

## A New Approach to Therapy for Hepatocellular Carcinoma?

*Geschwind JH, Ko YH, Torbenson MS, Magee C, Pedersen PL.* Novel therapy for liver cancer: direct intra-arterial injection of a potent inhibitor of ATP production. *Cancer Res* 2002;62:3909-3913. (Reprinted with permission.)

### Abstract

**Objective:** To assess the efficacy of 3-bromopyruvate (3-BrPA), a strong alkylating agent that inhibits hexokinase and mitochondrial oxidative phosphorylation, resulting in decreased cellular ATP production, on rabbit VX2 tumor cells.

**Methods:** Adult New Zealand White rabbits were used for the study. The rabbit VX2 tumor was originally grown on the hind leg of a carrier rabbit for two weeks and then excised, minced in Hank's solution and 0.1-0.2 ml of tumor cells were directly implanted into the left lobe of six rabbit livers and allowed to grow for 14 days. After appropriate anesthesia, 25 ml of 0.5 mM 3-BrPA was injected in bolus form, over 2 minutes, into the left hepatic artery through a transcatheter approach, under fluoroscopic guidance. Additionally, embolization of the left hepatic artery was then performed in a similar manner, using a mixture of Ethiodol and polyvinyl alcohol. Animals treated identically but not receiving 3-BrPA served as controls. Four days later, the liver was harvested and normal tissue and tumors were fixed in 10% formalin, sliced at 5-mm intervals, embedded in paraffin, and then sliced into 4- $\mu$ m sections and stained with H & E. Tumor viability was assessed by visual inspection.

**Results:** Compared with controls which had nearly 100% viable cells, the tumors treated with 3-BrPA contained essentially no viable cells, in other words nearly 100% necrosis. A small number of viable cells were noted in a few localized areas near arteries feeding the tumors, and at the tumor periphery, where sinusoidal blood flow was available. No damage to the normal tissue surrounding the tumor was noted. These results were reproduced in several experiments. Statistical evaluation revealed that tumors untreated with 3-BrPA contained  $74 \pm 5\%$  viable cells, and those treated with a single injection of 3-BrPA decreased the number of viable cells to  $16 \pm 5\%$ . The only apparent damage was seen occasionally in the peribiliary arteriolar complexes at much higher concentrations of 3-BrPA (5mM).

Furthermore, the effect of embolization of the VX2 tumor on adjacent liver tissue was assessed in a separate group of similar rabbits. It was found that embolization alone of the left hepatic artery caused significant injury to surrounding tissue manifested by extensive amounts of nonviable tissue when examined histologically. Interestingly, tissue from nine other organ systems was evaluated histologically 4 days after intra-arterial injection of 3-BrPA and no injury was seen. However, secondary tumors were found in the lungs of all rabbits implanted with VX2 tumors, in both the treated and control groups. Systemic delivery of 25 ml, 0.5 mM

3-BrPA was then performed. Animal toxicity was then assessed. Interestingly, the control rabbits that had been harboring liver implanted VX2 tumors for 14 days and now were given 3-BrPA systemically through an ear vein, demonstrated no obvious tissue damage in nine different organ systems examined. Moreover, there was no killing effect on the liver tumors. However, the lung tumors, the largest of which were several millimeters in diameter, were markedly decreased in size or completely obliterated.

**Conclusions:** 3-BrPA, when given through intra-arterial injection into VX2 tumor cells, appears to be effective in killing the great majority of tumor cells without any collateral tissue damage. Additionally, when given systemically, this potent inhibitor of cellular ATP production significantly decreases the size of metastatic tumors to the lung, again without evidence of native tissue damage.

### Comments

Treatment options for unresectable hepatocellular carcinoma (HCC) have traditionally consisted of percutaneous ethanol injection and radiofrequency ablation for the smaller tumors and arterial embolization, chemoembolization, intravenous, and intra-arterial chemotherapy for larger or disseminated tumors. Local ablative techniques appear to be quite successful in treating smaller tumors and patient survival after local ablation matches that seen with surgical resection. However, the benefits of the other treatments listed are not as clear. The beneficial effects of chemoembolization on patient survival are not clear<sup>1-3</sup> and this approach may be associated with significant hepatic injury, sometime precipitating liver failure. When HCC is very large or spread beyond the liver, traditional chemotherapy approaches appear to have little benefit. Thus, there is no universally agreed on standard treatment for unresectable HCC.

