Progress Report

Therapeutic efficacy of 3-bromopyruvate when Injected intratumorally on the growth of VM-M3 and CT-2A brain tumor cells

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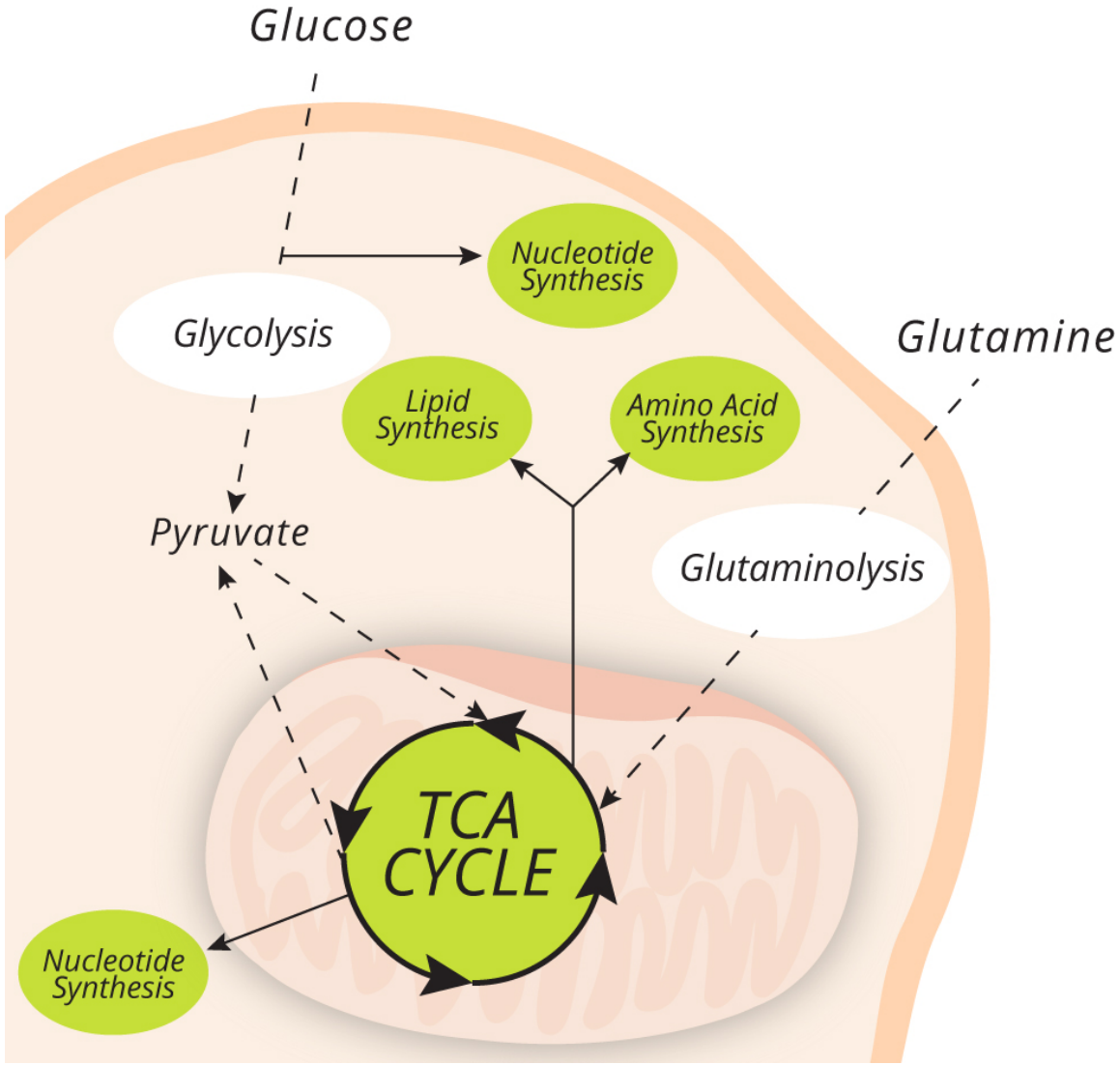
Overview

The previous progress reports showed that 3-BP was unable to produce a therapeutic effect against the VM-M3 tumor grown in its syngeneic VM/Dk mouse host. We suggested that this might be due to:

1) The greater dependence of the VM-M3 tumor on glutaminolysis than on glycolysis for energy production.

2) The low bioavailability of 3-BP after IP injection.

