Journal of the North for Basic and Applied Sciences, Vol. (9), Issue (1), (May 2024/ Shawwal 1445 H) 29-38

KINGDOM OF SAUDI ARABIA
Northern Border University (NBU)
Journal of the North for Basic & Applied Sciences(JNBAS)
p - ISSN : 1658 -7022 / **e-ISSN: 1658 - 7014**
www.nbu.edu.sa
s.journal@nbu.edu.sa

Inhibitory biochemical and pharmacological effects of β-hydroxypyruvate versus glycolysis inhibitors for treating C6 glioblastoma multiforme: An experimental study

Hussam H. Baghdadi*

(Received 20/6/2023 ; accepted 16/1/2024)

Abstract

Objectives: Glioblastoma multiforme is the most aggressive glioma carrying the worst prognosis. β-hydroxypyruvate is a metabolite of fructose (a hexose monosaccharide) and a biochemical analog of pyruvate, lactate and the promising anticancer drug 3-bromopyruvate (an inhibitor of the glycolysis enzyme Hexokinase II). This study aimed at investigating possible inhibitory biochemical and pharmacological effects of β-hydroxypyruvate as a potential anticancer agent.

Methods: C6 glioblastoma cells were grown on nutrient media *in vitro* and received different experimental treatments. (The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide, MTT) viability assay, ATP assay and hydrogen peroxide assay were used

Results: β-hydroxypyruvate significantly ($p<0.001$) decreased the energetics and viability of C6 glioblastoma cells. Lactate significantly ($p<0.001$) inhibited β-hydroxypyruvate effects stronger than pyruvate. β-hydroxypyruvate significantly ($p<0.001$) synergized citrate in inducing C6 glioblastoma cell death. β-hydroxypyruvate induced the production of significant quantities of hydrogen peroxide (H_2O_2) in a dose-dependent manner i.e. an oxidative stress effect that was more potent than relatively higher doses of both 3-bromopyruvate and sodium fluoride. However, pyruvate (but not lactate) significantly inhibited β-hydroxypyruvate-induced H_2O_2 generation. Likewise, pyruvate scavenged significantly ($p<0.001$) 3-bromopyruvate-induced H_2O_2 generation. The amino acid glycine (a structural analog of β-hydroxypyruvate) did not protect C6 glioma cells against β-hydroxypyruvate-induced cell death suggesting lack of a biochemical or pharmacological antagonism between β-hydroxypyruvate and glycine. Finally, pyruvate-induced scavenging of β-hydroxypyruvate-induced H_2O_2 generation takes place in a time-dependent manner. Lactate is devoid of any antioxidant activity.

Conclusion: β-hydroxypyruvate is an interesting analog of pyruvate, lactate and 3-bromopyruvate with significant promising inhibitory biochemical and pharmacological effects and moderate anticancer effects. β-hydroxypyruvate should receive further research studies.

Keywords: β-hydroxypyruvate, glycolysis inhibitors, C6 glioblastoma multiforme, lactate, pyruvate and 3-bromopyruvate

DOI: 10.12816/0061758

* **Corresponding Author:**
Department of Clinical Biochemistry and Molecular Medicine, Taibah Faculty of Medicine, Taibah University, Al-Madinah Al-Munawwarah, Saudi Arabia

e-mail: **Hussam.baghdadi@gmail.com**

المملكة العربية السعودية
جامعة الحدود الشمالية (NBU)
مجلة الشمال للعلوم الأساسية والتطبيقية (JNBAS)
طباعة ردمد: 1658-7022/ إلكتروني – ردمد: 1658-7014
www.nbu.edu.sa
http://jnbas.nbu.edu.sa

التأثيرات البيوكيميائية والدوائية لدواء بيتا هيدروكسي بيروفات مقابل مثبطات تحلل الجلوكوز في علاج الورم الأرومي الدبقي C6 متعدد الأشكال: دراسة تجريبية

حسام بغدادي

(قدم للنشر في 1444/12/2هـ؛ وقبل للنشر في 1445/7/4هـ)

مستخلص البحث:

الأهداف: الورم الأرومي الدبقي متعدد الأشكال (جلايوبلاستوما) هو الورم الأرومي الدبقي الأكثر شراسة والذي يحمل أسوأ تشخيص. و دواء بيتا-هيدروكسي بيروفات β-hydroxypyruvate هو مستقلب الفركتوز (سكر سداسي أحادي التسكر) و شبيه كيميائي حيوي لكل من البيروفات واللاكتات والعقار الواعد المضاد للسرطان 3-بروموبيروفات (مثبط لإنزيم أكسدة الجلوكوز هكسوكيناز Hexokinase II). و لقد هدفت هذه الدراسة إلى التحقق من بعض التأثيرات البيوكيميائية والدوائية لدواء بيتا هيدروكسي بيروفات كعامل محتمل مضاد للسرطان.

