

Hepatotoxicity and nephrotoxicity of 3-bromopyruvate in mice¹

Qiong Pan^I, Yiming Sun^{II}, Qili Jin^{III}, Qixiang Li^{IV}, Qing Wang^V, Hao Liu^{VI}, Surong Zhao^{VII}

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^IGraduate student, Department of Pharmacy, China. Technical procedures, manuscript preparation.

^{II}Graduate student, Department of Pharmacy, China. Technical procedures.

^{III}Assistant Professor, Department of Pathology, China. Technical procedures.

^{IV}Graduate student, Department of Pharmacy, China. Technical procedures.

^VAssistant Professor, Department of Pharmacy, China. Conception and design of the study, critical revision.

^{VI}Full Professor, Department of Pharmacy, China. Conception and design of the study.

^{VII}Assistant Professor, Department of Pharmacy, China. Conception and design of the study, critical revision.

ABSTRACT

PURPOSE: To investigate the hepatotoxicity and nephrotoxicity of 3-Bromopyruvate (3BP) in mice.

METHODS: Fifteen nude mice were grafted subcutaneously in the left flank with MDA-MB-231 cells, then all mice were divided into control group (PBS), 3BP group (8 mg/kg), positive group (DNR: 0.8 mg/kg) when tumor volume reached approximately 100 mm³. 28 days later, tumors, livers and kidneys were stored in 4 % formalin solution and stained with hematoxylin and eosin staining. The Kunming mice experiment included control group (PBS), 3BP group (4mg/kg; 8mg/kg; 16mg/kg), positive group (DNR: 0.8 mg/kg). 24 hours later, the blood were used for the determination of hepatic damage serum biomarkers. Livers were stored in 4 % formalin solution for the later detection.

RESULTS: 3BP at the dose of 8mg/kg had a good effect on inhibiting tumor growth in nude mice and did not damage liver and kidney tissues. Kunming mice experiment showed 3BP at the dose of 16mg/kg did damage to liver tissues.

CONCLUSION: 3-Bromopyruvate at the dose of suppressing tumor growth did not exhibit hepatotoxicity and nephrotoxicity in nude mice, and the effect on liver was confirmed in Kunming mice.

Key words: Bromopyruvate. Hepatotoxicity. Nephrotoxicity. Mice.

Introduction

Cancer cells acquire energy mainly through the glycolytic pathway rather than oxidative phosphorylation, while the normal cells depend mainly on mitochondrial oxidative phosphorylation to get energy, this particular phenomenon is called “Warburg effect” {Geschwind, 2002 #5} {Geschwind, 2002 #5} Warburg¹. Thus, we can inhibit the growth of tumor cells by inhibiting glycolytic pathway². As a glycolytic inhibitor, “3BP is a fast acting, promising, powerful, specific and effective anti-cancer agent”, said by Pedersen³. Also 3BP has been identified to inhibit tumor cells proliferation as a proposed widely used anti-cancer agent⁴, such as in breast cancer cells^{5,6}, liver cancer cells^{7,8}, lymphoma cells⁹, lung cancer cells¹⁰, pancreatic cancer cells^{11,12}, gastric cancer^{2,13}, colorectal cancer cells¹⁴. Those significant anti-tumor effects of 3BP has made it getting more and more attention^{15,16}.

Our group also pay attention to the anti-tumor activity of 3BP. For one paper, 3BP induced autophagy by stimulating ROS formation in human breast cancer cells, which could be inhibited by chloroquine¹⁷. The further research demonstrated that 3BP could induce apoptosis via AKT /protein kinase B signaling in breast cancer cells MDA-MB-231, and the knockdown of Mcl-1 plays an important role in 3BP-induced apoptosis¹⁸. Also, 3BP efficiently enhanced DNR-induced cytotoxicity in breast cancer cells by inhibiting the activity of ATP, especially, MCT1 overexpression increased the efficacy of 3BP in breast cancer cells MDA-MB-231⁶. Recently, we found that 3BP could also induce necroptosis and apoptosis at the same time and the same concentration in colon cancer cells SW480 and HT29¹⁴.

Toxicity is one of problems that must be considered during the treatment of disease. It correlates with excessive drug-dose or too long drug treatment for the body. Drug-related hepatotoxicity of some drugs has been defined in literature. Acetaminophen is the most widely used antipyretics, which is a safe drug at therapeutic doses. However, its overdose can lead to hepatic and renal damage¹⁹, which may be fetal. Ifosfamide is an anti-cancer agent, which is widely used in the treatment of solid tumors, such as gynecologic, head and neck cancers, lymphomas, and osteogenic sarcoma²⁰, but the nephrotoxicity particularly limited its application^{21,22}.

