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Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

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Misonidazole: A bioreductive marker of hypoxia.

By Annie Liu

Free Radical and Radiation Biology program
Department of Radiation Oncology
The University of Iowa
Iowa City, IA 52242-1181

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Abbreviations

ESR, electron spin resonance
^{18}FAZA, fluoroazomycinarabinofuranoside
^{18}FMISO, fluoromisonidazole, [^{18}F] 1-(2-nitro-1imidazolyl)-3-fluoro-2-propanol
HBO, hyperbaric oxygen
HPLC, high-pressure liquid chromatography
MISO, misonidazole
PET, positron emission tomography
RTOG, radiation therapy oncology group

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Abstract

Hypoxia in tumors can affect the outcome of chemotherapy and radiotherapy. Misonidazole (MISO), a hypoxic cell sensitizer, is used in the treatment of certain types of human tumors. The preferential toxicity of MISO to hypoxic cells is also of potential clinical significance in cancer chemotherapy. Several studies also showed increased cytotoxicity for normal tissues. In this review, I will discuss how MISO works to hypoxic cells and how to detect metabolites of MISO [3].

Introduction

Htpoxia contributes to tumor progression, and limits the response of tumor to radiotherapy and chemotherapy. Hypoxic cell sensitizers are groups of compounds that were developed to mimic oxygen in their sensitization of hypoxic tumor cells. Like many nitro compounds, for example, nitroimidazole, nitrofurans, and misonidazole. These compounds

are more toxic to mammalian cells under hypoxic condition than with oxygen. Bioreductive drugs undergo metabolic reduction to generate cytotoxic metabolites. These drugs also have been used in cancer therapy, as radiosensitizers and as cytotoxic agents with selectivity for hypoxic cells. The Radiation Therapy Oncology Group (RTOG) evaluated the use of the hypoxic cell sensitizer, misonidazole, in combination with irradiation for patients.

Properties of misonidazole

Misonidazole (NSC # 261037, molecular formula: $C_7H_{11}N_3O_4$, and molecular weight: 201.2 **)** is a hypoxic cell radiosensitizer **(Figure 1)** [2]. A side effect of MISO is to induce peripheral neuropathy in humans after exceeding a schedule-dependent cumulative threshold dose.

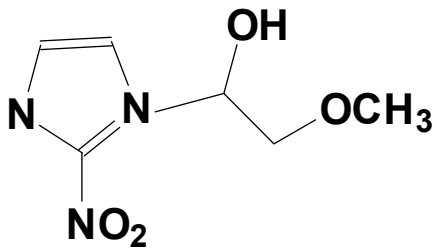
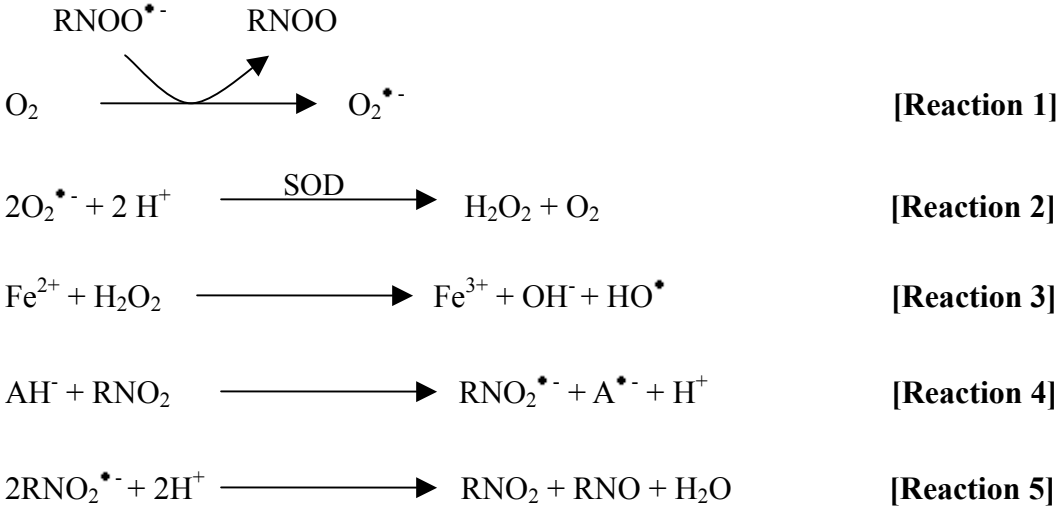


Figure 1: Chemical structure of MISO.

