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Tirapazamine causes vascular dysfunction

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Abstract

Tirapazamine exhibits preferential cytotoxicity towards hypoxic cells *in vitro*. Currently tirapazamine is undergoing Phase II/III clinical evaluation in combination with radiation and chemotherapeutic agents for the treatment of non-hematological cancers.

Tissue penetration studies using multicellular models have suggested that tirapazamine diffusion to distal, hypoxic cells is limited. However, animal studies show tirapazamine enhances the anti-tumor activity of radiation and chemotherapy, and clinical studies with tirapazamine, so far, have been promising.

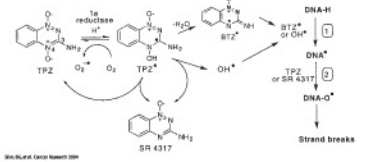
To investigate this apparent paradox we examined the histophysiology of tumors grown in mice after tirapazamine administration; the location of cell toxicity in relation to vasculature was observed using BrdUrd labeling as a marker of cell division. Effects on cells far from blood vessels were not seen, as might have been expected, instead we observed striking central vascular dysfunction after 1 day.

Tirapazamine (TPZ)

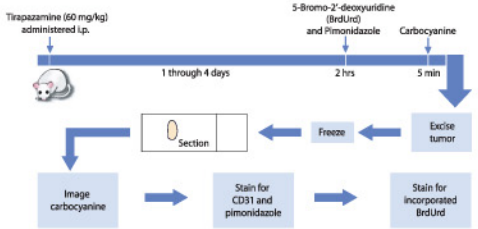
In vitro Tirapazamine is preferentially toxic to cells at low oxygen tensions.

It is activated under hypoxic conditions to a reactive free radical intermediate which in turn leads to double strand breaks in DNA.

The anti-tumor activity of radiation therapy and chemotherapy agents, such as cisplatin, has shown to be enhanced *in vivo* by tirapazamine.



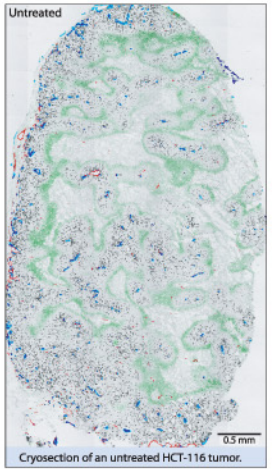
Experimental Procedure



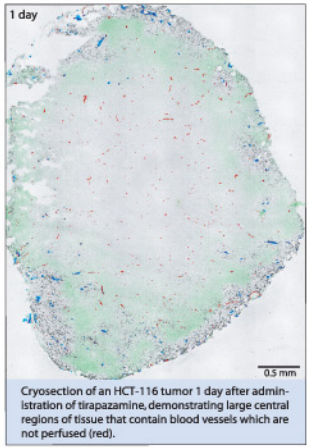
Summary

We observed extensive and permanent vascular dysfunction in a large proportion of tumors from mice treated with tirapazamine. In the affected tumors, blood flow ceased in the centrally located tumor vessels, leaving a rim of functional vessels around the periphery of the tumor. This vascular dysfunction commenced within 1 day after tirapazamine administration and was replaced by necrosis over the following 1-2 days.

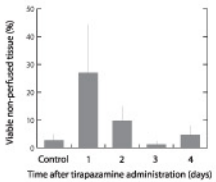
Tirapazamine causes central vascular dysfunction



Cryosection of an untreated HCT-116 tumor.

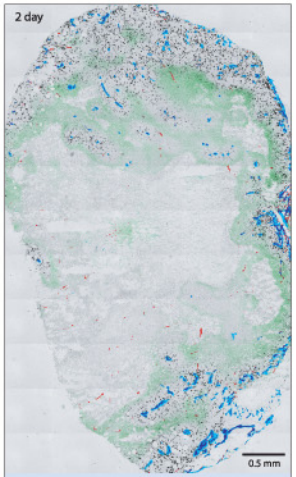


Cryosection of an HCT-116 tumor 1 day after administration of tirapazamine, demonstrating large central regions of tissue that contain blood vessels which are not perfused (red).

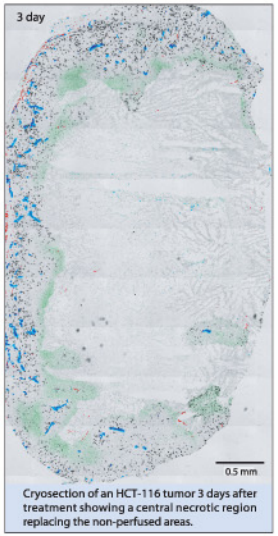


Tirapazamine causes vascular dysfunction in the central regions of tumors 1 day after treatment. The mean proportion of non-perfused, viable tumor tissue in untreated HCT-116 tumors is 2.6 %. One day after treatment with tirapazamine the mean value of non-perfused tissue increases to 27 %. Each vertical bar represents the mean data from several HCT-116 tumors (n= 7-10). Lines represent standard deviation.

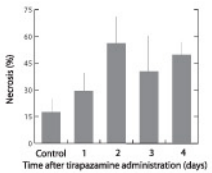
Areas of vascular dysfunction become necrotic



Cryosection of an HCT-116 tumor 2 days after treatment. Non-perfused areas are becoming necrotic.



Cryosection of an HCT-116 tumor 3 days after treatment showing a central necrotic region replacing the non-perfused areas.



Tirapazamine causes extensive central necrosis 2-4 days after treatment. The mean amount of necrosis in untreated tumors is 17 %. Each vertical bar represents the mean data from several HCT-116 tumors (n= 7-10). Lines represent standard deviation.

Data analysis

Images were cropped to remove necrosis and areas of non-perfused tissue. The total number of pixels for each selection was obtained and the proportion of necrotic or non-perfused tissue was calculated by dividing the number of pixels which were necrotic or non-perfused by the total number of pixels for each tumor.

Staining key

- Perfused blood vessels (CD31)
- Perfusion marker (Carbocyanine)
- Blood vessels that are not perfused
- Hypoxia (Pimonidazole)
- Proliferating cells (BrdUrd)
- Tissue

Hypothesis

We propose that the activity of tirapazamine *in vivo* may be related to its effects on hypoxic tumor vasculature located in the centre of tumors and that its activity against hypoxic cells located distal to functional blood vessels may not be as important as previously believed.

Other cell lines

Similar results have been observed in tumors from SiHa (human cervical carcinoma) and SCCVII (murine carcinoma) cell lines. Central vascular dysfunction is seen 1 day after tirapazamine administration and is replaced by necrosis over the following 1-2 days.

Acknowledgements
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