الطريقة: تم زرع خلايا الورم الأرومي الدبقي C6 على وسط غذائي في المختبر وتلقت علاجات تجريبية مختلفة. تم استخدام طريقة قياس حياة الخلايا MTT و قياس كمية الطاقة في الخلايا ATP و قياس تكون و تكسر مادة بيروكسيد الهيدروجين.

النتائج: أدى دواء بيتا-هيدروكسي بيروفات β-hydroxypyruvate إلى انخفاض ذي أهمية معنوية ($p<0.001$) في الطاقة الحيوية و حياة خلايا الورم الأرومي الدبقي C6 (جلايوبلاستوما). كما ثبطت اللاكتات معنويا ($p<0.001$) من تأثيرات دواء بيتا هيدروكسي بيروفات β-hydroxypyruvate ضد خلايا الورم الأرومي الدبقي و كانت أقوى من البيروفات بشكل كبير. كما أحثت السترات تأثيرات متآزرة ذات أهمية معنوية ($p<0.001$) لدواء بيتا-هيدروكسي بيروفات β-hydroxypyruvate في قتل خلايا الورم الأرومي الدبقي C6. كما تسبب دواء بيتا-هيدروكسي بيروفات β-hydroxypyruvate في إنتاج كميات كبيرة من بيروكسيد الهيدروجين (H_2O_2) بطريقة تعتمد على الجرعة ، أي أن هذا الدواء يحدث تأثير الإجهاد التأكسدي الذي كان أكثر فعالية من الجرعات الأعلى نسبيًا من كل من 3-بروموبيروفات وفلوريد الصوديوم. ومع ذلك ، فقد ثبطت البيروفات (و ليس اللاكتات) بشكل كبير من تكون H_2O_2 الناجم عن هيدروكسي بيروفات. وبالمثل ، فقد أزالت البيروفات بشكل كبير ($p<0.001$) كمية H_2O_2 الناجمة عن bromopyruvate-3. و لم يوفر الحمض الأميني جلايسين (يشبه في التركيب دواء بيتا-هيدروكسي بيروفات β-hydroxypyruvate) أي حماية لخلايا الورم الدبقي C6 ضد الموت الناجم عن التعرض لدواء هيدروكسي بيروفات β-hydroxypyruvate مما يشير إلى عدم وجود تناقس أو تضاد كيميائي حيوي أو دوائي بين دواء هيدروكسي بيروفات β-hydroxypyruvate والحمض الأميني جلايسين. و أخيرًا ، فقد أزالت البيروفات كمية بيرأوكسيد الهيدروجين H_2O_2 الناجمة عن التعرض لدواء هيدروكسي بيروفات بطريقة تعتمد على الوقت. و تأكد لدينا أن مادة اللاكتات خالية من أي نشاط مضاد للأكسدة.

الخلاصة: دواء بيتا-هيدروكسي بيروفات β-hydroxypyruvate هو شبيه تركيبي هام لكل من حمض البيروفات واللاكتات و دواء 3-بروموبيروفات مع تأثيرات واعدة و متوسطة بشكل كبير ضد الأورام، والتي يجب أن تلقى مزيدًا من الدراسات البحثية.

الكلمات المفتاحية:

بيتا هيدروكسي بيروفات، موانع أكسدة الجلوكوز، الورم الأرومي الدبقي متعدد الأشكال C6 ، اللاكتات، البيروفات و3-بروموبيروفات

للمراسلة:

قسم الكيمياء الحيوية السريرية والطب الجزيئي ، كلية طب طيبة ، جامعة طيبة ، المدينة المنورة ، المملكة العربية السعودية.

e-mail: Hussam.baghdadi@gmail.com

DOI: 10.12816/0061758

1. Introduction

Glioblastoma multiforme (GBM) is grade IV astrocytoma and is the most aggressive neurological malignancy having a poor prognosis in WHO 2016 classification of brain tumors. GBM stands for about 60%-70% of all astrocytomas with the worst prognosis. The prognosis of astrocytoma is quite variable and multifactorial depending on patients' age, disease stage, and histological type. Unfortunately, 5-years overall survival of GBM reaches approximately 4.8%-5.4% (Ostrom et al., 2020; Xie, Yang, Liu, & Zhao, 2018) Xie et al., 2018).