The first step in the metabolism of nitro compounds is to reduce to corresponding nitro anion radicals. This process is facilitated by flavoproteins such as xanthine oxidase and NADPH-cytochrome *P*-450 reductase. Under aerobic conditions, nitro anion radicals reduce

oxygen to superoxide and hydrogen peroxide **[Reaction 1 and 2]**. This can lead to the production of hydroxyl radicals by Fenton reaction **[Reaction 3]**. In the other way, under anaerobic conditions, these radicals can undergo reduction to form amines via nitroso and hydroxylamine. Josephy *et al.* observed that ascorbate enhance the cytotoxicity of MISO in Chinese hamster ovary cells. Because of reduction of the nitro group to form active metabolites is a possible mechanism for the cytotoxicity of these drugs. In low oxygen, the synergistic interaction between nitro compounds and ascrobate attributed to enhance cytoxic effects in hypoxic mammalian cells **[Reaction 4 and 5]** [2, 4].



How to detect misonidazole metabolites

Like many nitro compounds, MISO is more toxic in hypoxic conditions than in the presence of air. To detect the amine derivative and nitroreduction products bound to cellular macromolecules. A sensitive high-pressure liquid chromatographic (HPLC) assay for the detection and quantitation of the amine derivative of MISO in human urine is available.

Absorbance 365nm is monitored for the detection of dansyl derivative **(Figure 2)**. Otherwise, the analyses were also performed by collection before and after the administration of MISO. The results show that the amine is a urinary metabolite of MISO **(Figure 3)** [6, 7].

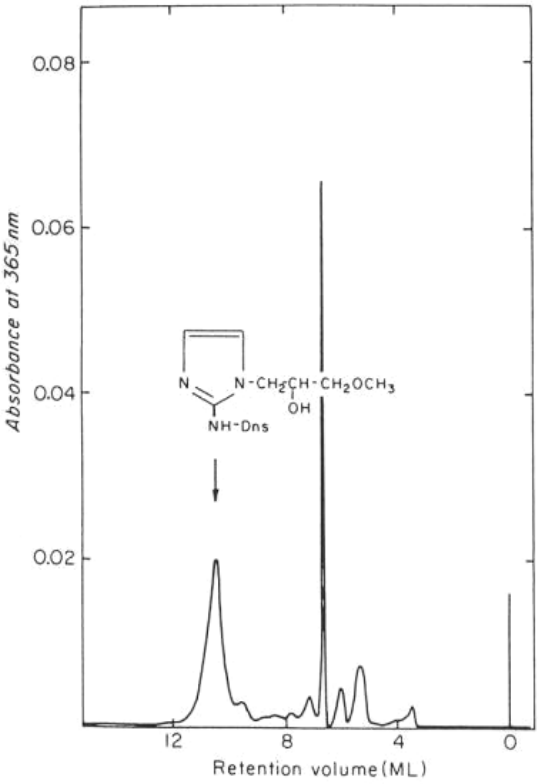


Figure 2: HPLC profile of the dansylation product of MISO [14].

