Magnetic Resonance Microscopy in Cardiac Development

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KEY WORDS MRM; mouse development; embryo; imaging; visualization

ABSTRACT Magnetic resonance microscopy (MRM) is a fast and non-destructive imaging technique that can analyze the three-dimensional structure of the embryonic heart both qualitatively and quantitatively. Intravascular contrast agents have been developed to accentuate the anatomy of cardiac chambers, the cardiac outflow tract, and major arteries and veins throughout the embryonic body. MRM generates non-distorted three-dimensional data of vascular anatomy in a fraction of the time required by conventional optical image reconstruction techniques. The three-dimensional nature of these data allows the creation of visual models that can be manipulated for fast and easy interpretation of the complex relationships between heart chambers and aortic arches. This is particularly helpful because these relationships change in complex ways during development. The non-destructive nature of MRM makes it well suited for investigating rare or valuable specimens and live subjects. MRM techniques have been developed for imaging the embryo in utero and in vitro, although MRM studies of fixed embryo specimens are easier to perform and produce data with better contrast and higher resolution. *Microsc. Res. Tech. 52: 323–330, 2001.*

INTRODUCTION

Investigators of heart development depend on imaging technology to observe, measure, and manipulate the complex series of events leading to formation of the adult heart. Investigating heart development is challenging because the heart is inaccessible, its three-dimensional architecture is complex, it is derived from several different embryonic cell lineages, and it undergoes major restructuring in a short period of time. Furthermore, the animal models used to study heart development are often difficult to produce. Imaging is used to record gene expression patterns directly or to document the morphological consequences of gene transcription. These demands have driven important advancements in the imaging technologies that are now available to investigators for studying heart development.

Magnetic resonance microscopy (MRM) is one of many imaging techniques used to study the role of gap junctions in normal and abnormal development. It is similar to clinical magnetic resonance imaging (MRI) in its use of superconducting magnets and pulses of radiofrequency energy to excite the water protons in the subject, generating three-dimensional arrays of image data. The minimally invasive nature of MRM and its ability to see deep within a specimen has made MRM a promising new tool for analyzing the morphological consequences of altered gene expression in the developing cardiovascular system. Cx43 (a gap junction gene) is one such example.

This review describes proton-imaging MRM where the images represent the quantity of water in the specimen, the mobility of the water in tissues, and the interaction of water with its chemical environment. This review also discusses contrast agents that are used to alter the chemical environment sufficiently to either enhance or suppress the amount of magnetic resonance signal coming from the protons within the specimen.

HISTORY OF MRM

The first magnetic resonance studies on live animals are believed to have been performed by Jasper Jackson in 1967 (Wehrli, 1992). These initial studies produced one-dimensional recordings of the nuclear spins in the subjects. It was in the early 1970s that Paul Lauterbur produced the first images of live animals based on magnetic resonance (Lauterbur 1974, 1975). This important breakthrough led to the rapid development of MRI devices used clinically in medical imaging. Early developers of MRI soon applied this new capability to the microscopic domain (Mansfield and Grannell, 1975). As MRI was refined for clinical studies, researchers found a wide range of additional applications for MRM in the life sciences as well as the material sciences: diffusion and flow studies of liquids through porous media, studies of plant physiology, and animal pathology (Blümich and Kuhn, 1992; Johnson et al., 1987; Johnson and Maronpot, 1989). High-field magnets and specialized imaging coils were further developed to study histology, pathology, and toxicology in small animals (Johnson et al., 1993).

MRM has been used to study a broad range of biological questions in small animals. Its non-invasive features make it particularly effective for longitudinal time studies. This has led to its use in cancer studies to track tumor growth, changes in metabolism associated with apoptosis, changes in tissue pH, changes in vascularity, and to characterize tumor re-oxygenation (Evelhoch et al., 2000). Longitudinal studies have been

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performed with MRM for toxicology studies in the liver (Johnson and Maronpot, 1989) and kidney (Morehouse et al., 1995) in small animals. Studies of ischemia in the rat brain have benefited from this ability to make repeated measurements (Benveniste et al., 1991), as have studies tracking cell fates in the developing frog embryo (Jacobs and Fraser, 1994). MRM has been used to investigate the lungs in live animals with the use of hyperpolarized gases as contrast agents to enhance signal in the airways (Moller et al., 1999). The non-invasive nature of this technique has also led to its use in developmental biology. MRM has been used to analyze the phenotypes of normal embryos and embryos from transgenic and knockout animal models (Johnson et al., 1986; Smith et al., 1992, 1996).