Though previous research showed that 3BP had no hepatotoxicity when inhibiting lung tumor genesis¹⁰. Whether 3BP is safe in other tumors is still a pending issue. In the work here we focus on hepatotoxicity and nephrotoxicity of 3BP in nude mice and Kunming mice.

Methods

This study was approved by the Ethics Committee of Bengbu Medical College.

The compound 3BP was purchased from Sigma-Aldrich (St. Louis, MO, USA). Daunorubicin (DNR) was obtained from Zhejiang Hisun Pharmaceutical Company Limited (Zhejiang, China). One step TUNEL apoptosis assay kit was purchased from Beyotime Institute of Biotechnology (Wuhan, China). Solution of 3BP was prepared in phosphate-buffered saline (PBS), after adjusting the pH to 7.0 with NaHCO₃, the solution was sterilized with 0.22 μm Millipore filter before immediate use.

Fifteen female nude mice (BALB/c) aged 4-5 weeks and weighed 18-20 g were used in this study, which were purchased from Suzhou Industrial Park, Ireland Matt Technology Co. Ltd. All mice were injected with human breast cells MDA-MB-231 (10⁸ cells/ml; 0.2ml/mouse) subcutaneously to induce tumor formation, then they were randomly divided into three groups (n=5 per group) when tumor volume reached approximately 100 mm³: control group, drug group and positive group, which were intraperitoneally injected every second day with 0.2 ml of PBS, 3BP (8 mg/kg) and DNR (0.8 mg/kg) for 28 days, then the mice were sacrificed after quickly removing blood, liver and kidney for the subsequent analysis.

Twenty-five Kunming mice (18-20g) were obtained from the Experiment Center of Bengbu Medical College. All animals were divided into four groups: control group (PBS; 0.2ml), drug group (3BP; 4mg/kg, 8mg/kg, 16mg/kg), positive group (DNR, 0.8mg/kg), which were treated with single intraperitoneal injection, 24 hours later, blood and liver were collected for the subsequent analysis.

All animals were kept at room temperature and subjected to a natural day/night light cycle and allowed free access to standard diet and water.

Blood chemistry analysis

Animals were sacrificed after quickly removing liver and kidney, and blood were collected from eyes. The samples of blood were stewing for half an hour, taking the upper serum after centrifugation (1500 r/m, 10 min). The serum was detected by automatic biochemical analyzer (Beckman, USA).

Hematoxylin and eosin staining assay

Tumors, livers and kidneys were stored in 4% formalin

solution, embedded in paraffin, which were cut into sections and stained with hematoxylin and eosin according to the manufacturer's instructions.

Immunohistochemistry assay

Liver tissues were stored in 4% formalin solution, embedded in paraffin, which were cut into tissue sections and were used for immunohistochemistry according to the manufacturer's instructions.

TUNEL assay

The slices of liver tissues were stored in 4% formalin solution, embedded in paraffin, which were cut into sections and were analyzed by one step TUNEL apoptosis assay kit. The analysis was carried out according to the manufacturer's instructions.

Statistical analysis

All data were expressed as mean ± standard deviation (SD). SPSS v.16.0 software (SPSS Inc., Chicago, IL, USA) was used for data analysis. * $p < 0.05$ was considered statistically significant.

Results

The effect of 3BP in nude mice

3BP clearly suppressed tumor growth compared with the control group (Figure 1 a, b). The results of H&E staining demonstrated that 3BP caused part of necrosis tumor issues (Figure 1c). The analysis of blood chemistry showed that activity of AKP, ALT and AST changed indistinctively ($p > 0.05$), which means that 3BP at 8mg/kg did not damage liver tissues (Figure 2a), also the same results of H&E staining on liver (Figure 2b). The analysis of blood chemistry in nude mice showed that the concentration of BUN, UA and CER changed indistinctively ($p > 0.05$), which means that 3BP at 8mg/kg did not damage significantly to kidney tissues (Figure 2c). The results of H&E staining showed that 3BP at 8mg/kg did not damage kidney tissues (Figure 2d).

