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Measurement of hypoxia in human tumours by non-invasive spect imaging of iodoazomycin arabinoside

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Summary Tumour oxygenation status in individual patients may be assessed using the bioreduction and linkage of 2-nitroimidazole markers to viable hypoxic cells *in vivo* with subsequent detection by conventional nuclear medicine techniques. Iodoazomycin arabinoside (IAZA) was radiolabelled with Iodine-123 and administered i.v. to 51 patients with newly diagnosed malignancies whose tumours were subsequently imaged by planar and single-photon emission computed tomographic (SPECT) procedures. Quantitative analyses of radiotracer avidity were performed at 24 h post-injection and tumour-normal tissue ratios of greater than 1.10 were deemed positive for tumour hypoxia. By this criterion, the frequencies of hypoxia in small-cell lung cancer, squamous cell carcinomas of head and neck and malignant gliomas were 60% (9/15), 40% (6/15) and 0% (0/11) respectively. The correlation of positive IAZA scans with tumour control and survival in patients with lung cancer and head and neck tumours is currently under study. Preliminary observations in neck metastases from squamous cell carcinoma of head and neck tumours indicates decreased local control at 3 months post-treatment in tumours with IAZA avidity. This study concludes that: (1) ^{123}I-IAZA can be administered safely and repeatedly as an outpatient routine imaging procedure in cancer patients during initial work-up and follow-up; (2) that retained drug can be detected by conventional nuclear medicine procedures in inaccessible deep-seated tumours; and (3) that this technique could prove useful for identifying those patients for whom hypoxia-directed therapy is indicated.

Keywords: hypoxia; iodoazomycin arabinoside; bioreductive drug; nitroimidazole; imaging

In addition to cell kinetics, tumour microenvironment and inherent cell sensitivity, chronic and intermittent tumour hypoxia is currently being considered as one of the multifactorial causes of tumour treatment resistance. The oxygenation status of individual human tumour is not routinely measured today. Recently, efforts have been made to define procedures for measuring this tumour property in individual patients. Of several procedures under current development which were reviewed at a tumour oxygenation workshop at the National Institute of Health, USA (Stone *et al.*, 1993), direct tumour measurements of pO_2 with oxygen electrodes and indirect measurements by sensitiser bioreduction and linkage procedures are undergoing clinical evaluation. The most popular investigational method used currently to measure tumour hypoxia in humans is the computer-driven Eppendorf pO_2 microelectrode. In this procedure, a microelectrode needle is inserted into the tumour and sequential pO_2 measurements are made as the needle advances in stepwise fashion through the tissue. Preliminary results on tumours of the cervix have shown that median values of pO_2 predict for tumour response to combined modality treatments (Höckel *et al.*, 1991). These Results were reported earlier (Gatenby *et al.*, 1988) using computerised tomography-guided pO_2 electrodes in tumours of head and neck. While these results are extremely encouraging, electrode measurements of tumour oxygenation are convenient only for accessible tumours. Furthermore, electrode measurements are invasive in nature, do not distinguish between viable and necrotic zones within tumours, and are technically demanding. Therefore, considerable interest remains in the use of non-invasive methods of determining oxygenation status using intracellular binding of nitroimidazoles such as fluorinated fluoromisonidazole as measured by positron emission tomography (PET) and the ^{123}I-IAZA method using conventional gamma camera and nuclear medicine scanning.

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Of tumours resected from 27 patients to whom ^{3}H-misonidazole had been administered 24 h before surgery, 12 tumours showed positive labelling by autoradiography and liquid scintillation counting techniques; 8/12 and 3/3 of the positives were small-cell lung cancers (SCLC) and melanomas respectively (Chapman *et al.*, 1989). This research prompted the synthesis and radiolabelling of bioreductive hypoxic markers which could be detected by planar and SPECT nuclear medicine imaging techniques. Iodoazomycin arabinoside (Mannan *et al.*, 1991) is metabolically activated within viable cells to reactive intermediates which covalently link to cellular molecules at rates inversely proportional to intracellular oxygen concentrations (Mercer *et al.*, 1991). There is animal laboratory evidence that ^{123}I-IAZA does not cross the blood-brain barrier readily. However, it is known that malignant gliomas have a disrupted blood-brain barrier.

