactate transporter (monocarboxylate transporters). Up-regulation of these transporters results in enhanced

3BP uptake in tumor cells[37]. Thus, 3BP inhibits LDH by competing with its substrates[22].

Paracetamol (acetaminophen, N-acetyl para-amino phenol) is widely used in pediatric practice and adults. Paracetamol is a GSH depletor and is safer than acetyl salicylic acid (aspirin, Aspegic) as an antipyretic because Aspegic may induce Rey's syndrome[27]. Paracetamol is tolerated at high doses[28]. Indeed, there was no increase in hepatic toxicity in alcoholic patients who were given the maximum therapeutic dose of paracetamol (4 g/day)[29]. Lack of maximum decrease in serum LDH level with 3BP might be due to high cellular GSH content, i.e., high tumor content of GSH may inhibit 3BP-induced anticancer effects. When the GSH depletor paracetamol was used with 3BP, LDH dramatically decreased. Notably, this decrease was not due to 3BP-mediated inhibition of serum LDH as evidenced by the lack of a maximum decrease in serum LDH level with 3BP alone. Maximum LDH decrease upon combined treatment confirmed that tumoral GSH was antagonistic to 3BP-induced melanoma cell death. That might indicate a shut down in glycolysis in melanoma cells and signal metabolic cure of metastatic melanoma. Similarly, Qin *et al.*[12] reported that some melanoma cells were resistant to 3BP due to their high cellular content of GSH, an antioxidant and inhibitor of 3BP. Depletion of GSH in melanoma using L-Buthionine sulfoximine (BSO), a selective inhibitor of GSH biosynthesis, sensitized resistant melanoma cells to 3BP and induced necrotic cell death. Thus, when initial response to 3BP treatment is weak, it may be advisable to combine a GSH depletor with 3BP.

BSO is another GSH depletor that was studied *in vitro* but has not been studied in humans; paracetamol is safer than BSO for human use. Interestingly, paracetamol inhibited growth and decreased tumor size in experimental models[30]. Melanoma cells using tyrosinase enzyme used paracetamol as a substrate for tyrosinase. In addition, paracetamol killed melanoma cells by depleting GSH, increasing reactive oxygen species levels, and inducing mitochondrial toxicity[31]. Paracetamol was also recently reported to increase LDH activity[38]. Combination of paracetamol with 3BP seems promising, as 3BP targets cancer cells at many points. We recently reported that 3BP

targets the energetic arm, metastatic arm (hyaluronan synthesis through uronic acid pathway), and the mitotic arm of malignancy (DNA synthesis) in addition to targeting phosphohexose isomerase, an autocrine motility factor[39]. In the case reported here, unformulated 3BP (Sigma, USA) was administered through slow intravenous infusion to minimize any possible adverse events. This approach was tolerable with minimal toxicity. The patient's condition was stable under supportive treatment. 3BP and paracetamol were given when the patient's condition and laboratory investigations were stable. Close medical supervision was offered at all times and no treatment (except supportive treatment) was given when acute conditions were present. Normal renal functions, liver functions, and hematologic indices during treatment may indicate that 3BP is a safe anticancer agent. The moderate elevation in serum urea might be due to the antibiotics given for severe chest infection, infection state, or 3BP.

Conclusions

3BP can be regarded as an antimetabolite, being a structural analog of pyruvate and lactate, that can be administered by slow intravenous infusion with minimal hepatic, renal, and hematologic toxicity. Anticancer efficacy of 3BP can be antagonized by high tumor GSH content but can be potentiated on concurrent administration of GSH depletors such as paracetamol. Future clinical trials using 3BP as an anti-melanoma agent and as a general anticancer agent are strongly recommended. When the anticancer effect of 3BP needs to be potentiated, combination with paracetamol may be considered.

Acknowledgment

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