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Motexafin Gadolinium

(Gd-Tex^{2+})

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Abbreviations

Gd-Tex: Motexafin gadolinium

MRI: Magnetic Resonance Imaging

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Abstract

There is a growing need to increase the efficacy of various modalities used as cancer therapies, to achieve complete tumor regressions and cure. Radiation therapy, one of the common modality is limited by amount of dose received to normal tissues. Hence, it is necessary to develop better radiosensitizers that will improve tumor response to radiation and reduce normal tissue morbidity. A new class of prophyrin-like synthetic radiosensitizers has been developed, known as texaphyrins. Motexifin gadolinium previously known as gadolinium texaphyrin, is a novel radiosensitizer that is presently undergoing Phase III clinical trials for brain metastases. In addition to its selectivity to tumors, it is detectable by MRI. These properties make Gd-Tex an almost ideal radiosensitizer . However recent reports have cast doubts on its radiosensitizing ablilities. This report reviews the molecule and its proposed mechanism of action.

Introduction

The two most widely used treatment modalities for cancer are radiotherapy and chemotherapy. However the benefits of these treatments are severely restricted by normal tissue damage coupled with tumor cell resistance. Radiosensitizers are compounds that improve the efficacy of delivered radiation. A new class of radiosensitizers is being extensively explored, called lathanide texaphyrins [1]. Texaphyrins are metal coordinating expanded porphyrins represented by complexes I (Gd-Tex, motexafin gadolinium) and II (Lu-Tex, motexafin lutetium) as seen in Figure 1. Like porphyrins, texaphyrins are fully aromatic and highly colored (Absorbance >700 nm).

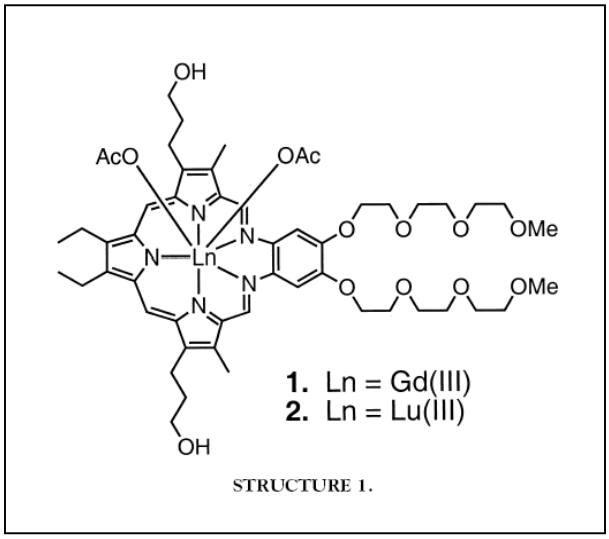

Figure 1. Structure of lanthanide III texaphyrins showing porphyrin-like aromatic rings and five coordinating nitrogens in the core. OAc represents axially coordinated acetate ions [2].

Inaddition, texaphyrins complexes I and II are easier to reduce than most metalloporphyrins {$E_{1/2}$ = –0.041 V and –0.044 V *vs* hydrogen electrode}. These synthetic porphyrin analogues now are being studied, for their use as drugs in a wide range of medical therapies including radiosensitizers, photodynamic therapy of tumors, and atherosclerosis. Another feature of these compounds is their ability to be detected in MRI. All these factors have lead to extensive studies on its mechanism of action and use in therapy. In fact XCYTRIN (Gd-

Tex) in already in Phase III clinical trials as a radiosensitizer for patients with metastatic cancers of the brain.

Mechanism of Action

The mechanism under which Gd-Tex increases radiosensitivity of cells is still under investigation. The role of reactive oxygen species in modulating radiation response of cells is a topic of great interest. Recent evidences have shown that Gd-Tex may exert its effects through intracellular redox changes [3]. A possible explanation for its activity can be found in the discovery that Gd-Tex produces H_2O_2 *via* superoxide production even in the absence of ionizing radiation using reducing metabolites *e.g*. ascorbate and NADPH (Figure 2).

