CuTira Brachytherapy: A New Combination of Radioactive Copper Isotopes and the Hypoxic Cytotoxin, Tirapazamine, for Targeted Tumor Therapy

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We previously showed a significant enhancement of tirapazamine-induced cytotoxicity and DNA damage after binding with copper. This result suggests that conjugates of tirapazamine with radioactive copper, i.e., ^{64}Cu and ^{67}Cu, may offer potential for targeted therapy of a wide range of advanced stage tumors including a possible treatment for patients with solitary hepatocellular carcinoma by intrahepatic arterial infusion. Major supporting considerations include: (a) tirapazamine having a high selective toxicity against hypoxic cells; (b) the nature of radioactive decay of these copper isotopes and obtainable high specific activity; and (c) simple procedure for the production of copper-tirapazamine complex.

Key Words: copper isotopes; hypoxic cytotoxin, tirapazamine, tumor therapy

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There is an increasing interest in therapeutic applications of copper isotopes in nuclear medicine (*1–6*). These recent articles emphasize the tumor therapeutic potential of a ^{64}Cu-labeled monoclonal antibody. While Connett et al. (*6*), in a radioimmunotherapy study, demonstrated encouraging experimental results against small tumors, they also advocate developing other strategies to deliver higher tumor doses and dose fractionation. We have recently reported a new cytotoxic mechanism for tirapazamine, a hypoxic cytotoxin, in which it binds with metal ions, particularly the copper ion, resulting in an increased cytotoxic effect (*7*). We propose to use this copper-tirapazamine complex forming property to combine tirapazamine and radioactive copper ions for intratumor therapy. We call this application CuTira brachytherapy. This form of therapy may provide antitumor activity due to the copper-tirapazamine complex in combination with the radioactivity of the copper isotope. The therapeutic dose of CuTira brachytherapy, therefore, may be lower than those used in individual conventional applications of these agents. The development of this form of therapy should benefit from the substantial existing experience with tirapazamine (*8,9*) and in vivo application of radioactive copper isotopes (*5,10*) in tumor therapy. The design of CuTira brachytherapy is based on the following:

1. Radiotherapy is an effective modality for treating solid tumors. Efforts to increase the therapeutic gain of radiotherapy by reducing the influence of hypoxia on radioresistance of tumor cells has been a major research focus for many investigators and clinicians. This approach has changed dramatically since the realization that low oxygen tension, which is common in many tumor tissues and rarely occurs in normal tissues, can actually be exploited by using hypoxic cytotoxins (*11*). These agents act like pro-drugs exerting little cytotoxic effect on cells under oxic conditions but undergoing bioreductive transformation to cytotoxic forms under low oxygen conditions. Tirapazamine is currently the most actively studied of these bioreductive agents. It has been used in a variety of experimental models and has advanced to Phase III clinical trials to determine its ability to enhance the effectiveness of radiotherapy (*8,9*).
2. The energies of the β-particles emitted from ^{64}Cu and ^{67}Cu are very similar to those of ^{131}I. Thus, copper isotopes should provide advantages similar to those of ^{131}I in tumor therapy. Copper-64 may be a better choice in this application than ^{67}Cu because it has a shorter half-life (12.8 hr compared to 61 hr), can be produced with a higher specific activity (>500 mCi/μg of Cu) and has a greater potential availability (*6,12*). Moreover, ^{64}Cu may be less radio-toxic to normal tissues than ^{131}I because its γ-ray abundance is lower.
3. Copper-64 and ^{67}Cu have similar cytotoxic effects. The mean lethal activity (D_{37}) of ^{64}Cu for two mammalian cell lines tested were 105 and 190 μCi/ml (*13*). More importantly, the efficacy of ^{64}Cu has been demonstrated in vivo (*6*). Even in microgram quantities of high specific activity, ^{64}Cu might be expected to kill a substantial proportion of tumor cells.
4. Copper-64 tirapazamine complex has all the features of a conventional unsealed radioisotope source used for therapy including the type of radiation emitted and its energy characteristics and half-life, cost, availability and ease of production (*6,14*).
5. The doses of tirapazamine used in experimental tumor therapy ranged from 20–50 mg/kg body weight (*15,16*). We estimate 20 μg in 20 μl to be an appropriate dose for a 1-cm^3 tumor. Since tirapazamine can complex with copper ions at a 2:1 ratio (*7*), this amount of tirapazamine should be able to complex with up to several micrograms of ^{64}Cu.
6. To achieve a high level of distribution within the tumor, the radioactive complex should be injected directly into the tumor mass once or in several injections. Such intralesional injections have been used in cytokine, drug and gene therapies. Quantities of 10–20 μg/10–20 μl of tirapazamine would first be complexed with 1–2 μg (or higher)/1–2 μl ^{64}Cu and then infused under positive pressure at a rate of 1–2 μl/min into a tumor mass of 1-cm^3 or smaller. This dose of CuTira therapy would achieve a therapeutic level of tirapazamine and, even assuming a ^{64}Cu specific activity as low as 50 mCi/μg,