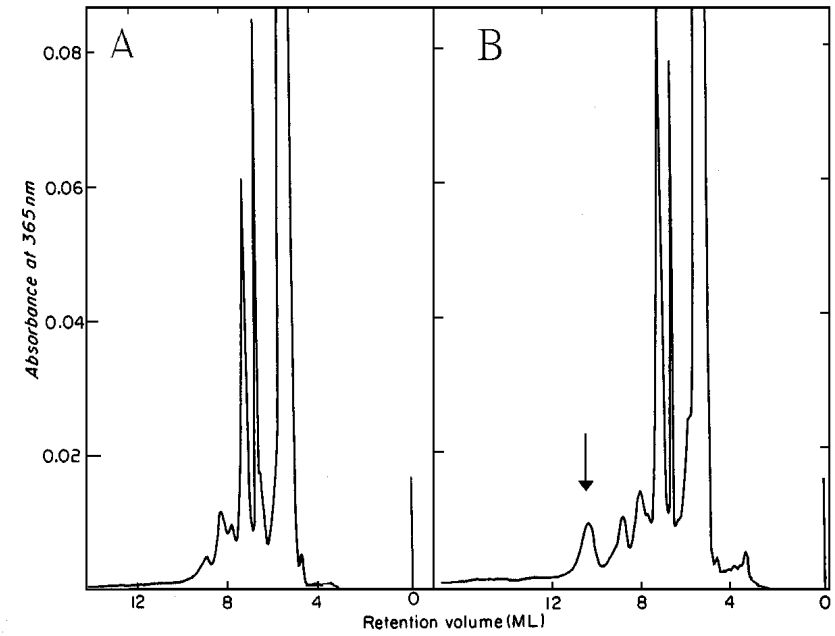


Figure 3: HPLC separation of dansylation products of before (A) and after (B) administration of MISO. The arrow indicated the dansylated amine [14].

How to detect the nitro anion radical of misonidazole

ESR (electron spin resonance), the measurement owes the magnetic properties of the electron which is associated with the electron spin. The ESR spectrum of MISO anion radical is well-resolved. This extremely resolved spectrum allowed us to obtain accurate hyperfine splittings **(Figure 4).**

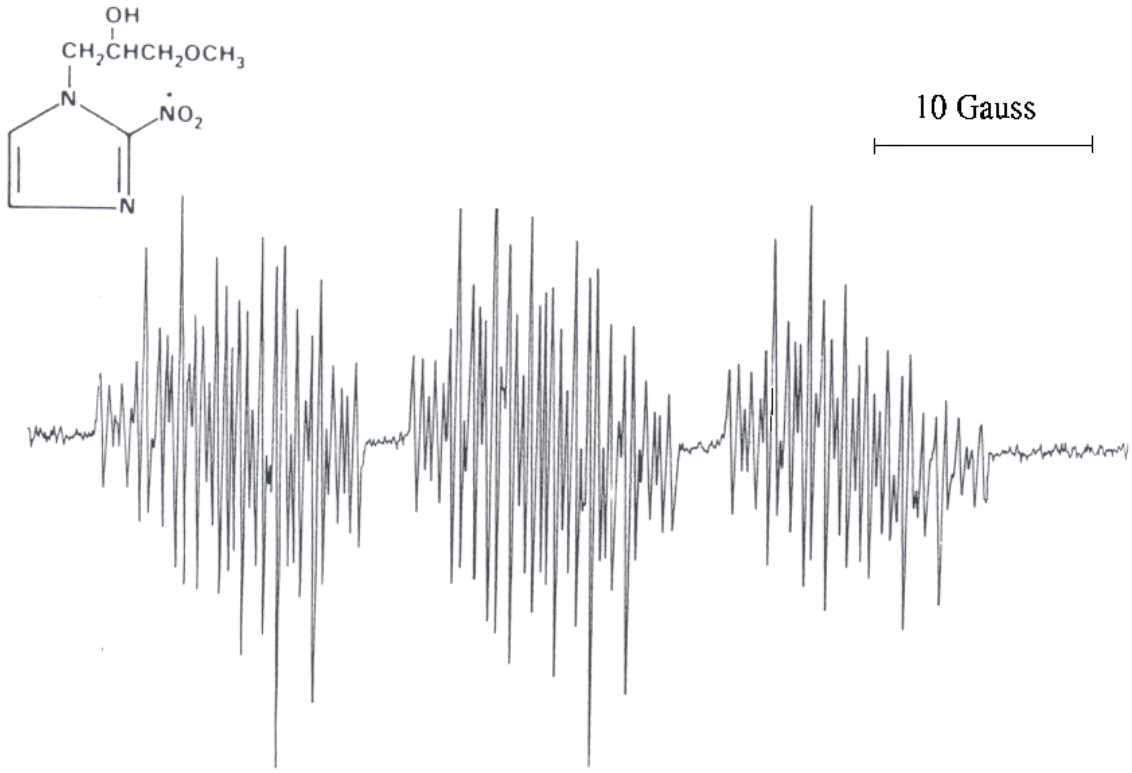


Figure 4: The ESR spectrum of the nitro anion radical of MISO [2].