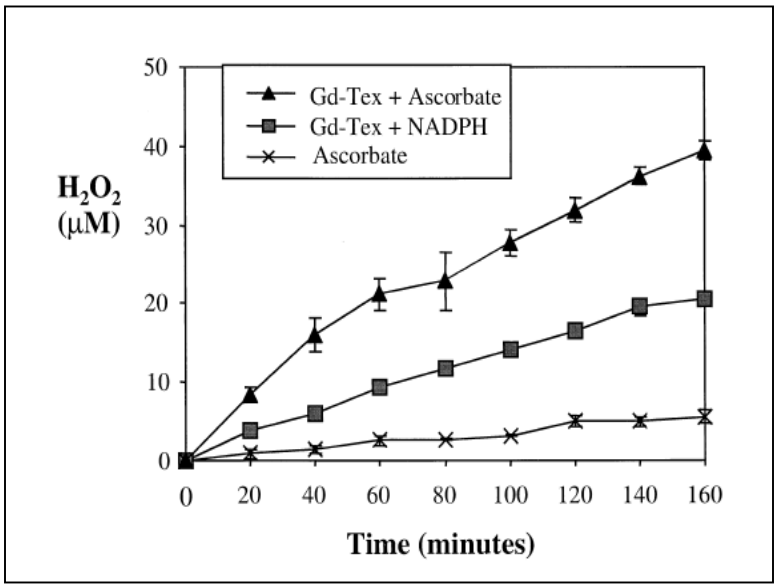

Figure 2: Gd-Tex can oxidize NADPH and ascorbate, forming hydrogen peroxide. Colorimetric method was used to measure hydrogen peroxide formation. NADPH or ascorbic acid (250 uM) and Gd-Tex (12.5 uM) were mixed and incubated. Amount of hydrogen peroxide formed was measured at indicated intervals of time [3].

Pulse radiolysis studies have been used to elucidate the Gd-Tex mechanism of action [4]. A large excess of oxygen was allowed to react with hydrated electrons for the selective production of superoxide anions (reaction 1). It was found that under these conditions there was equilibrium between Gd-Tex and superoxide anion (reaction 2) and the Gd-Tex radical cation as

formed in the 0.5-1 ms time scale. Likewise the kinetic rate constants for the forward and reverse reaction were calculated as $k = 9.8 \times 10^6\ M^{-1}\ s^{-1}$ and $k_{-1} = 3.4 \times 10^6\ M^{-1}\ s^{-1}$.

$$e^-_{(aq)} + O_2 \longrightarrow O_2^{\bullet -} \qquad \text{(reaction 1)}$$

$$O_2^{\bullet -} + \text{Gd-Tex}^{2+} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{Gd-Tex}^{\bullet +} + O_2 \qquad \text{(reaction 2)}$$

Where $k_1 = 9.8 \times 10^6\ M^{-1}\ s^{-1}$ and $k_{-1} = 3.4 \times 10^6\ M^{-1}\ s^{-1}$.

These findings suggest that Gd-tex treatment leads to oxidative stress as a result of redox cycling. For example as seen in Figure 3, superoxide would be formed by after ascorbate oxidation by Gd-tex. Further reduction of superoxide would lead to hydrogen peroxide formation. The reduction of hydrogen peroxide would consume the reduced GSH and form GSSG.