A pilot clinical study was initiated in June 1990 to determine the safety and feasibility of measuring human tumour hypoxia non-invasively by ^{123}I-IAZA. The early experience with the first ten patients on this protocol has been reported (Parliament *et al.*, 1992). We now describe the measurements made with this hypoxic nuclear medicine marker in 51 patients recruited to this study over 4.5 years. A large proportion of tumours investigated were deep-seated and, consequently, were unsuitable for characterisation by oxygen electrode procedures.

Materials and methods

Preparation and administration of radiolabelled IAZA

IAZA (1-[5'-iodo-5'-deoxy-β-D-arabinofuranosyl]-2-nitroimidazole) was prepared in the Pharmacy and Pharmaceutical Sciences laboratories at the University of Alberta. ^{123}I as sodium iodide (Nordion International Limited, Vancouver, Canada) was used in an iodine-exchange reaction with cold IAZA to produce ^{123}I-IAZA by procedures described previously (Parliament *et al.*, 1992). The purified radiola-

belled marker was prepared in sterile saline which contained 10 mg of unlabelled IAZA and sterilised by filtration (Millipore, 0.22 μm pore) into a multidose vial. The radiopharmaceutical was dissolved in 5.7 ml of sterile saline for i.v. injection. The purified sample had no detectable chemical impurities and over 99% radiochemical purity when analysed by high performance liquid chromatography (HPLC). The range of radioactivity dose was 145–343 MBq. This was given by a slow i.v. infusion over 5–10 min initially and later, after no toxicity had been observed, the radiopharmaceutical was given by bolus injection. Lugol's iodine was administered 24 h before imaging, to block thyroid uptake of the free radio-iodine.

Patient selection and procedures

This imaging protocol was reviewed and approved by the local Institutional Review Board. Appropriate written informed consent was obtained from all patients. The objectives of this study were: (1) to determine the safety and feasibility of administering ^{123}I-IAZA to patients with primary or metastatic solid tumours; (2) to determine the frequency of hypoxic marker avidity in patients with small-cell lung cancer (SCLC), squamous cell carcinoma of the head and neck (SCC of H and N), glioblastomas and soft-tissue sarcoma: (3) to correlate IAZA tumour avidity with local tumour control and survival. Patients were eligible for the study if they were $\leqslant 75$ years of age, had a Karnofsky performance status of $\geqslant 60\%$, had satisfactory haemato-logical parameters (platelet count $> 100 \times 10^9\,l^{-1}$, WBC $> 3 \times 10^9 l^{-1}$, haemoglobin > 100 g l^{-1}, and hepatic and renal function within 1.5 times the normal range.

The above specific solid tumours were chosen because of our previous observations demonstrating high avidity for adducts of nitroimidazoles using both ^{3}H-misonidazole and ^{123}I-IAZA (Urtasun *et al.*, 1986; Parliament *et al.*, 1992).

Blood pharmacokinetics were assessed for the second, sixth and ninth patients on protocol and showed an elimination half-life of 9.8 ± 4.1 h while the physical half-life of the isotope is 13 h (Parliament *et al.*, 1992). Hypoxic marker imaging was performed at 17–24 h after marker administration (at least two serum clearance half-lives) when adequate ^{123}I was available for gamma camera detection.

Imaging procedures

The imaging was performed using a General Electric 400 AC gamma camera system linked to a Picker PCS 512 computer for image acquisition, storage and processing. Anterior and posterior planar static images were obtained at 1 h and late images at between 17 and 24 h after injection. Coronal, axial and sagittal reconstructions of SPECT images were obtained from the late scans. The images were evaluated qualitatively and quantitatively. Comparative analyses of tumour–normal tissue (T/N) ratios at 24 h after injection were obtained by region of interest (ROI) analyses of tumour site *vs* a mirror-image contralateral normal tissue. The T/N ratio of 1.10 was chosen as the lower range to report positivity, since below this ratio it was difficult to image areas of hypoxia reliably.

In a subset of patients, ^{99m}Tc-HMPAO was administered i.v. 24 h before the hypoxic marker procedure and measurements of tumour perfusion were acquired 30–90 min after injection.

Results

No unacceptable toxicities resulting from this radiopharmaceutical were observed in any patient. Transient somnolence and transient mild chills experienced by some patients were rare events (Parliament *et al.*, 1992). The imaging procedures were well accepted and were performed on an outpatient basis as routine imaging in the Department of Radiology at the Cross Cancer Institute. The total scanning time was approximately 1 h for each procedure on 2 consecutive days.