The glycolytic phenotype of the CT-2A glioma tumor makes this an attractive tumor model for studying 3-BP. Also, we will inject 3-BP directly into the VM-M3 and CT-2A tumors grown subcutaneously in flank.



F**igure 1**. Relationship between glycolysis and glutaminolysis in cell metabolism (Image courtesy of FORMA Therapeutics)

Experimental Overview

I*n vivo* experiments are presented to evaluate the therapeutic efficacy of 3-BP against the VM-M3 mouse glioblastoma and the CT-2A malignant astrocytoma when the tumors are grown subcutaneously in the flank of the syngeneic of the VM/Dk and C57BL/6, respectively. 3-BP will be injected directly into the flank grown tumors. The treatment protocol will be done according to the instruction of Dr. Young Ko.

Methods

VM-M3 tumor fragments were implanted subcutaneously into the flank of the VM/Dk host strain. CT-2A cells were grown in culture and harvested with 0.25% trypsin containing 1 mM EDTA. The cells were washed twice and resuspended in DMEM. VM/Dk or C57BL/6J mice were anesthetized with isoflurane. The tumor fragments (VM-M3) or cells (CT-2A) were then injected s.c. with 1 million cells in PBS in the flank using a prechilled tuberculin syringe (27-gauge needle). After approximately 7 days (when tumor nodules appeared), 3-BP was injected several times (2-3 times a week) into the tumors and around the tumor periphery. Tumor volume was measured every other day. Tumors were collected and weighed after approximately 22 days of treatment.

The study involved two groups of VM/Dk or C57BL/6 mice:

- **Vehicle (saline buffer) injection.**
- **3-BP (10 mg/kg 3-BP + saline buffer).**

Figure 2. 3-BP reduces VM-M3 tumor growth in flank

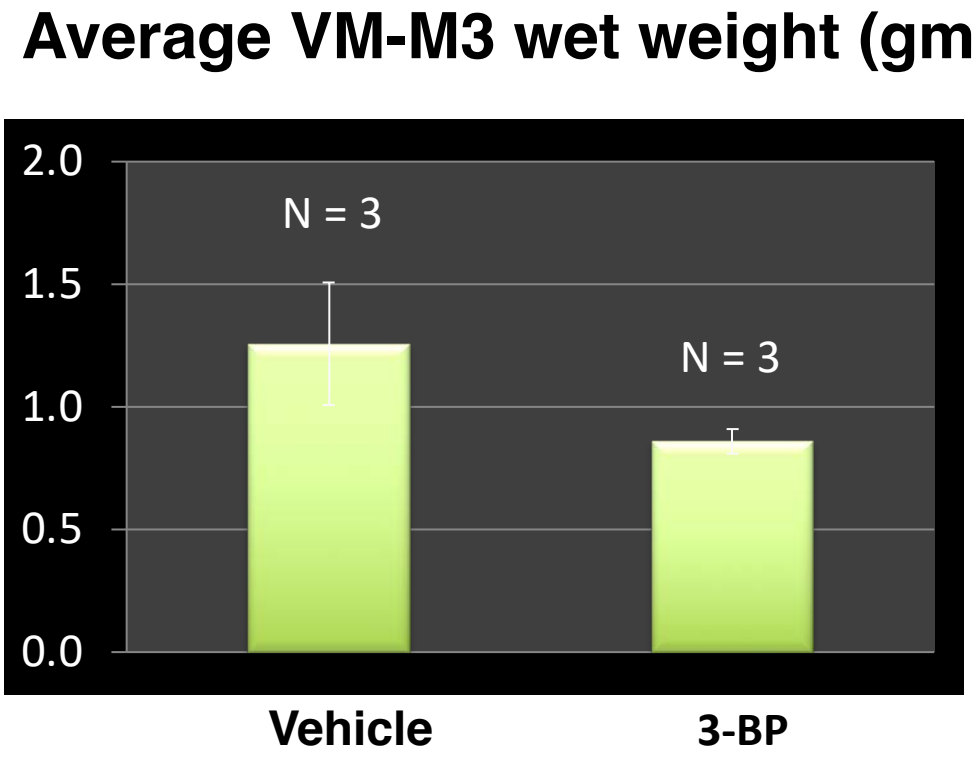


Figure 3. 3-BP reduces VM-M3 tumor metastasis from flank to liver and spleen in VM/Dk mice.