Combined with other potent cytotoxic agents e.g., histone deacetylase inhibitors, glycolysis inhibitors as 2-deoxyglucose and its derivatives were reported to synergistically eliminate glioblastoma cells. Histone deacetylase inhibitors as sodium butyrate and sodium valproate exerted synergistic cytotoxic effects against GBM cells (Pająk et al., 2021). The glycolysis inhibitor 3-bromopyruvate alone or combined with other anticancer agents or strategies was reported to significantly impair glioblastoma cells (and other types of cancer) survival, proliferation, metastasis, migration and angiogenesis (El Sayed et al., 2012-a; El Sayed et al., 2012-b? & El Sayed et al., 2012-c; El Sayed et al., 2013.

; El Sayed et al., 2014)

β-hydroxypyruvate is a metabolite of fructose (a hexose monosaccharide) and a biochemical analog of pyruvate, lactate and the promising anticancer drug 3-bromopyruvate (an inhibitor of the glycolysis enzyme Hexokinase II) (Figure 1). β-hydroxypyruvate was reported to exert promising anticancer effects. β-hydroxypyruvate was at least 2-fold significantly enhanced in hyperoxia was hydroxypyruvate. When cells were treated with Roxadustat to induce hypoxia-inducible factor stabilization, hydroxypyruvate was metabolized by endothelial cells and resulted in a 20-fold increase in 3-phosphoglycerate and a 4-fold increase in serine. In endothelial cells, hydroxypyruvate but not pyruvate increased proline hydroxylation and destabilized hypoxia-inducible factor. In choroidal explant experiments, hydroxypyruvate had angiostatic properties. A special metabolite produced by hyperoxia called hydroxypyruvate destabilizes HIF by a classical mechanism, at least in part (Singh et al., 2018).

In this study, the biochemical and pharmacological effects of β-hydroxypyruvate were investigated on the rat C6 glioblastoma cells.

Figure 1

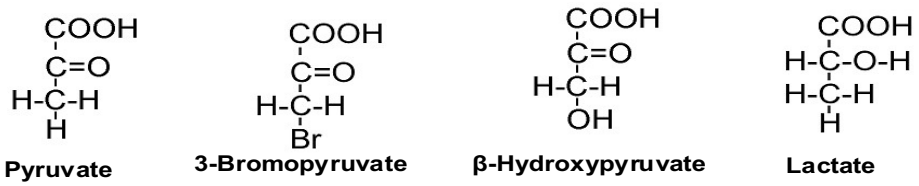

Figure 1. Lactate, hydroxypyruvate and 3-bromopyruvate are structural analogs of pyruvate.

2. Methods:

Experimental reagents and chemical agents

3-bromopyruvate, pyruvic acid (sodium salt), sodium fluoride (NaF), β-hydroxypyruvate, sodium L-lactate, fetal bovine serum (FBS), Dulbecco's modified Eagle's medium (DMEM), 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenylyltetrazolium bromide (MTT) (St. louis, MO, USA). Sodium acetate was purchased from Katayama Chemical Industries (Osaka, Japan). Citrate, H_2O_2, Dimethyl sulfoxide (DMSO) and agar were purchased from Wako (Osaka, Japan). DMEM/F12 and penicillin-streptomycin antibiotic mixture were from Invitrogen life technologies (Carlsbad, CA, USA). EDTA was from Dojindo molecular technologies (Kumamoto, Japan). Rabbit polyclonal anti-caspase-3 antibody was from Santa Cruz biotechnology (CA, USA).

Cells culture and maintenance

As previously reported in the methodology (El Sayed et al., 2012a), C6 rat glioma cells (purchased from Dainippon Pharmaceutical Co., Osaka, Japan) were seeded in plastic dishes DMEM/F12 with 15% (v/v) horse serum, 2.5% (v/v) FBS, and 1% penicillin-streptomycin at 37 °C in a humidified environment with 5% CO_2. The antibiotics 1% penicillin-streptomycin and horse serum with the nutrient DMEM were used to sustain the proliferation of glioblastoma cell line at 37 °C in a humidified environment with 5% CO_2.

MTT viability assay

As previously reported in the methodology (El Sayed et al., 2012a), C6 glioma cells were added to the 96-well plates for 24 hours or until cells achieved 80% confluency. Then, fresh stimulating media (DMEM/F12 with 1% FBS) was used containing the added treatment to the stimulating media. A 21-hour duration (incubation period) at CO_2 incubator was permitted. After adding MTT reagent (50 µl of 1 mg/ml solution) to all wells using a multichannel micropipette, the mixture was incubated for an additional 3–4 hours. DMSO addition (150 µl/well), centrifugation, and supernatant aspiration were carried out. In order to ensure the best possible dissolution, plates were shaken in a microplate shaker until all of the insoluble formazan crystals had been completely dissolved. OD was measured for all samples at 550 nm using Biotek Synergy multimode microplate reader. The standard curve was drawn and concentration values were assayed. Figure 2 describes the endogenous metabolic pathway of β-hydroxypyruvate.