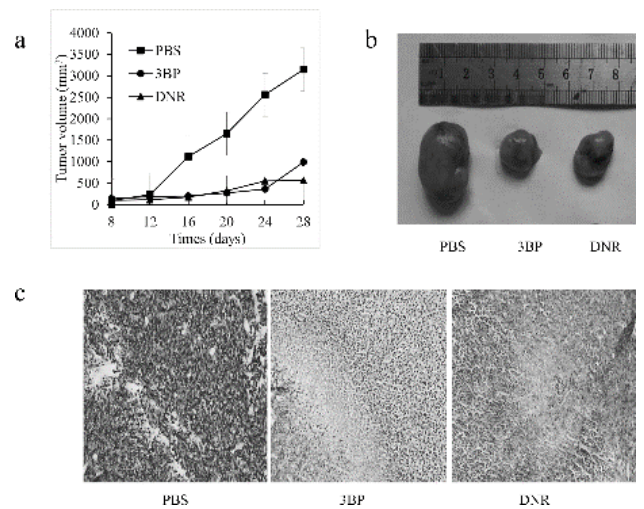


FIGURE 1 - Antitumor effect of 3BP in nude mice. Breast cancer cells MDA-MB-231 were inoculated subcutaneously to induce tumor formation. Mice with tumors (100mm³) were randomly divided into three groups (5 mice/group). The tumor volume was calculated from day 8. **a.** Comparison of tumor volumes. Tumor growth was monitored every 4 days and calculated with the formula: length x width²/2. **b.** Comparison of the transplanted tumor. Tumors were quickly removed after animals were sacrificed, take a photograph of them for comparison. **c.** H&E staining of tumor tissues *in vivo*. 3BP at 16mg/kg caused part of necrosis tumor issues compared with the control group.

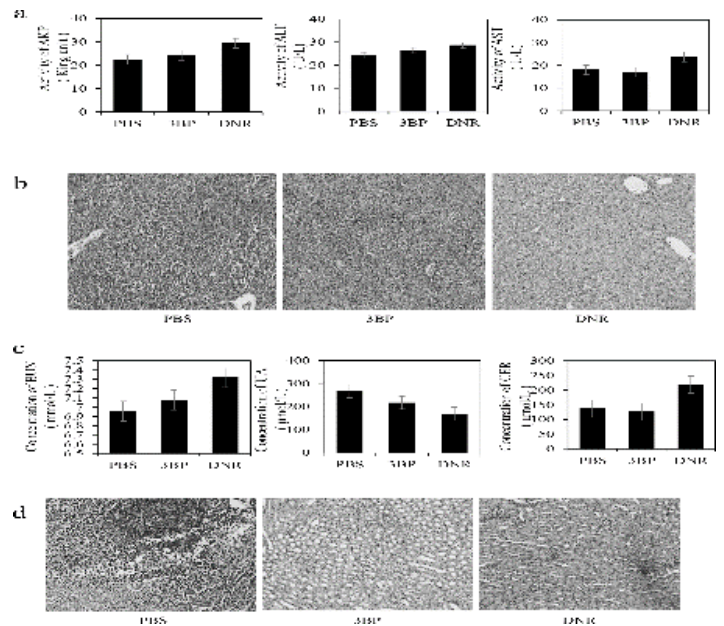


FIGURE 2 - The effect of 3BP on liver and kidney in nude mice. The analysis of blood chemistry indicator. The blood was collected from animal eyes. The samples of blood were stewing for half an hour, taking the upper serum after centrifugation (1500 r/m, 10 min), then analyzed by automatic biochemical analyzer. **a.** The analysis of the activity of AKP, ALT and AST in serum changed indistinctively in drug groups compared with the control group ($p > 0.05$). **b.** H&E staining of liver tissues *in vivo*. 3BP at 8mg/kg caused no hepatotoxicity compared with the control group. **c.** The concentration of BUN, UA and CER in serum changed indistinctively ($p > 0.05$). **d.** H&E staining of kidney tissues *in vivo*. 3BP at 8mg/kg caused no nephrotoxicity compared with the control group.

The effect of 3BP on liver and kidney in Kunming mice

Liver index in drug groups were higher ($p>0.05$) than that in control group (Figure 3a). The activity of AST and ALT both increased in three drug groups, 3BP at 4 mg/kg and 8mg/kg did not change significantly ($p>0.05$), while 3BP at 16mg/kg group changed significantly ($p<0.05$) compared with the control group (Figure 3b). The results of H&E staining showed that 3BP at 8mg/kg did not make damage to liver tissues (Figure 3c). As an endoplasmic reticulum stress protein, the expression of GAPDH will be increased when tissues were injured. Compared with the control group, the expression of GAPDH at 4mg/kg and 8mg/kg 3BP group did not changed obviously, while its expression in 16mg/kg 3BP group (Figure 3d). The result of TUNEL assay showed that there were few apoptotic cells in 4mg/kg and 8mg/kg 3BP group, while more apoptotic cells could be seen in 16mg/kg 3BP group and positive group (Figure 3e).

These results indicated that 3BP at the dose of suppressing tumor growth did not exhibit hepatotoxicity and nephrotoxicity in nude mice.