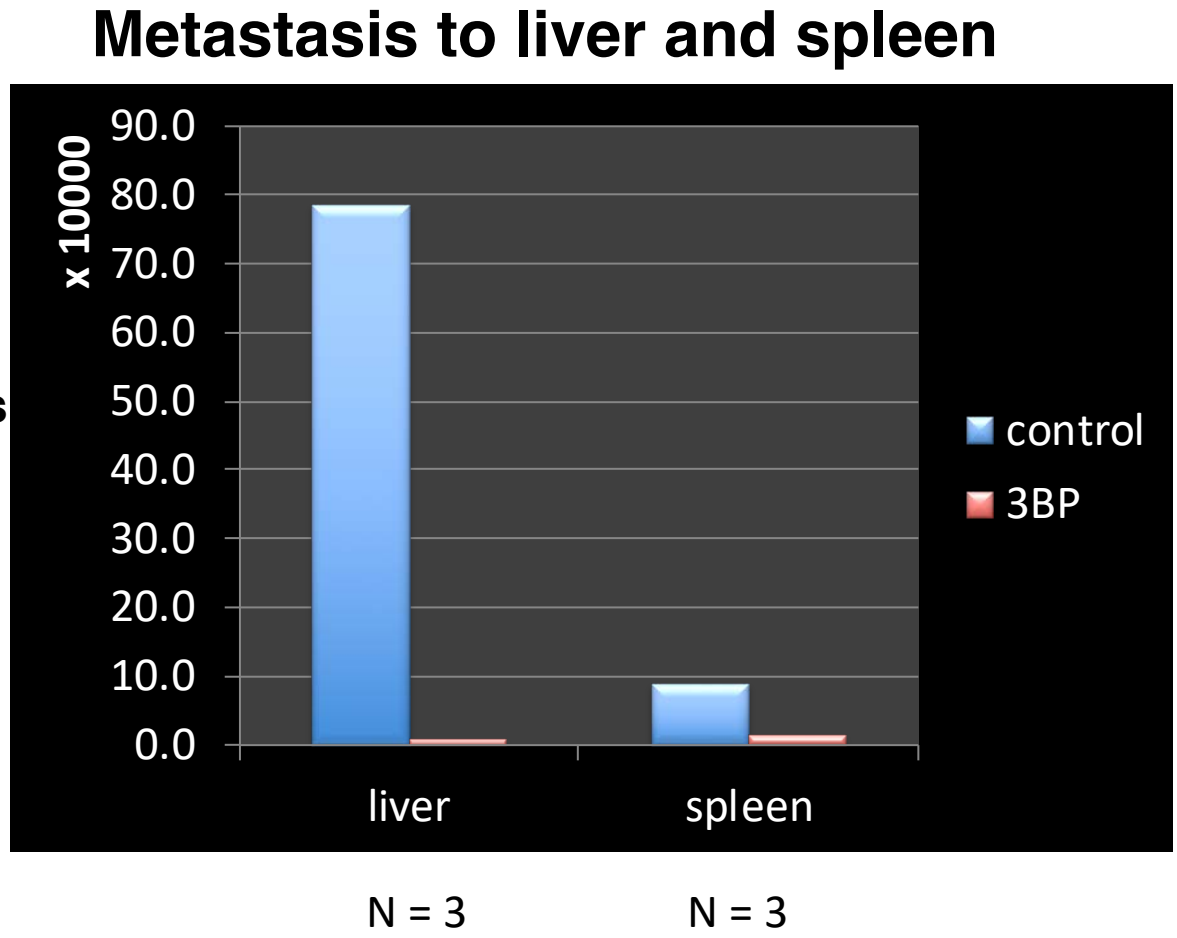


Figure 4. 3-BP reduces CT-2A tumor volume in flank

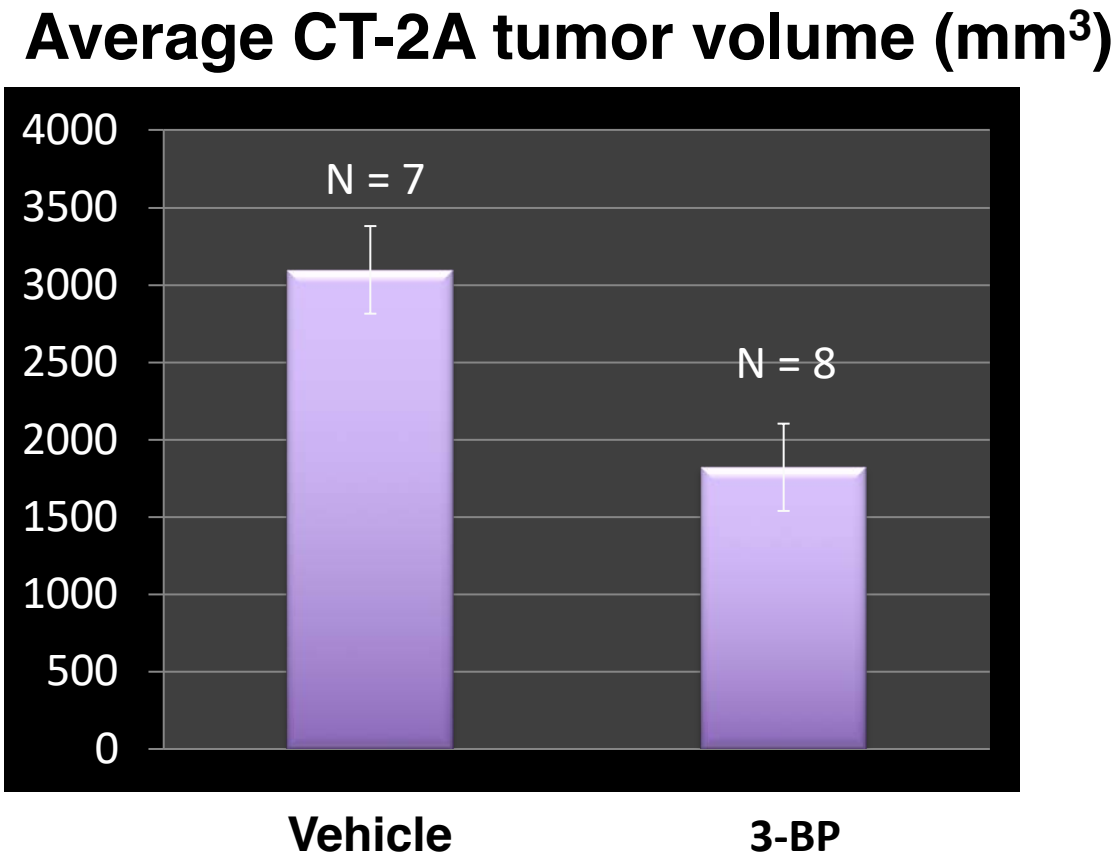


Figure 5. 3-BP reduces CT-2A tumor weight in flank

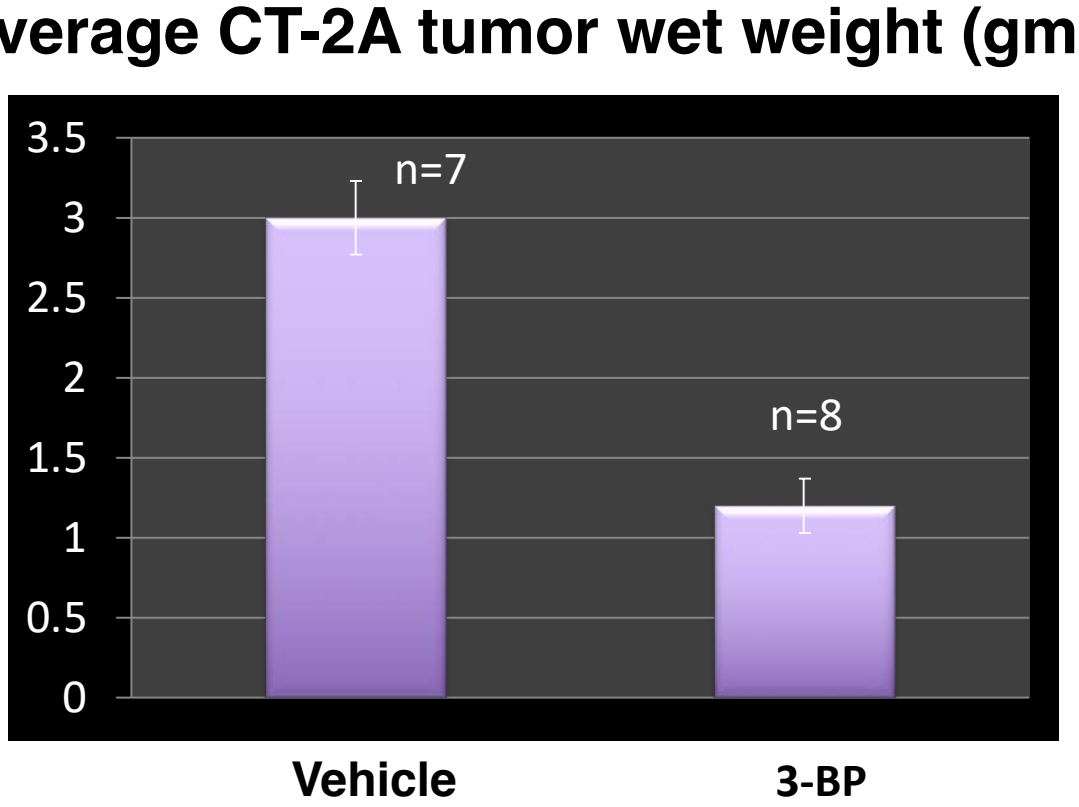
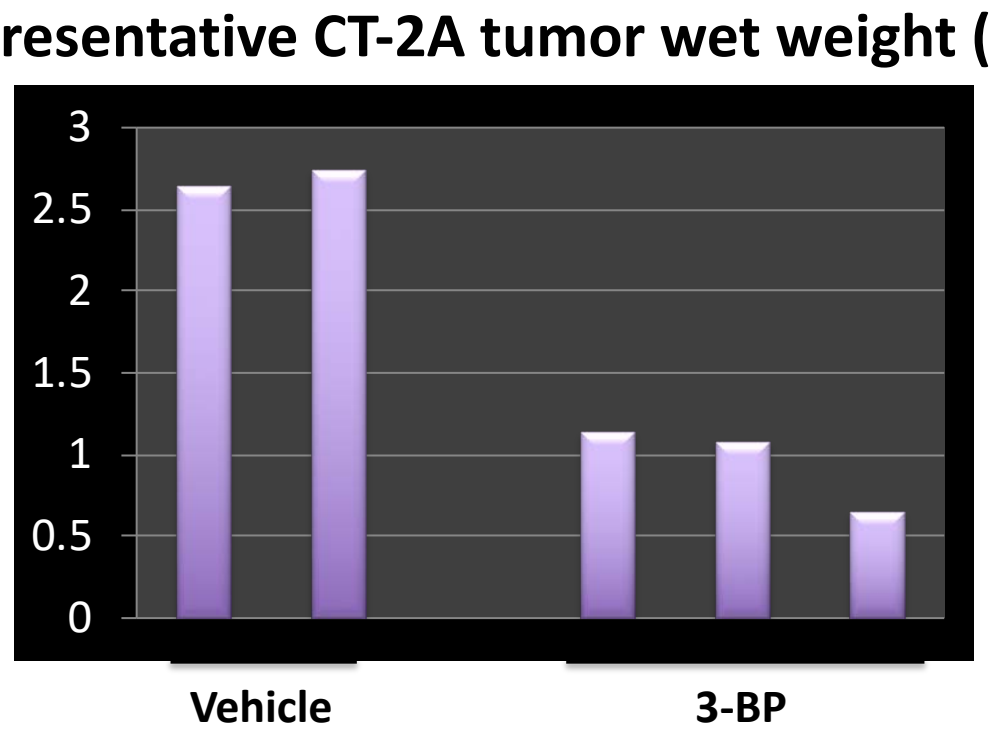
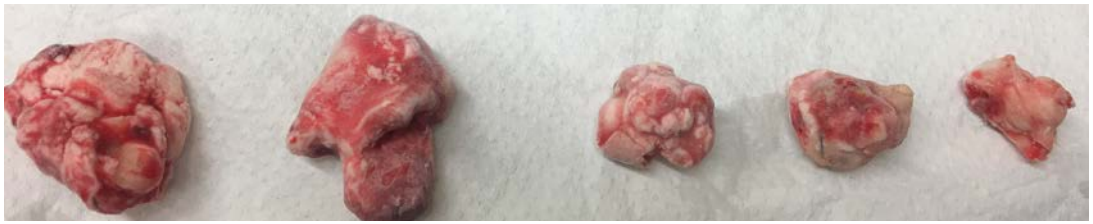


Figure 6. 3-BP reduces CT-2A tumor weight in flank





Conclusions

- Intratumoral injections of 3-BP caused significant reductions in the wet weight or volume of the VM-M3 glioblastoma and CT2A astrocytoma.

- Intratumoral injections of 3-BP caused a reduction of VM-M3 metastasis to liver and spleen.

- Intratumoral injections of 3-BP caused no apparent toxicity or a reductions in body weight of either VM/Dk mice or C57BL/6 mice.

- These observations are consistent with the *in vitro* data that 3-BP can kill tumor cells if 3-BP can make direct contact with the tumor cells.

Further studies will be needed, using larger numbers of mice, to validate the significance of the observations.