Figure 2

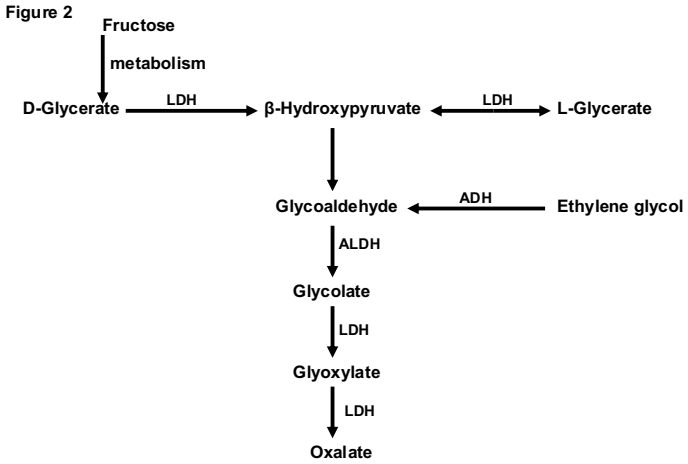

Figure 2. Fructose catabolism resulted in the formation of hydroxypyuvate via glycolysis and formation of D-glycerate. L-glycerate also can form fructose. Lactate dehydrogenase catalyzes many steps in the synthesis and catabolism of hydroxypyuvate.

Assaying hydrogen peroxide levels

As previously reported in the methodology (El Sayed et al., 2012a), estimation of $H_2O_2$was done in accordance with the manufacturer's instructions using the Amplex® red H_2O_2 assay kit (Molecular Probes, Invitrogen, CA, USA). To test the effectiveness of pyruvate and lactate in scavenging H_2O_2. H_2O_2 was added to cell-free media treated with exogenous H_2O_2 (250 µM). To examine the effects of pyruvate and lactate on ROS-steady state, an H_2O_2 assay was also performed after serially administering pyruvate and lactate to cultivated C6 cells. Using the same equipment, it was determined whether pyruvate and lactate had any impact on scavenging H_2O_2 generation.

Assaying ATP levels

As previously reported in the methodology (El Sayed et al., 2012b), Using an ATP determination kit from Molecular Probes (Eugene, OR, USA), the energetics of a C6 glioma were assessed 24 hours after therapy. 96-well plates containing C6 cells were seeded with 10.000 cells per well before being incubated for 24 hours. Fresh medium (DMEM/F12 with 1% FBS) was used for the treatment for 24 hours. After removing the medium, the ATP standard reaction solution was added and shielded from light. In C6 cells, ATP levels were estimated using the luminometer function of Biotek Synergy multimode microplate reader. The standard curve was drawn and concentration values were assayed.

Statistical Evaluation:

Data collection and analysis was done followed by data processing using SPSS software to determine the mean and standard error of the mean. The outcomes of the experimental groups were compared using the paired samples t test. Significant statistical indicators, such as *, **, and ***, indicated p-values less than 0.05, 0.01 and 0.001 respectively, when compared to the negative control. # indicated p< 0.05, ## indicated p< 0.01, and ### indicated p< 0.001 for comparing significant differences between several groups.

Results:

All of the above mentioned 2 paragraphs are not results, but biochemical facts that could be mention in the introduction or discussion.

Lactate, more than pyruvate, significantly antagonizes hydroxypyruvate

Hydroxypyruvate (2 mM) significantly (p<0.001) decreased C6 glioma energetics by about 50%. Lactate, more than pyruvate, significantly protected the energetics of C6 glioblastoma cells against hydroxypyruvate-induced decrease in their energetics. Pyruvate minimally but significantly (p<0.05) decreased hydroxypyruvate-induced decrease in C6 energetics. Lactate maximally and significantly (p<0.001) decreased hydroxypyruvate-induced decrease in C6 glioma energetics (Figure 3).