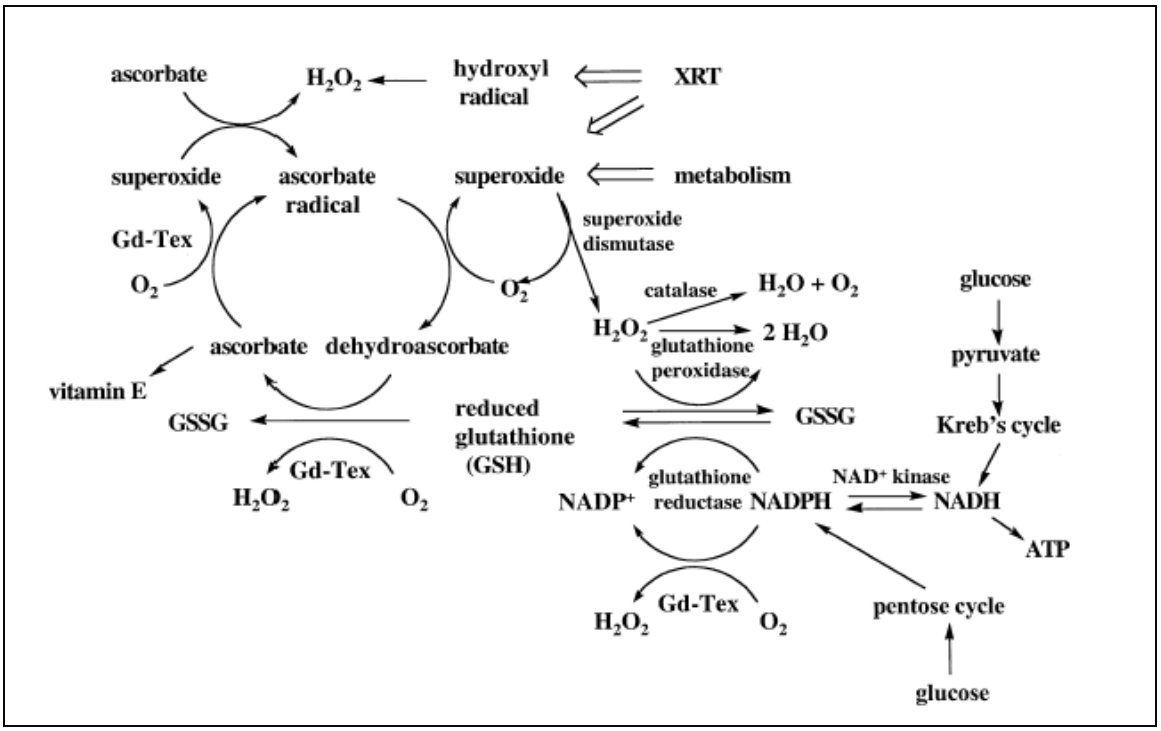

Figure 3. Proposed mechanisms of Gd-Tex induced radiosensitivity by redox cycling. Electron transfer to Gd-Tex can occur in the presence of metabolites with more negative reduction potential *e.g*. ascorbate GSH and NADPH. In the presence of oxygen, more reactive oxygen species like hydrogen peroxide and regenerate Gd-Tex. Thus such a futile redox cycling finally causes oxidative stress in the cells and thereby making the cell more sensitive to radiation [4].

This GSSG would in turn be reduced by NAPDH. NADPH is also utilized in the redox cycling of Gd-Tex. All these reactions finally lead to depletion of NADPH pools, which can be replenished by the pentose cycle. However, it is this futile redox cycling initiated by GD-Tex which causes oxidative stress to the cell and ultimately enhances radiation induced cell killing.

In 1996 Young *et al* first reported the use of Gd-Tex as a radiosensitizer that is preferentially taken up by tumor cells as well as detected by MRI [5]. However recent reports have raised questions about the efficacy of Gd-Tex as a radiosensitizing agent [6]. Figure 4 compares results obtained from two studies that looked at the radiosensitizing effect of Gd-Tex on HT-29 cells. While Young et al reported a sensitization ratio of about 1.92 with Gd-Tex; Bernhard et al reported no significant radiosensitization. Hence further studies need to be done to evaluate Gd-Tex mechanism of action.

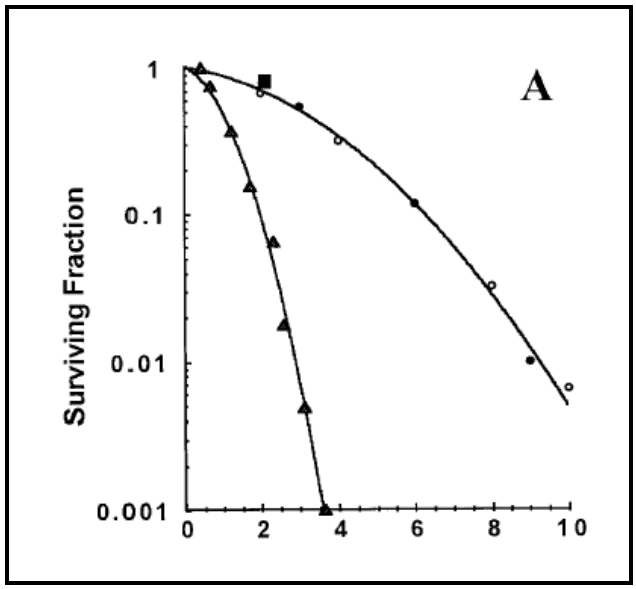

Figure 4. Surviving fractions of HT29 cells treated with Gd-Tex compared between two contradictory datas. (▲): Surviving fraction obtained from Young et al data show a sensitization ratio of almost 2. (■, ○, ●): Represent survival data obtained from three independent investigators which report no significant increase in radio sensitization of HT29 cells with Gd-Tex pretreatment [6].