^{18}FMISO for imaging tumor hypoxia

The assessment of tumor oxygenation before and after therapy is a helpful evaluation of tumor response to treatment. The most encouraging way is non-invasive approach to detect

tumor hypoxia. Using radiotracers selectively accumulate in hypoxic tumors and can be applied together with functional PET will be an excellent way. Among the potential PET hypoxia tracers, ^{18}FMISO is the most widely used PET radiotracer for imaging tumor hypoxia. Over the past two decades, the radiolabeled MISO as a probe for quantifying hypoxia in tumors was been used. FMISO, a radiofluorinated analogue of MISO, has been successfullyused for imaging oxygen deficiency tumor with PET. Under a hypoxic atmosphere for 20 min, the uptake of both ^{18}FAZA and ^{18}FMISO increased significantly in comparison to normoxic conditions. After 100 min incubation of tracer with cells, the quotient of tracer cell uptake between hypoxia and normoxia further increased **(Figure 5).** Many paper have demonstrated that ^{18}FMISO uptake is significantly higher in tumor tissue than normal tissue [11, 12].

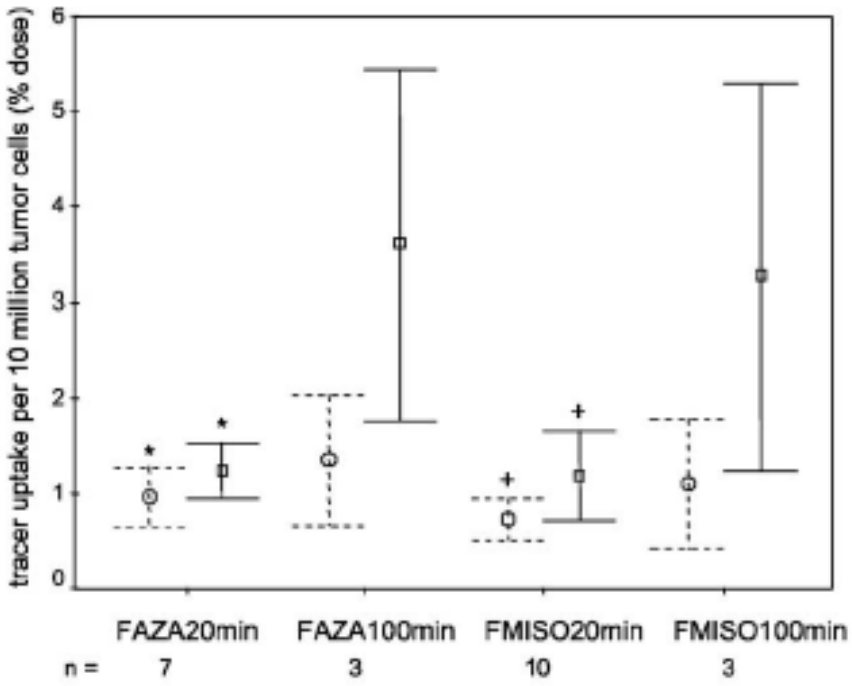


Figure 5: Uptake of ^{18}FAZA and ^{18}FMISO in Walker 256 rat tumor cells maintained in

normoxic (------) and hypoxic (——) atmosphere [11].

Summary

Hypoxic radioresistance is a real problem in solid tumors. It has been demonstrated to be a problem after conventional fractionation. Some better results have been reported with the combination of HBO and MISO than control groups, but not in all. These findings were presented in several papers. This may explain in many ways. For example, HBO introduced technical limitations to the radiotherapy delivery or the dose of MISO used was suboptimal for sensitization because of dose-limiting neurotoxicity. Since MISO was not present in tumors in sufficient concentration to be an effective radiosensitizer. We need the new compounds appear sufficiently better than MISO to sensitizing hypoxic cells.

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