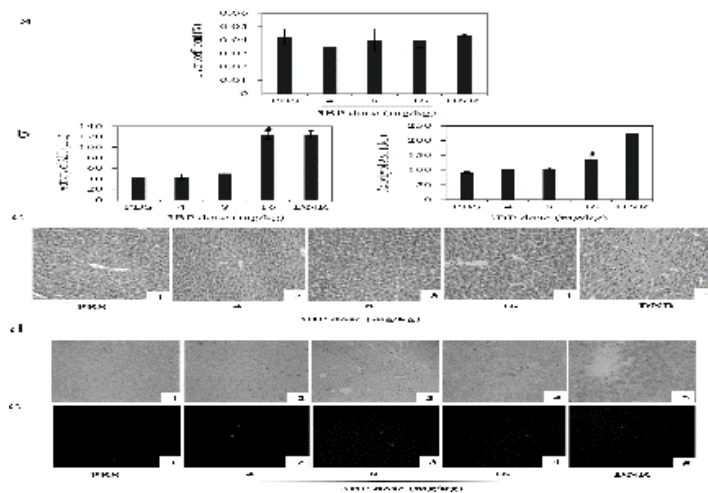


FIGURE 3 - The effect of 3BP on liver in Kunming mice. **a.** Comparison of liver index. Liver was collected after animals were sacrificed and were calculated with the formula: liver index weight/body weight×100%. Liver index in 3BP (4mg/kg) group were higher compared with the control group ($p>0.05$). With the increasing drug dose, the liver index in 3BP (8mg/kg) group and 3BP (16mg/kg) group was almost equal. **b.** Comparison of activity of ALT and AST in serum increased in drug groups, 3BP at 4 mg/kg and 8mg/kg did not change significantly compared with the control group ($p>0.05$), while 3BP at 16mg/kg group changed significantly ($p<0.05$). **c.** H&E staining of liver tissues *in vivo*. 3BP at 4mg/kg and 8mg/kg did not cause hepatotoxicity compared with the control group. **d.** The effect of 3BP on expression of GAPDH in liver. The expression level of GAPDH at 4mg/kg 3BP and 8mg/kg 3BP group changed fairly compared with control group. **e.** The effect of 3BP on apoptosis. There were few apoptotic cells in 3BP at 4mg/kg and 8mg/kg group, while 16mg/kg group were changed significantly compared with the control group.

Discussion

As a glycolytic inhibitor, 3BP has been widely used in anti-cancer researchers in various tumor cells⁹, particularly in liver cancer cells²³. Our group have done some researches about 3BP anti-tumor effect *in vivo*¹⁸. The researches of anti-cancer effects of 3BP had a good foundation of papers. However, there were few researches about the toxicity of 3BP.

Liver is the major organ to cope with metabolic substances, which has various functions including detoxification, bile secretion, glycogen storage, immune defenses etc. What's more, kidney is an important excretory organ. The Function of liver and kidney indicator the toxicity of drugs²⁴. The Function of liver and kidney is one of important indicator of toxicity²⁴. As a sign of liver damage, oxidative stress causes multiple lesions in the plasma membrane^{25,26}. One of the important targets of oxygen is glyceraldehyde-3-phosphate (GAPDH), which is also an important index of liver damage^{27,28}. The other important targets of liver damage are the changes of enzyme activity. AST and ALT are important enzymes in liver, both are vital to evaluate liver injury. ALT mainly exists in cytoplasm of liver cells, while AST in liver cytoplasm and mitochondria. Both ALT and AST will release into the blood when the liver cells are damaged. Cell degeneration, necrosis permeability of cell membranes or broken cell membrane increases in liver cells contained ALT and AST will be released into the blood, increased the activity of ALT and AST. Also, the concentration of BUN, CER and UA has been used to analyze the function of kidney, which will be changed when the tissues were damaged.

In previous reports, anti-tumor effects of 3BP in animals have been gradually found by the world^{29,30}. The present study demonstrated that 8mg/kg 3BP presented no hepatotoxicity and nephrotoxicity in nude mice, which also had a good effect of inhibiting tumor growth. For Kunming mice experiment, there was no significant hepatotoxicity in liver tissues for 3BP group (8mg/kg). In summary, these results may remind us that as a potent anti-tumor agent, 3BP has no hepatotoxicity and nephrotoxicity under the dose of inhibiting tumor cell growth. The extensive toxicity of 3BP need to be evaluated in further researches. The results of this study may have an implication for future study of 3BP.

Conclusion

3-Bromopyruvate at the dose of suppressing tumor growth did not exhibit hepatotoxicity and nephrotoxicity in nude

mice, and the effect on liver was confirmed in Kunming mice.

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Correspondence:

Hao Liu
Bengbu Medical College, Anhui - China
Phone: +865523175230
Fax: +865523175234
liuhao6886@foxmail.com

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