Detection

One of the most unique properties of the Gd-Tex is the ability to detect the compound using magnetic resonance imaging. Such a ready visualization provides ways to determine biolocalization properties (both temporal and spatial). Figure 5 shows an MRI scan obtained from tumor and Gd-Tex localization in the tumor after 10 min of injection. There was almost 94% increase in tumor signal intensity after 10 min of Gd-Tex injection. This also correlated with enhanced sensitivity of the tumor to ionizing radiations. Additionally new and sensitive high performance liquid chromatographic assays have been developed that can detect and quantitate Gd-Tex amounts in human plasma [2].

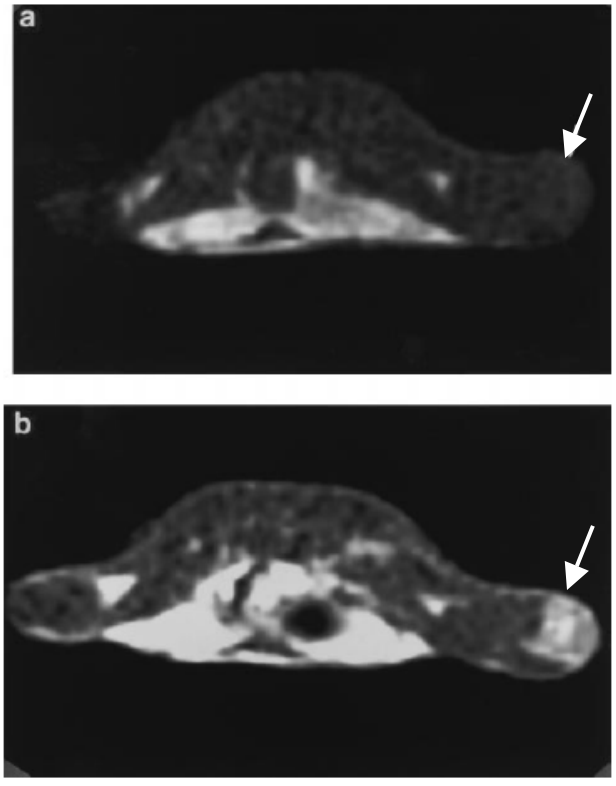

Figure 5. Axial MRI scans obtained from tumor of the leg. Arrow shows position of the tumor. In (b), localization of Gd-Tex is seen primarily in the tumor within 10 min of injection [5].

In vivo studies

Inspite of the exact mechanism of action not yet clearly understood, in vivo animal studies have shown Gd-Tex to be an effective radiaosensitizer [8,9]. Figure 6 shows the radiation enhancement with Gd-Tex treatment, in a mouse mammary carcinoma tumor model, which is known to be a highly radiation-resistant tumor. Radiation enhancement with Gd-Tex was evaluated using a multifraction radiation treatment regimen [8].