As with many diseases, basic molecular research has provided new insights into the metabolic characteristics of HCC and has opened up new possibilities for treatment. Previous studies have shown that HCC cells are rapidly growing and have a high rate of glucose catabolism. Use of glucose is regulated, in part, by the glycolytic activity of hexokinase. Four isoforms of hexokinase have been identified in human tissue, and these seem to have a tissue-specific distribution.<sup>4</sup> Type I is most abundant in brain and erythrocytes, type II in both skeletal muscle and adipocytes (this isoform is highly sensitive to insulin), and type IV is typically expressed in normal liver and pancreatic cells.<sup>5</sup> Types I and II have unique characteristics in that they have the ability to bind to mitochondria, which

allows the cell to preferentially use adenosine triphosphate (ATP) produced by oxidative phosphorylation within the cell.<sup>4</sup> Additionally, protein synthesis is increased through the use of adenosine diphosphate in the tricarboxylic acid to generate specific amino acids.

Molecular studies have shown that HCC cells, as distinct from normal hepatocytes, have an increased expression of type II hexokinase relative to type IV hexokinase. Rempel et al.,<sup>6</sup> using Southern blot analysis and fluorescence *in situ* hybridization studies in rat HCC cells, have shown that hexokinase activity is significantly up-regulated relative to normal hepatocytes. This work was corroborated by Shinohara,<sup>7</sup> who used Northern blot analysis. Moreover, when exposed to hypoxic conditions, hepatoma cells activate the type II hexokinase promoter up to 7-fold.<sup>8</sup> These adaptive modifications within HCC cells confer a survival advantage in hypoxic conditions over noncancerous hepatocytes by increasing the affinity for glucose by up to 100-fold.<sup>5</sup> Geschwind et al. have therefore made use of this predilection of HCC for ATP by infusing an inhibitor of hexokinase (3-BrPA) directly into the hepatic artery. They found that this resulted in very specific necrosis of implanted HCC.

The rabbit VX2 tumor has been shown in previous studies to be an ideal model for evaluating HCCs. This tumor exhibits rapid growth, increased glycolysis, and increased expression of hexokinase.<sup>9</sup> When exposed to 3-BrPA, hexokinase is directly inhibited. Similarly, when HCC cells in culture are exposed to 3-BrPA, hexokinase was inhibited, facilitating cell death.<sup>9</sup>

Several lessons can be learned from this intriguing report. First, it appears that 3-BrPA is quite effective at killing malignant hepatocytes in an animal model, without causing damage to adjacent, normal liver tissue or to other organ-specific systems. This is a significant finding because the available therapeutic modalities involving chemoembolization to date almost uniformly cause surrounding tissue damage. In patients with minimal hepatic reserve, this can have devastating consequences.

Secondly, the VX2 rabbit animal model appears to be a good model for studying growth and treatment of HCC. The VX2 tumor, grown on rabbits, seems to have several similar characteristics to human HCC and appears to be an easy model to study. Also, the size and vascular anatomy of the rabbit allow for easy cannulation and access to hepatic vasculature, making investigational studies possible and reproducible.

Finally, hexokinase has been shown to be of vital importance to hepatoma cell cellular respiration. Hexokinase is necessary to convert glucose to glucose-6-phosphate, which leads to generation of ATP. HCC cells, as previously mentioned, have significantly up-regulated

hexokinase genes that allow the cells to survive in hypoxic conditions as they continue to grow and outstrip the blood supply. The alkylating agent, 3-BrPA, inhibits this process and also inhibits ATP production by oxidative phosphorylation. By blocking this process, cells are not able to survive.

However, several questions remain to be answered. How is it that the noncancerous tissue is not killed or injured as well, especially skeletal muscle tissue that also has the type II isoform of hexokinase that HCC cells express? Is it simply because of the significant up-regulation of type II hexokinase activity in tumor cells? Or could it be that the natural antioxidant system (*i.e.*, superoxide dismutase and glutathione) inherent to native cells may be more robust than HCC cells? It is interesting to note that there remained some viable cells adjacent to arterioles, suggesting that in areas of high oxygen exposure, hexokinase expression is not enhanced. Also, what will be the impact of interfering with energy metabolism in cirrhotic liver as opposed to normal surrounding liver in these animal studies? Will the injury to adjacent, nontumorous liver be greater if the underlying liver is diseased?

A second important question relates to the systemic injection of 3-BrPA that seemed to have a beneficial effect on metastatic tumors in the lung, but no effect on liver tumors. Does it matter where the systemic injection was given? In this study the systemic 3-BrPA was given via an ear vein, meaning that the alkylating agent would first pass through the lung before being exposed to the liver. Is the dose taken up entirely by the metastatic pulmonary tumors? Clearly, studies of pharmacokinetics and pharmacodynamics of 3-BrPA are needed.

In summary, it appears that 3-BrPA is an effective treatment for reducing tumor cell viability in the rabbit VX2 tumor model, without subjecting the adjacent liver tissue to damage. Furthermore, metastatic tumors also have been shown to respond to this therapy. These data are very encouraging, and although there are still questions to be answered, this therapeutic modality offers promise in a disease for which new treatments are sorely needed, and further investigation seems warranted.

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