MRM PROTOCOLS FOR EMBRYO STUDIES

MRM studies of fixed embryo specimens can be performed more easily than studies of live embryos in utero. Imaging embryos outside of the uterus allows the use of smaller imaging coils that can be placed in close proximity to the subject and improves signal-to-noise in the resulting images. Fixed embryos imaged outside the uterus can also be immobilized to avoid motion artifacts that plague in vivo imaging schemes. In a typical ex vivo preparation, the embryo and placenta are dissected together from the uterus and extraembryonic membranes and the umbilical vessels are isolated and immobilized. A small opening is then made in the umbilical artery and vein and the vein is cannulated with a finely drawn glass pipette that is connected to a micro-peristaltic pump (the hole in the artery acts as a vent to prevent inflation of the vasculature). The blood is replaced first by infusing warmed Verapamil in a cacodylate buffer (to relax the heart in diastole), then by a buffered formalin and glutaraldehyde fixative, and finally by the MRM contrast agent BSA-DTPA-Gd (Smith et al., 1994; Smith 2000). The umbilical vessels are tied off, the embryo is separated from the placenta, and the embryo is immersion fixed overnight. The prepared embryo is immobilized in an airtight container, placed in an imaging coil that matches the embryo's size, and then put into a superconducting magnet for imaging. Imaging embryo specimens is typically performed with static magnetic field strengths from 4 to 12 Tesla. By comparison, most clinical MRI magnets operate from 0.5 to 1.0 Tesla. Specialized gradient inserts for the magnet are also critical for obtaining high-resolution images. Typical imaging times for single slice images can be fractions of a second, but three-dimensional data acquisitions are typically 20 minutes to 2 hours in length. T_1-weighted and diffusion-weighted pulse sequences provide excellent image contrast with short imaging times for specimens prepared this way.

Three-dimensional image arrays for fixed embryo specimens typically consist of 256 image slices with 256×256 pixels per slice. In-plane image resolution varies with the field-of-view for the specimen, from about 20 μ per pixel in smaller embryos, to about 120 μ per pixel for larger specimens. This assumes a field-of-view of 5 and 31 mm, respectively.

Real-time visualization of the three-dimensional data is typically done on computer workstations running Unix or NT operating systems with 128 to 512 MB of RAM. The software combines the image slices into stacked cubes and allows the investigator to manipulate the way the resulting three-dimensional image is displayed. The viewer can rotate the image stack, change the apparent opacity of structures based on signal intensity, slice into the stack from any plane and view the new surfaces, or segment (isolate) structures of interest based on similarities of signal intensity. The resulting visualizations can be saved and displayed on standard desktop computers as single images, simple QuickTime® animations, or more complex animated "fly-through" animations.

The non-invasive nature of MRM permits in utero imaging of embryos with its consequent ability to track embryos over time (Fig. 1). However, in utero imaging in animals is complicated by the presence of the mother and by numerous sources of motion that can degrade the quality of the image data. Imaging coils must accommodate the large size of the mother rather than fitting snuggly around each embryo. Measures must be taken to minimize the motion in the mother-embryo pair, or to compensate for it with appropriate pulse sequences and data processing. The mother is typically anesthetized and immobilized to control large-scale movement. The anesthesia is administered with a ventilator as a mixture of isoflurane gas and air in scan-synchronous pulses to cause the mother to breathe between imaging pulses and thereby minimize movement of her abdomen during the imaging cycle. The mother's heartbeat, the embryo's heartbeat, maternal blood flow, and embryonic blood flow also introduce motion during the imaging sequence. Although the MRM pulse sequence can be gated to the maternal EKG, it is not practical to gate the pulse sequence to the heartbeats of 12 to 14 embryos in the typical litter. Instead, it is important to use well-designed pulse sequences and specialized post-processing of the image data to minimize the artifacts imposed by heart and blood motion (Smith et al., 1998). Typical imaging times for in utero studies are about 30 minutes for complete three-dimensional data sets. In utero studies require staff specialized in animal handling and anesthesia as well as high-field magnets and specialized coils.

In vitro imaging of an embryo in a controlled culture chamber provides an intriguing compromise between in utero and fixed embryo imaging. The mouse embryo can maintain normal growth in culture chambers for more than 24 hours (Flynn, 1987; Picard, 1991; Yavarone et al., 1993). The culture chamber can be designed to accommodate an imaging coil that is just slightly larger than the embryo. The embryo is more accessible in vitro than in utero, it can undergo normal growth during interesting times of organogenesis, and it is free from many of the motion artifacts that hamper imaging the embryo in utero. However, in order to maintain oxygenation and nutrient levels, the culture medium must be refreshed by maintaining a slow and continuous flow (Smith, 1996).