Figure 3

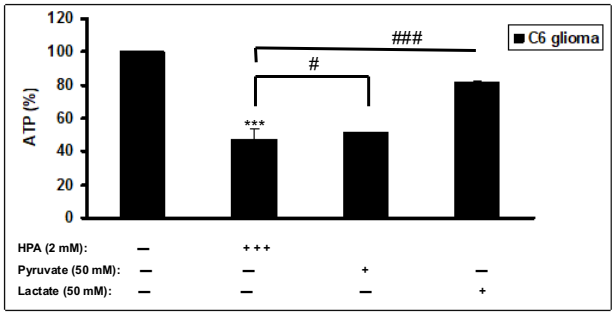

Figure 3. Hydroxypyruvate decreased C6 glioma energetics. Pyruvate slightly protected against hydroxypyuvate effects while lactate strongly protected.

Citrate has a synergistic effect with serial doses of β-hydroxypyruvate

Using MTT assay, citrate (3mM) decreased the viability of C6 glioblastoma cells by 10%. Hydroxypyruvate (0.25 mM) decreased the viability of C6 glioblastoma cells by about 20%. Adding serial doses of hydroxypyruvate (0.25, 5 and 1 mM) reduced the viability of C6 glioblastoma cells by 50%, 55% and 55%, respectively. Citrate has a synergistic effect with serial doses of hydroxypyruvate till reaching a maximal anti-glioma effect (Figure 4).

Figure 4

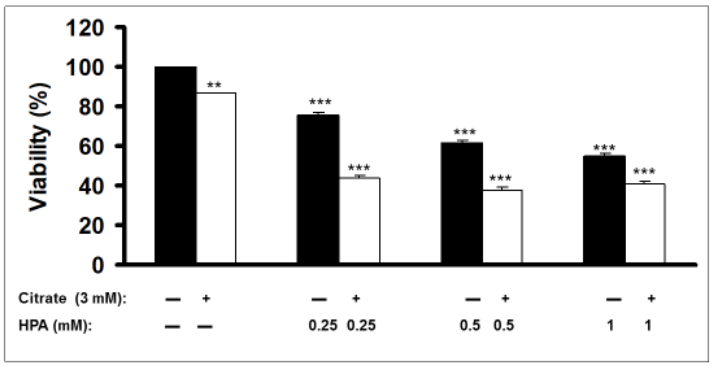

Figure 4. Citrate (a glycolysis inhibitor of phosphofructokinase) exerted a significant synergistic effect to hydroxypyuvate in glioma cells killing.

3-bromopyruvate, sodium fluoride and serial doses of hydroxypyruvate induce significant production of H_2O_2 in C6 glioma

Serial doses of hydroxypyruvate caused the generation of increasing quantities of hydrogen peroxide in a dose-dependent manner. Hydroxypyruvate (0.5, 1 and 2 mM) caused the generation of about 15000, 25000 and 45000 relative fluorescence units (RFU) of hydrogen peroxide. Relatively high doses of 3-bromopyruvate (100 µM) and sodium fluoride (15 mM) produced 5000 RFU and 6000 RFU of H_2O_2, respectively (Figure 5).

Figure 5

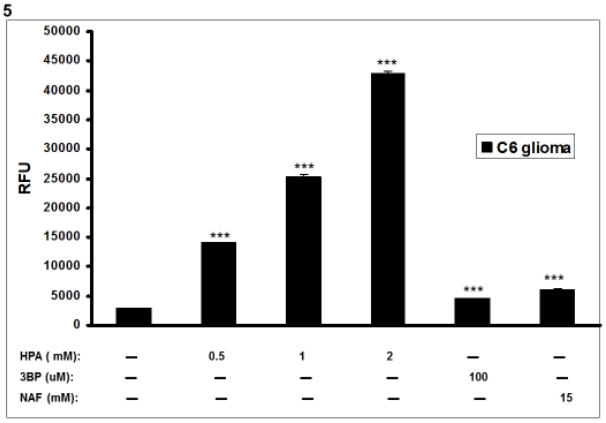

Figure 5. Hydroxypyuvate caused a significant dose-dependent generation of hydrogen peroxide more than that induced by the glycolysis inhibitors 3-bromopyruvate (hexokinase inhibitor) and sodium fluoride (enolase inhibitor).

Pyruvate, not lactate, significantly scavenged H_2O_2 generated due to 3-bromopyruvate and hydroxypyruvate in C6 glioma

Both 3-bromopyruvate and hydroxypyruvate caused the generation of H_2O_2 in C6 glioma cells as was previously reported (Figure 5). 100 µM 3-bromopyruvate caused the generation of H_2O_2 (about 5000 RFU) while 2mM hydroxypyruvate caused the generation of H_2O_2 (about 45000 RFU) (Figure 6). Adding lactate (10 mM and 100 mM) with both 3-bromopyruvate and β-hydroxypyruvate did not affect H_2O_2 scavenging in C6 glioma cells. However, adding pyruvate (100 mM) with both 3-bromopyruvate and hydroxypyruvate maximally and significantly decreased H_2O_2 generation in C6 glioma cells i.e. a scavenging effect (Figure 6).

