

## Energy-dispersive X-ray Microscopy to Trace Gadolinium in Tissues<sup>1</sup>

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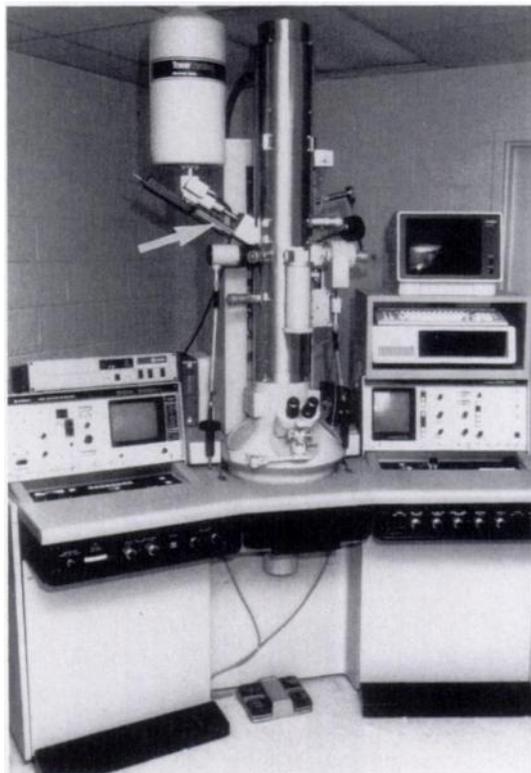
An electron microscopic technique, energy-dispersive x-ray (EDX) analysis, was applied to rat tissue specimens containing gadolinium diethylenetriamine-pentaacetic acid (DTPA). EDX spectroscopy was able to detect the characteristic radiation spectra of gadolinium from tissues in concentrations as low as 0.005 mmol per gram of tissue. A mapping algorithm allowed the detected spectra to be assigned to a specific site of origin within the tissue. This technique allows direct visualization of the location of Gd-DTPA complexes in tissues.

**Index terms:** Gadolinium • Magnetic resonance (MR), contrast enhancement

**Radiology 1989;** 173:868-870

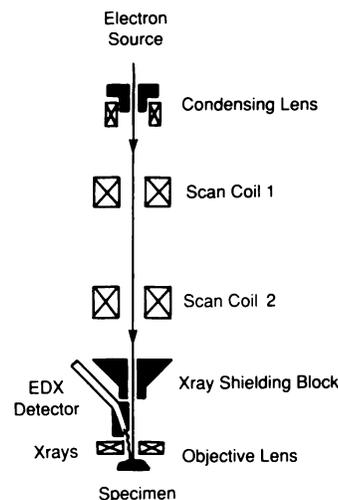
**G**ADOLINIUM diethylenetriamine-pentaacetic acid (DTPA) and other paramagnetic contrast agents for magnetic resonance (MR) imaging have generated considerable excitement within the radiologic community. While there is great interest in these compounds from a clinical and radiologic viewpoint, relatively little is known about their distribution at the cellular level. No satisfactory methods presently exist for localizing or tracing Gd-DTPA in tissue specimens microscopically.

Over the past decade, several techniques for elemental microanalysis with electron microscopy have been developed (1-5). One of these, energy-dispersive x-ray (EDX) spectroscopy, is now widely available in most major medical centers. EDX spectroscopic systems record characteristic x-ray spectra emitted when the electron microscopic beam encounters a heavy metal atom (such as that of gadolinium) within a tissue. The location of the element emitting such a spectrum can then be assigned to a specific site on the electron microscopic image. The feasibility of using EDX spectroscopy to trace Gd-



a.

b.



**Figure 1.** Electron microscope modified for EDX analysis. (a) Photograph of the electron microscopic EDX unit used in this project. The x-ray detector is marked with an arrow. (b) Diagram of the top half of the instrument shows the electron beam striking a target specimen. Characteristic x irradiation is detected by a field-effect transistor cooled in liquid nitrogen.

DTPA in tissues was therefore undertaken.

### Materials and Methods

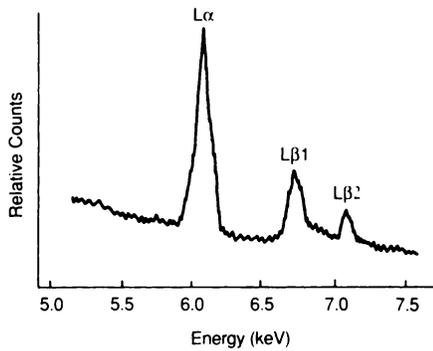
EDX microanalysis was performed with a commercially available system (H-600 scanning electron microscope with backscattered x-ray detectors; Hitachi, Tokyo). A 25-kV accelerating voltage was used on all specimens with a working distance of approximately 15 mm in the scanning fast-raster mode (magnification,  $\times 500$ -1,000). Spectral peaks were acquired for 90-120 seconds and analyzed on a Tracor Northern (Middleton, Wis) EDXA 5500 EDX computer and analysis system. The EDX spectroscopic apparatus and its sectional diagram are shown in Figure 1.