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would add 100 mCi of radioactivity. Repeated treatments (perhaps with a smaller dose volume over a period of days) could further improve the distribution.

This approach offers some potential advantages over a ^{64}Cu-labeled monoclonal antibody (*6*). First, since the preparation of CuTira is simpler than radiolabeling a monoclonal antibody, using CuTira to deliver multiple doses of ^{64}Cu will be easier. Second, unlike monoclonal antibody application that is limited to tumors having the matching antigen, CuTira may be used to treat many types of tumors. Finally, CuTira is not expected to trigger any immune reactions.

The principles of CuTira application we have outlined may be applied to a wide range of advanced stage tumors. CuTira brachytherapy also may provide a unique application in combination with Lipiodol/alcohol for treating patients with solitary hepatocellular carcinoma (HCC) by intrahepatic arterial infusion. While most of the conventional therapies including chemotherapy, radiotherapy and conformal radiotherapy, radioimmunotherapy and, with a small number of exceptions, various surgical approaches are ineffective in HCC, intrahepatic arterial injection of Lipiodol-based radio/chemotherapy has improved tumor response and effectively delivered therapeutic agents to liver metastases (*17,18*). This improvement is because Lipiodol administered through the hepatic artery is selectively retained in HCC for a prolonged period of time (*19*). Although enhanced tumor responses were noted from this procedure, an increase in patient survival has not yet been established, which may indicate significant regrowth of residual HCC cells. Alcoholization has been used to treat renal cell carcinoma (*20*) and liver cysts (*21*) as well as to achieve emergency hemostasis of or prevent the rupture of HCC (*22*). Intratumor injection of ethanol alone has been shown to be effective in the destruction of HCC (*23*). CuTira brachytherapy is technically compatible with intrahepatic arterial infusion for treating HCC. CuTira prepared in 95% ethanol and mixed with Lipiodol can be infused via the intrahepatic artery. In this application, both radioactive and nonradioactive copper complexed with tirapazamine may exert a significant antitumor effect. This expectation is based on the fact that acute alcohol application in the liver can produce pericentral hypoxia (*24*), which in turn can promote the hypoxic cytotoxicity of tirapazamine (*11*) against residual viable tumor cells. Liver cells are known to contain the high concentration of tirapazamine reductase necessary for this bioreductive transformation. It has been shown in some HCC patients that copper accumulates to a greater degree in the tumor than in the surrounding liver parenchyma (*25*), which may further enhance the therapeutic effect from HCC by the copper tirapazamine action (*7*).

The use of radiolabeled agents to treat liver tumors, e.g., ^{131}I-Lipiodol or ^{131}I-labeled antiferritin antibody, has shown encouraging therapeutic effects (*26,27*). CuTira brachytherapy offers two additional features: a new therapeutic mechanism and compatibility with the technique of intrahepatic arterial infusion.

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