Figure 1 shows a positive axial SPECT scan of the neck of a patient with right-sided nodal metastases from a squamous cell carcinoma of the tonsil. The areas of high counts in the midline anteriorly represent the regions of the salivary glands and the nasopharynx. Similarly, there are moderate counts on the normal tissues of the left neck, representing salivary glands. Figure 2 shows a positive axial SPECT scan through the thorax of a patient with SCLC. The areas marked in each image identify the regions of interest (ROI) over tumour and normal tissue sites from which total counts were obtained to define T/N ratios of radioactivity. The range of marker uptake on delayed images (in general 2 half-lives after marker administration) in sites qualitatively avid was 1.10–1.97. We defined tumour hypoxia on these scans as ratios greater than 1.1. By this criteria, 40% of tumours in this study contained measurable levels of viable hypoxic cells. None of the patients with avid IAZA scans had haemoglobin levels lower than 10.6% at the time of the scans, the majority had haemoglobin levels equal to or higher than 12.9%. The T/N ratios were consistently higher in patients with SCLC (1.476 ± 0.208) than in patients with SCC of head and neck (1.273 ± 0.169). A total of 52 different tumour sites in 51 patients have been included for analysis and the number and percentage of those with measurable hypoxia are shown in Table I. Again, tumours which labelled at the highest frequency were primary

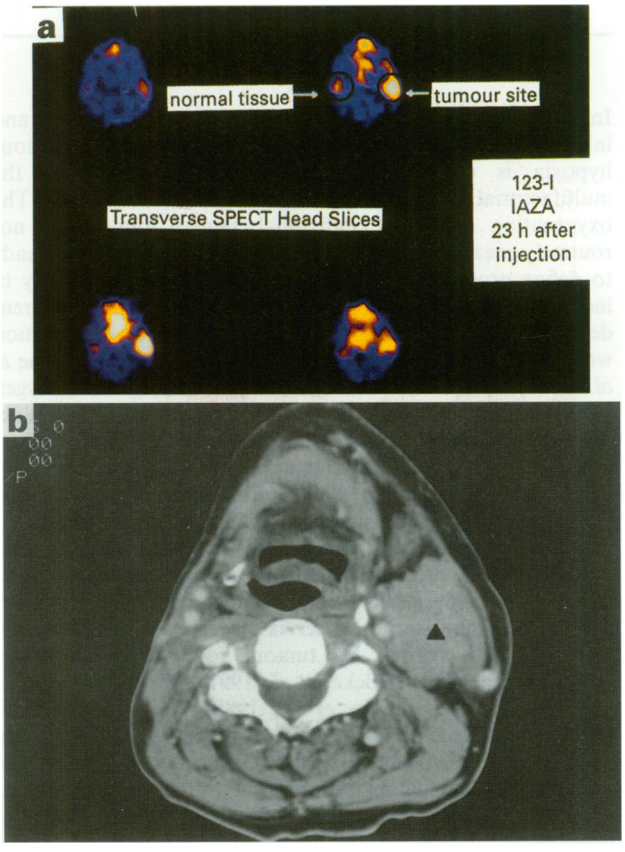

Figure 1 **a**, Positive axial SPECT scan of the neck of a patient with a right-sided neck nodal metastasis from a squamous cell carcinoma of the tonsil. The circles on the right node metastasis and on the opposite side of the neck represent the regions of interest (ROI) from which total counts were obtained to define T/N ratios of radioactivity. The area of high counts in the midline anteriorly represents salivary glands and nasopharingeal region. The ROI on the normal tissues of the left neck also includes some activity in the salivary glands. **b**, Computerised tomography scan of the lower neck with ▲ representing metastatic solid tumour in lymph nodes of the lower neck. This coincides with the area of positive uptake in the SPECT IAZA scan.

SCLC (60%), metastases from SCLC to the brain (75%) and soft-tissue sarcomas (60%). Squamous cell carcinoma of the head and neck with neck metastases showed a lower frequency of hypoxic labelling (40%) while in a group of 11 patients with intact, untreated glioblastoma, none demonstrated IAZA uptake, except for one which was marginal. It is of interest that in four of these 11 patients, tumour perfusion studies with ^{99m}Tc-HMPAO demonstrated striking blood perfusion deficits. Except for malignant glioma, a significant inverse correlation between tumour perfusion and IAZA avidity was found and this has already been reported (Groshar *et al.*, 1993).