A dilution experiment was undertaken to determine an upper limit for the minimum concentration of gadolinium detectable by our system. Freshly harvested cubes of brain tissue from a rat (approximately 1 mg) were soaked for 10 minutes in isotonic saline solutions

containing Gd-DTPA in varying concentrations (0.001-0.50 mol/L). The cubes of brain were next air dried on filter paper for 10 minutes and then flash frozen by dipping them in liquid nitrogen for 45 seconds. The frozen tissues were transferred to a freeze-drying apparatus (Labconco Freeze Dry 5, Kansas City, Mo) initially cooled to  $-50^{\circ}\text{C}$ ; thawing and dehydration of samples was performed over a 24-hour period (6). The dried specimens were mounted on grids and carbon coated for electron microscopic and EDX analysis.

Next, the feasibility of using EDX to detect intravascularly administered Gd-DTPA was investigated. Mature Fisher 344 rats under general anesthesia received intracardiac injections of Gd-DTPA (0.1 mmol/kg). Within 5 minutes of injection, the animals were killed by guillotining. Specimens of liver, spleen, kidney, muscle, dura, optic nerve, pituitary gland, choroid plexus, frontal lobe, cerebellum, and pons were rapidly obtained and sectioned by hand with a  $\times 20$  dissecting microscope.

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**Figure 2.** Characteristic spectrum from a tissue example containing Gd-DTPA. Note  $L_{\alpha}$ ,  $L_{\beta 1}$ , and  $L_{\beta 2}$  peaks, which have been broadened by backscattering.

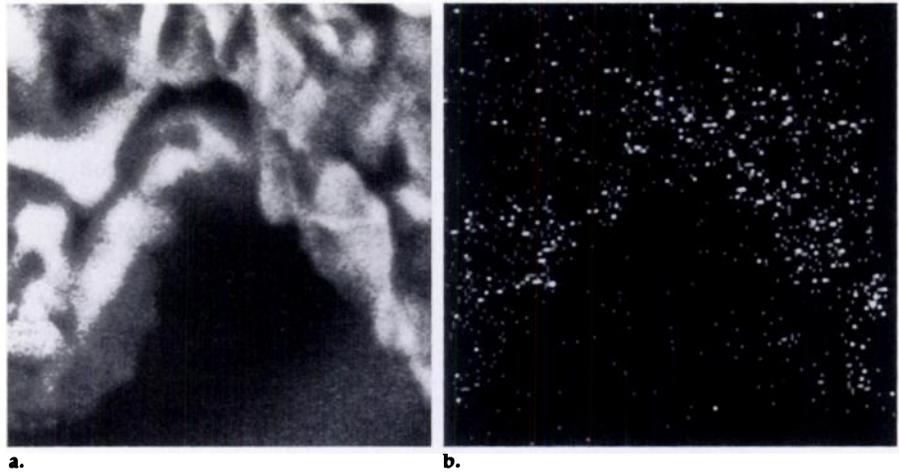
These specimens were flash frozen, freeze-dried, and carbon coated for electron microscopic and EDX analysis. A second group of rats received injections of saline only and served as controls.

## Results

Figure 2 illustrates the typical EDX spectrum obtained from a brain specimen soaked with gadolinium. The peaks correspond to known L-shell characteristic emission radiations for gadolinium ( $L_{\alpha 1} = 6.06$  keV,  $L_{\beta 1} = 6.72$  keV,  $L_{\beta 2} = 7.10$  keV). M-shell emissions (at 1.19 keV) and K-shell emissions (at 42.76 keV) were not detectable with our EDX system.

In the dilution experiment, L-shell radiations specific for gadolinium were detected from all tissue specimens soaked in Gd-DTPA solutions with a concentration of 0.005 mol/L or stronger. While we do not know the resultant gadolinium concentration present inside each cube of soaked tissue, we may presume that it was no greater than that of the soaking solution. On the basis of these assumptions, we conclude that a reasonable upper limit for the minimum concentration of gadolinium detectable by our system was 0.005 mol/L, or approximately 0.005 mmol per gram of tissue.

EDX spectral analysis was next applied to the tissue specimens harvested from rats after intracardiac injections of Gd-DTPA. Definitive spectra were obtained from tissues known to have a large blood pool and to enhance with Gd-DTPA at MR: liver, spleen, kidney, muscle, dura, pituitary gland, and choroid plexus. Trace or equivocal spectra were obtained from the optic nerves. No Gd-DTPA was detected in the frontal lobe, cerebellum, or brain stem (ie, areas excluded by the blood-brain barrier and with low blood pool fractions). No gadolinium spectra were obtained from the tissues of control animals, which received saline injections only.



**Figure 3.** Choroid plexus of a rat that had received an intravascular injection of Gd-DTPA. (a) Scanning electron microscopic image (original magnification,  $\times 1,000$ ) of the freeze-dried choroid plexus. The anatomy is somewhat distorted due to the relatively crude method of tissue preparation employed. (b) Gadolinium image map. Dots represent the loci from which gadolinium radiations were emitted.