Figure 6

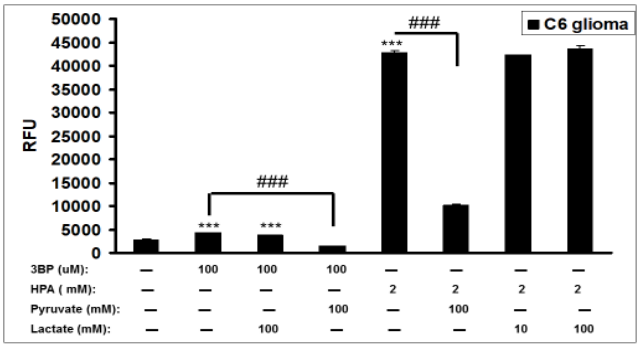

Figure 6. Pyruvate significantly and maximally scavenged the hydrogen peroxide generated due to hydroxypyuvate and 3-bromopyruvate. Lactate had no scavenging effects.

Glycine did not protect the viability of C6 glioma cells treated by hydroxypyruvate

Treatment of C6 glioma cells with 1 mM β-hydroxypyruvate resulted in about 40% decrease in C6 glioma viability. Adding serial doses of glycine (10, 50 and 100 mM) did not antagonize hydroxypyruvate-induced glioma cells death (Figure 7).

Figure 7

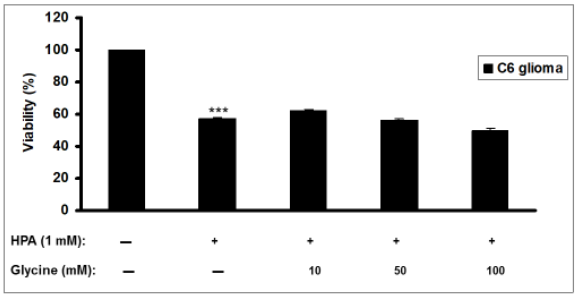

Figure 7. Glycine (a structural analog of hydroxypyuvate) exerted no protective effects against hydroxypyuvate-induced glioma cell death.

β-Hydroxypyruvate-induced H_2O_2 generation decreases with time

A time-dependent decrease in β-hydroxypyruvate-induced H_2O_2 production in C6 glioma took place. Pyruvate, not lactate, significantly and maximally scavenged H_2O_2 (Figure 8). Lactate exerted no H_2O_2 scavenging effects.

Hydroxypyruvate-induced timely H_2O_2 generation is scavenged by pyruvate

Pyruvate significantly and maximally scavenged β-hydroxypyruvate-induced timely H_2O_2 generation (Figure 8).

Figure 8

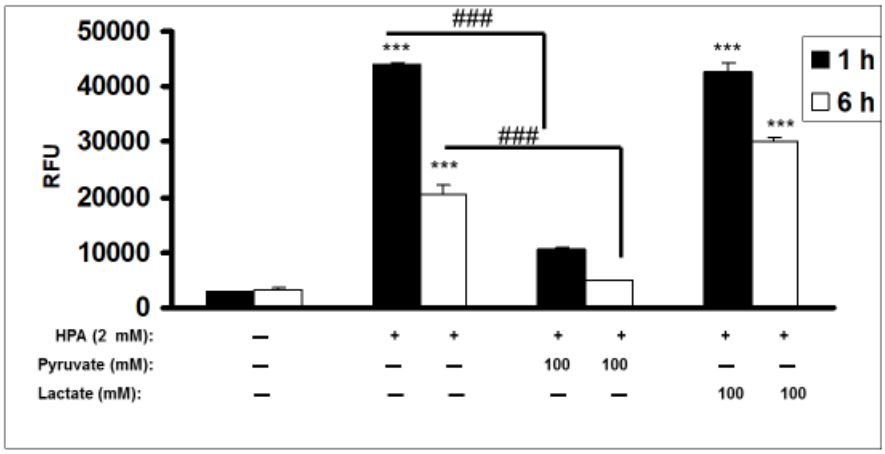

Figure 8. A significant timely decrease in hydrogen peroxide generation occurs due to hydroxypyuvate. That was significantly scavenged by pyruvate but not lactate

Statistical analysis

Data were collected and analyzed statistically using SPSS software (version 20 SPSS Inc., Chicago, Illinois, USA). Mean± SD were presented. One-way ANOVA analysis of variance was done. p value indicates significance differences from control group (*p <0.05, ** indicates p< 0.01 and *** indicates p< 0.001). #, # # and # # # indicate significance differences among different treatment conditions within the same group (# p <0.05, # # indicates p< 0.01 and # # # indicates p< 0.001).