In order to assess for evidence of reoxygenation during fractionated treatments with radiation, IAZA scans were repeated in three SCLC patients halfway through their course of radiation to the primary thoracic lesion. All three patients initially had positive initial IAZA scans and in all three the scans became negative after 25 Gy in 13 fractions and in an overall time of two and a half weeks. In contrast, in three patients with head and neck tumours with initial IAZA avid neck nodes, the repeated IAZA scans at 66, 33 and 40 Gy continued to be IAZA-avid. Two of these three patients subsequently died of uncontrolled neck metastases.

The correlation of radiation treatment response in patients with neck nodes from head and neck tumours was performed in 14 patients. The complete response at 3 months was 1/4 patients with avid IAZA scans. In contrast, 7/10 with negative IAZA scans had evidence of complete response at 3 months (Table II). Further patients are being accrued to this study in an attempt to determine if this trend is significant. In our experience there is no evidence to suggest that the outcome of patients with higher T/N ratios is worse than that for patients with T/N ratios approaching 1.10.

Table I Paitent composition and avid IAZA scans with median T/N of 1.47 (range 1.10–1.97) by quantitative analysis

Small-cell lung cancer	9/15	(60%)
SCC of head and neck	6/15	(40%)
Malignant gliomas	0/11	(0%)
Brain metastases	3/4	(75%)
Soft-tissue sarcomas	3/5	(60%)
Prostate carcinoma	0/1	(0%)
Melanoma (metastatic to neck)	0/1	(0%)
Total	21/52	(40%)

Table II Response at 3 months after radiotherapy in 14 squamous cell carcinomas of the head and neck patients

Response	*Avid IAZA*	*Non-avid IAZA*
CR	1/4	7/10

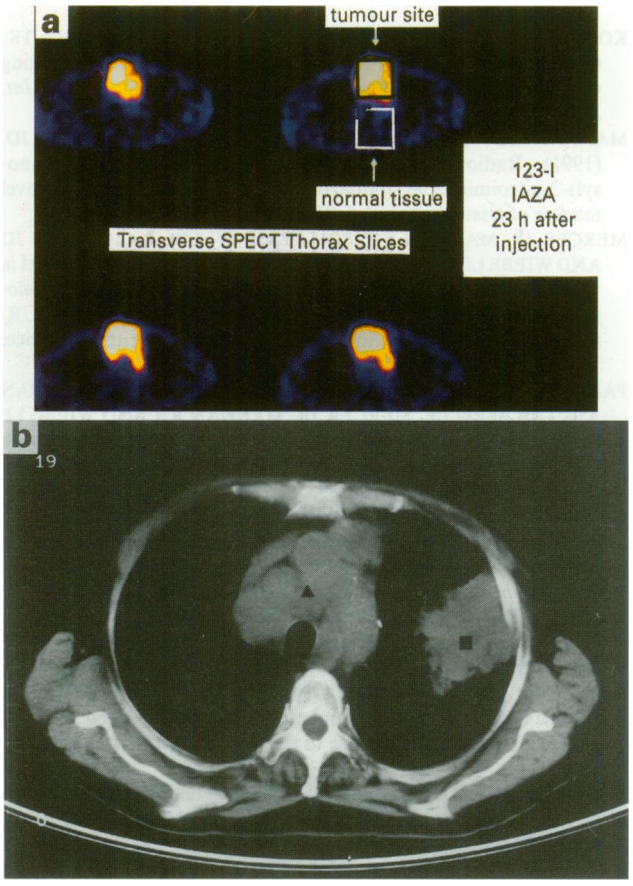

Figure 2 **a**, Positive axial SPECT scan of the thorax of a patient with midline mediastinal nodes from small-cell lung cancer. The squares over the tumour and over the normal tissues posteriorly over the midline represent the regions of interest (ROI). **b**, Computerised tomography scan of the thorax demonstrating the anterior mediastinal mass corresponding to the increased uptake on the SPECT IAZA scan on the same patient. ▲ mediastinal small-cell lung cancer. ■, post-obstructive pneumonitis.

Discussion

The proportion of human tumours investigated which show avidity for IAZA (40%) is similar to the previously published proportion of tumours with avidity for ^{3}H-misonidazole (44%) (Urtasun *et al.*, 1986; Chapman *et al.*, 1989). Recent studies of tumour oxygenation using microelectrodes also showed 40–50% of SCC of the head and neck and cervical cancers to have low median pO_2 levels (Terris and Dunphy, 1994; Fleckenstein *et al.*, 1992; Höckel *et al.*, 1991). The sum of this clinical evidence indicates that tumour hypoxia can be predicted by these procedures and is unlikely to be predicted by tumour histology, volume or growth rate (Chapman, 1991; Höckel *et al.*, 1995). These results suggest that if this tumour property is to be used for predicting radiation response or for selecting subsets of patients for whom hypoxia-directed therapies are indicated, it will have to be measured for each tumour individually.