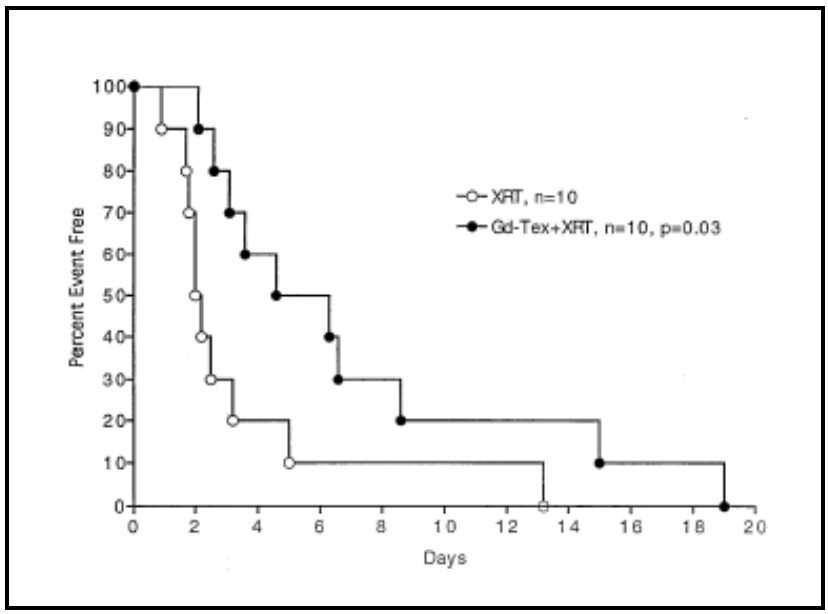

Figure 6. Radiosensitization of MCa tumor model with Gd-Tex. Animals were given 40 uM/kg of Gd-Tex, 2 hours before treatment with 32 Gy (•). Control untreated animals are shown (○) [8].

Summary

Gd-Tex is a new class of radiosensitizers that have been developed to potentiate the efficacy of delivered radiations. These compounds are already in phase III clinical trials in the treatment of brain tumors. An additional benefit of these sensitizers that are not seen with previous sensitizers is that Gd-Tex can be detected by MRI methods. This would be beneficial in treatment planning and subsequent monitoring of the tumor response to radiation. The proposed mechanism of action of Gd-Tex as a radiosensitizer is the futile redox cycling reactions leading to oxidative stress in cells that take up Gd-Tex. However there have been some contradictory

results on the radiosensitization abilities of Gd-Tex. Hence further mechanistic understanding of Gd-Tex as a radiosensitizer is required.

References

1) Sessler JL, Miller RA. (2000) Texaphyrins: New drugs with diverse clinical application in radiation and photodynamic therapy. *Biochem Pharmacol*. **59**; 733-739.
2) Parise RA, Miles DR, Egorin MJ.(2000) Sensitive high-performance liquid chromatographic assay for motexaphin gadolinium and motexaphin lutetium in human plasma. *J Chromatography*. **749**; 145-152.
3) Magda D, Lepp C, Gerasimchuk N, Lee I, Sessler JL, Lin A, Biaglow JE, Miller RA. (2001) Redox cycling by motexafin gadolinium enhances cellular response to ionizing radiation by forming reactive oxygen species. *Int J Radiat Oncol Biol Phys*. **51**; 1025-1036.
4) Sessler JL, Tvermoes NA, Guldi DM, Hug GL, Mody TD, Magda D. (2001) Pulse radiolytic studies of metallotexaphyrins in the presence of oxygen: Relevance of the equilibrium with superoxide anion to the mechanism of action of radiation sensitizer, motexaphin gadolinium (Gd-Tex, Xcytrin). *J Phys Chem B*. **105**; 1452-1457.
5) Young SW, Qing F, Harriman A, Sessler JL, Dow WC, Mody TD, Hemmi GW, Hao Y, Miller RA. (1996) Gadolinium (III) texaphyrin: A tumor selective radiation sensitizer that is detectable by MRI. *Proc Natl Acad Sci USA*. **93**; 6610-6615.
6) Bernhard EJ, Mitchell JB, Deen D, Cardell M, Rosenthal DI, Brown JM. (2000) Re-evaluating gadolinium (III) texaphyrin as a radiosensitizing agent. *Cancer Res*. **60**; 86-91.
7) Rockwell S, Donnelly ET, Liu Y, Tang LQ. (2002) Preliminary studies of the effects of gadolinium texaphyrin on the growth and radiosensitivity of EMT6 cells *in vitro*. *Int J Radiat Oncol Biol Phys.* **54**; 536-541.
8) Miller RA, Woodburn K, Fan Q, Renschler MF, Sessler JI, Kautcher JA (1999). In vivo animal studies with gadolinium (III) texaphyrin as radiation enhancer. *Int J Radiat Oncol Biol Phys.* **45**; 981-989.
9) Xu S, Zakian K, Thaler H, Matei C, Alfiers A, Chen Y, Koutcher JA (2001). Effects of motexaphin gadolinium on tumor metabolism and radiation sensitivity. *Int J Radiat Oncol Biol Phys*. **49**; 1381-1390.