Discussion:

There is structural similarity arising between pyruvate and its analogs 3-bromopyruvate, lactate and hydroxypyruvate. Lactate has the same structure of pyruvate plus 2 hydrogen atoms, 3-bromopyruvate is the same structure of pyruvate with replacing one hydrogen atom by a bromide ion. Hydroxypyruvate is the same structure of pyruvate with one hydrogen atom with a hydroxyl group (Figure 1).

Metabolic origin and fate of hydroxypyruvate

Main metabolic pathway of β-hydroxypyruvate arises mainly from the catabolism of the sugar fructose. Hydroxypyruvate is produced endogenously from fructose catabolism through glycolysis pathway till reaching 3-phosphoglycerate. Then, the enzyme lactate dehydrogenase (LDH) catalyzes multiple steps in the synthesis of hydroxypyruvate from fructose as well as from L-glycerate till reaching the final catabolite oxalate (Figure 2). Fructose is a

monosaccharide that contains a ketone group unlike glucose that contains an aldehyde group. Fructose-coated Angstrom silver particles was reported to suppress the gastric cancer growth by activating gasdermin D-mediated pyroptosis (Li et al.). The anti-tumor efficacy of fructose-coated Angstrom silver particles (F-AgÅPs) against lung and pancreatic cancer was confirmed. Fructose-coated Angstrom silver inhibited osteosarcoma growth and metastasis via promoting ROS-dependent apoptosis through the alteration of glucose metabolism by inhibiting the enzyme pyruvate dehydrogenase kinase (Hu et al., 2020). Interestingly, hydroxypyruvate can result endogenously from metabolism of fructose and d-glycerate (Figure 2). The close structural similarity between hydroxypyruvate and the well-known anticancer agent and glycolysis inhibitor (3-bromopyruvate, a hexokinase II inhibitor) (Figure 1) may confer anticancer effects to hydroxypyruvate. Moreover, our data confirmed that hydroxypyruvate exerted moderate anti-glioma effects in a dose-dependent manner at molar concentrations where 1-2 mM can decrease the glioma energetics (ATP %) (Figure 3) and kill almost 50% of glioblastoma cells (Figure 4).

Interestingly, hydroxypyruvate-induced hydrogen peroxide generation was confirmed in this study (Figure 4). It is dose-dependent and much more than the quantity of hydrogen peroxide generated by the effect of the other glycolytic inhibitors 3-bromopyruvate (a hexokinase II inhibitor) and sodium fluoride (enolase inhibitor) (Figure 5). Both of the glycolysis inhibitors 3-bromopyruvate and sodium fluoride significantly generated hydrogen peroxide ($p<0.001$) (Figure 5). Hydrogen peroxide generation may explain hydroxypyruvate-induced glioma cell death. Compared to previous studies where 3-bromopyruvate effectively killed glioma cells at micromolar concentrations (El Sayed et al., 2012-a; El Sayed et al., 2012-b & El Sayed et al., 2012-c, hydroxypyruvate moderately killed glioma cells using more concentrations (in millimolar range). This confirms superiority of 3-bromopyruvate over hydroxypyruvate as an anticancer agent. Metabolism of 3bromopyruvate was strongly suggested to be through glutathione conjugation (El Sayed et al., 2017). No report is there to confirm if a similar biochemical role is occurring to hydroxypyruvate.

Decreasing cellular energetics in cancer cells may reflect decreased viability. Being a structural analog to hydroxypyruvate, this study investigated a possible antagonistic effect of its two naturally occurring structural analogs: pyruvate and lactate. This study revealed that lactate was better than pyruvate as an inhibitor of hydroxypyruvate effects on C6 glioma energetics. At equal molar concentrations, lactate rescued C6 glioma cells energetics maximally and was stronger than the effects of pyruvate. However, this did not go in line with the effects of both lactate and pyruvate against hydroxypyruvate-induced hydrogen peroxide generation (Figure 6) where pyruvate maximally inhibited hydroxypyruvate-induced generation of hydrogen peroxide while lactate had no effect. This gives the impression that hydroxypyruvate kills glioma cells partially through an oxidative stress mechanism. In other words, although hydroxypyruvate resulted in generation of enormous oxidative (large quantities of hydrogen peroxide) reaching about 45000 RFU, this was not translated into a corresponding increase in C6 glioma cells killing. So, hydroxypyruvate-induced glioma cells death is partly not totally due to an oxidative stress mechanism.