The paradox observed in the 11 patients with glioblastoma multiforme with no avidity for ^{123}I-IAZA but with impaired tumour blood perfusion is of interest. This absence of tumour metabolic binding of the radiopharmaceutical in this subgroup of patients with malignant glioma is at odds with some previously reported laboratory evidence indicating that human brain tumours contain the bioreductive enzymes (DT-diaphorase and cytochrome P450 reductase), necessary for the metabolic binding of nitroimidazoles (Rampling *et al.*, 1993). On the other hand, the impaired tumour blood perfusion observed in four of these 11 patients correlates well with previous reports of a high percentage of low pO_2 values (<2.5 mm Hg) in patients with glioblastoma using the invasive computer-driven Eppendorf microelectrode, although general anaesthesia could have affected the readings (Rampling *et al.*, 1993). There is recent evidence from animal experimental models with transplanted human glioma cell lines that not all necroses are surrounded by nitroimidazole-labelled cells. This could explain in part the absence of IAZA binding in our patients (Parliament *et al.*, manuscript in preparation). In addition, some iodoazomycin nucleosides have not been found to cross the intact blood–brain barrier of rodents readily (JD Chapman, personal communication).

Measurements of tumour hypoxia with fluoromisonidazole, whose presence in tumours was quantified by positron emission tomography (PET) have been performed (Rasey *et al.*, 1989; Koh *et al.*, 1992). The proportion of tumours with

measurable hypoxia was higher in that study (60–70%) than that reported with IAZA. There are several differences between the two studies which can account for this discrepancy. Firstly, the tumour histologies investigated in these studies were different. Previous hypoxic marker investigations showed that tumours of different histology present with widely different frequencies of measurable hypoxia (Chapman *et al.*, 1989). Secondly, tumour–plasma ratios of F-18 marker were determined 3 h after marker administration and were considered positive at values of $\geqslant 1.3$. Metabolically linked marker would necessarily be measured at this early time against a high background of unbound marker which had not yet been excreted. Our pharmacokinetic model (Parliament *et al.*, 1992) of this bioreductive imaging procedure suggests that T/N ratios of hypoxic markers should be maximum after 8–10 elimination half-lives of unbound drug. The determination of T/N ratios of IAZA at 2–3 elimination half-lives in this study was a practical compromise resulting from the 13 h half-life of ^{123}I. Optimal imaging procedures with bioreductive markers may require compounds with faster elimination half-lives and/or radioactive isotopes with longer decay half-lives. An analysis of tumour hypoxia and tumour perfusion with ^{99m}Tc-HMPAO in a subset of these patients (Grosher *et al.*, 1993) indicated that some tumours were hyperperfused relative to surrounding normal tissues. Such tumours might give a falsely positive measure of tumour hypoxia if tissue levels of marker are determined at earlier times after administration. Investigations are now required in humans to correlate tumour hypoxia determined by bioreductive markers with an independent measurement of tumour oxygenation (possibly pO_2 electrodes). The positive correlation of these two methods has already been made in multicellular spheroids (Gross *et al.*, 1995). Ultimately, it will be necessary to determine whether tumour hypoxia measured non-invasively by these procedures truly predicts treatment resistance. Currently, there are new gamma-emitting nitroimidazole radiopharmaceuticals under development for human use and a novel ^{99m}Tc labelled compound is currently undergoing a phase I clinical trial in Edmonton, Alberta, Canada.

The definition of a new procedure which predicts for tumour resistance will have little clinical value unless novel therapies to overcome the basic mechanism of resistance are developed. In the case of tumour hypoxia, radiosensitisers such as etanidazole or hypoxic cell cytotoxins such as tirapazamine may improve the treatment response of those tumours with measurable hypoxic fractions. The administration of carbogen during radiotherapy of tumours with viable hypoxic cells could produce improved oxygenation and improved tumour response (Falk *et al.*, 1992). It appears that, after many years of research into mechanisms of tumour hypoxia and radioresistance, both the techniques and scientific interest are at hand to answer this lingering question definitively.

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