Spatial mapping of the gadolinium spectra to tissue loci was also successful. The EDX spectroscopic system not only allowed identification of the characteristic spectrum of gadolinium but also recorded the precise position of the electron beam on the specimen when the spectrum was emitted (1). It was therefore possible to spatially map over the electron microscopic image the exact locations from which spectra of gadolinium were emitted.

In Figure 3 a scanning electron microscopic image of the rat choroid plexus is shown with its gadolinium map. Although the quality of the images is relatively poor, the spectrum is clearly shown to be localized to the fronds of the choroid plexus. Cell boundaries are not identified, since this is a scanning (not transmission) electron microscopic study and because the tissue is both unfixed and crudely sectioned. These difficulties of tissue handling for EDX microscopy are addressed in the Discussion.

## Discussion

EDX microanalysis is a relatively new electron microscopic technique that allows identification of specific elements with an atomic number greater than 9. Several detailed reviews describing the physical principles underlying this technology are available (1-5). EDX spectroscopic systems detect and record characteristic radiations emitted by a specimen that has been bombarded by an electron beam. The electrons used to bombard the sample tissue for electron microscopy dislodge resident electrons in inner orbitals (K, L, and M shells) of the specimen atoms. Characteristic x rays are then emitted by the histologic sample, a process

### Approximate Sensitivities of Various Analytic Methods for Detecting Gadolinium

Method	Detection Limit ( $\mu\text{mol/L}$ )
Inductively coupled-plasma (ICP) atomic emission spectrometry (7)	1-2
Polarized x-ray fluorescence excitation analysis (FEA) (8)	3-5
High-performance liquid chromatography (HPLC) (7)	20
EDX spectroscopy*	<5,000

\* Results from this study.

identical to that which occurs in conventional x-ray tubes. The characteristic radiation is detected by a supercooled field-effect transistor, and an identity is definitively assigned to the emitting element. Spatial localization of the site in the specimen from which the characteristic radiation originated is also possible; the result is an element map that can be superimposed on the electron microscopic image.

Several other analytical techniques for detecting and measuring gadolinium in tissue specimens exist (Table). The most sensitive technique, ICP atomic emission spectroscopy, can detect gadolinium in concentrations as low as 1  $\mu\text{mol/L}$  (7). Polarized x-ray FEA may be used to assay either solid or liquid samples containing gadolinium. Its sensitivity is slightly less than that of ICP spectroscopy (8). HPLC represents a less expensive (and less sensitive) alternative to the other analytic methods; its detection limit for gadolinium is a factor of 10 higher (but the price of the equipment is a factor of 10 lower) than that of ICP spectroscopy or FEA (7).

The EDX system we have used is at least 1,000 times less sensitive than ICP spectroscopy or polarized x-ray FEA for the detection of gadolinium. However, EDX spectroscopy is not a quantitative analytic technique like the previously described methods. EDX analysis should be considered an innovative adjunct to electron microscopy rather than a new method for chemical assay. The EDX system is capable of not only detecting gadolinium but also localizing it within tissues at the microscopic level. ICP spectroscopy, polarized x-ray FEA, and HPLC, while extremely sensitive for detecting small amounts of gadolinium, are useless for precise localization. The tissue specimens must first be homogenized, digested, or vaporized before they can be analyzed by the ICP, FEA, or HPLC instruments.

While our EDX images are relatively crude, they demonstrate the feasibility and potential of this technique for studying the distribution of contrast agents containing gadolinium and other metals at the cellular level. Until now the application of microscopic techniques to the localization of water-soluble contrast agents has been difficult. Traditional methods of tissue fixation for either light or electron microscopy can significantly dislocate these diffusible compounds or wash them completely from the specimen (9).

For this reason we have used only unfixed, flash-frozen, and freeze-dried specimens for our EDX microanalysis. This freezing technique, while it is relatively easy to perform and ensures little dislocation of diffusible substances, is associated with several disadvantages (6). First, significant disruption of cel-

lular architecture may result from ice-crystal formation if care is not taken to make the sample sufficiently small. Second, the freeze-drying process can also distort cellular relationships at the ultramicroscopic level. Finally, this method limits us to surface imaging (scanning electron microscopic mode), since the electron beam cannot penetrate the relatively thick chunk of tissue prepared.

Optimal use of EDX microanalysis for localization of gadolinium in tissue will therefore require significant further refinements in our methods of tissue handling. We are presently implementing a "slam-freeze" technique coupled with cryoultramicrotomy and viewing the specimens in an electron microscope fitted with a cold stage (10). This will allow transmission electron microscopic imaging to be performed and will, it is hoped, allow better preservation of cellular architecture than we obtain with our current methods.

In conclusion, the feasibility of electron microscopy with EDX microanalysis to detect Gd-DTPA in tissues has been proved. Further technical refinements that are now being developed should allow direct visualization of a variety of radiologic contrast agents in target tissues. This research may lead to the more intelligent design of future contrast agents, as well as aid our understanding of how radiologic contrast agents distribute at the microscopic level. ■

**Acknowledgments:** The author thanks Hank Chilton, PharmD, for his help with tissue preparation and Debbie Jennings for her assistance and expertise with the EDX system.

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