Combining hydroxypyruvate (0.25 mM) with the natural glycolysis inhibitor citrate (3 mM) produced a dose-dependent synergistic effect in decreasing C6 glioma viability till reaching a maximal glioma killing effect (Figure 4). Interestingly, both hydroxypyruvate and citrate are antagonistic regarding the oxidative stress effect. Citrate is antioxidant while hydroxypyruvate is a pro-oxidant.

In this study also, adding glycine amino acid did not protect the viability of C6 glioma cells against hydroxypyruvate effects despite the presence of a structural similarity between glycine and

hydroxypyruvate (Figure 7). In other words, feeding glycine or glycine-rich proteins to glioma patients may not inhibit hydroxypyruvate-induced glioma cells killing.

Finally, generation of hydrogen peroxide due to treatment of C6 glioma cells with hydroxypyruvate decreased in a significant timely fashion. It started high then declined. Pyruvate-induced scavenging of hydrogen peroxide was evident while lactate did no scavenging effects (Figure 8). This data confirmed the antioxidant cytoprotective effects conferred by pyruvate that are lacking in lactate.

Conclusion

This study concluded that β-hydroxypyruvate, an analog of the glycolysis inhibitor 3-bromopyruvate induces the generation of large quantities of hydrogen peroxide, which can not by itself explain its moderate antiglioma effects. Despite lacking an antioxidant effect, lactate is a potent inhibitor of β-hydroxypyruvate-induced glioma cell killing. The opposite is true for pyruvate that exhibits minimal inhibition of β-hydroxypyruvate despite being a potent antioxidant. Glycine amino acid exerts no protective effects against β-hydroxypyruvate-induced glioma cell death.

Conflict of interest

The author declares that there is no conflict of interest with any one

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial or not from profit sectors.

Authors' contribution

The author (HHB) is single in this article. All manuscript issues (design, drafting, approving the submission and revision) were done. All criteria were met:

Substantial contributions to the conception or design of the work; analysis, interpretation of data for the work.

* Drafting the work or revising it critically for important intellectual content.
* Final approval of the version to be published.
* Agreement to be accountable for all aspects of the work in ensuring that questions related.

Ethical approval statement: Not applicable as this is an *in vitro* study on cancer cell lines

References

El Sayed, S. M., Baghdadi, H., Zolaly, M., Almaramhy, H. H., Ayat, M., & Donki, J. G. (2017). The promising anticancer drug 3-bromopyruvate is metabolized through glutathione conjugation which affects chemoresistance and clinical practice: An evidence-based view. *Medical hypotheses, 100*, 67-77.

Hu, X.-K., Rao, S.-S., Tan, Y.-J., Yin, H., Luo, M.-J., Wang, Z.-X., . . . Du, W. (2020). Fructose-coated Angstrom silver inhibits osteosarcoma growth and metastasis via promoting ROS-dependent apoptosis through the alteration of glucose metabolism by inhibiting PDK. *Theranostics, 10*(17), 7710.

Li, Y. Y., Xia, K., Liu, Y. W., Tan, Y. J., Li, H. M., Wang, Y. Y., . . . Cao, J. Fructose-coated Ångstrom silver particles suppress gastric cancer growth by activating gasdermin D-mediated pyroptosis. *Advanced Therapeutics*, 2200100.

Ostrom, Q. T., Patil, N., Cioffi, G., Waite, K., Kruchko, C., & Barnholtz-Sloan, J. S. (2020). CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2013–2017. *Neuro-oncology, 22*(Supplement_1), iv1-iv96.

Pająk, B., Siwiak-Niedbalska, E., Jaśkiewicz, A., Sołtyka, M., Zieliński, R., Domoradzki, T., . . . Priebe, W. (2021). Synergistic Anticancer Effect of Glycolysis and Histone Deacetylases Inhibitors in a Glioblastoma Model. *Biomedicines, 9*(12), 1749.

Singh, C., Sharma, A., Hoppe, G., Song, W., Bolok, Y., & Sears, J. E. (2018). 3-Hydroxypyruvate destabilizes hypoxia inducible factor and induces angiostasis. *Investigative Ophthalmology & Visual Science, 59*(8), 3440-3448.

Xie, J. C., Yang, S., Liu, X. Y., & Zhao, Y. X. (2018). Effect of marital status on survival in glioblastoma multiforme by demographics, education, economic factors, and insurance status. *Cancer medicine, 7*(8), 3722-3742.