APPLICATIONS OF MRM FOR EMBRYO STUDIES

MRM was used to analyze the hearts and outflow tracts of Cx43 knockout mice and transgenic mice (CMV43) over-expressing the Cx43 gap junction gene

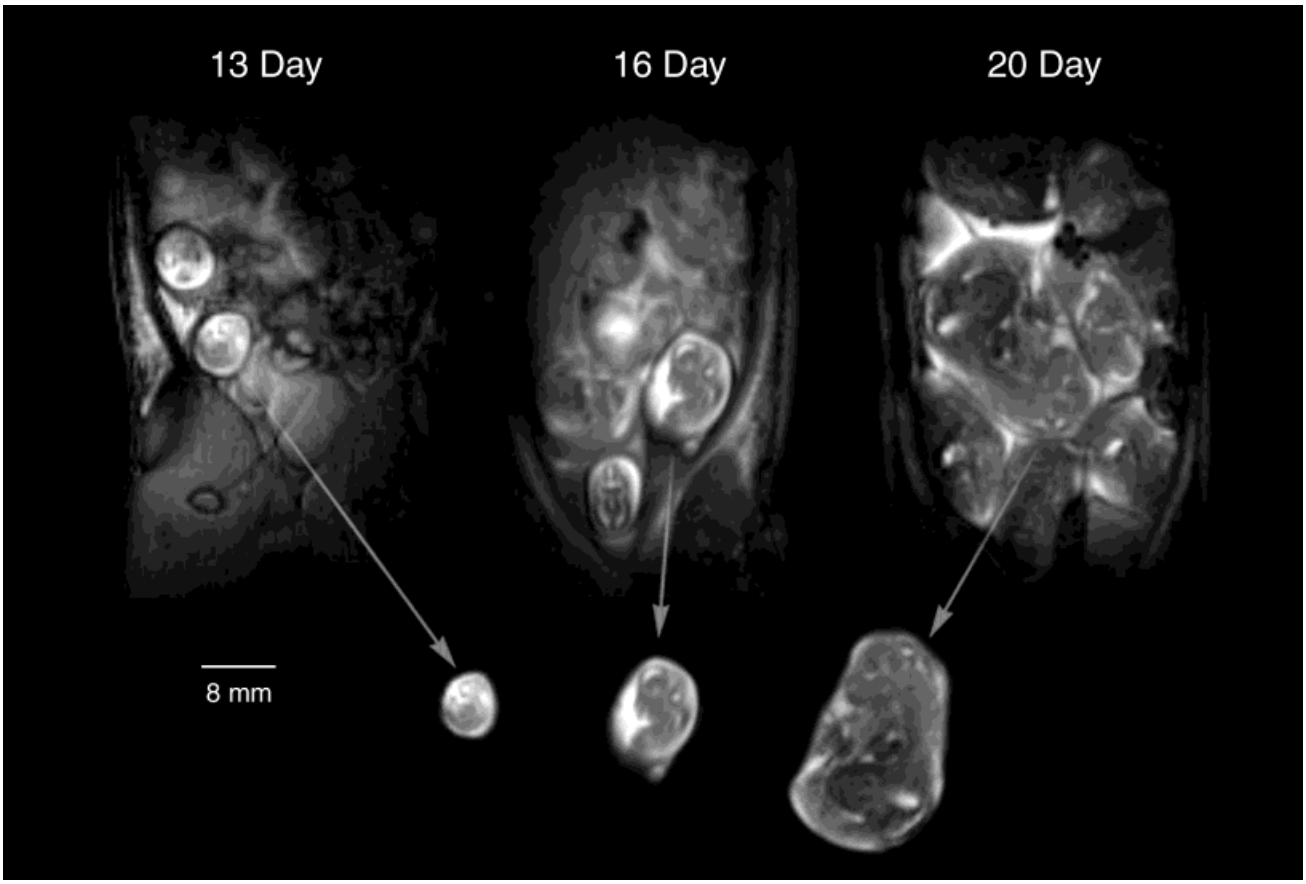

Fig. 1. In utero MRM images of a single litter of rat embryos at 13, 16, and 20 days of gestation. The large images are coronal sections through the female's lower abdomen and pelvis. Isolated embryos have been reoriented and placed below to show growth during this time period.

(Huang et al., 1998). The genetic manipulations were targeted to neural crest cells known to contribute to development of the cardiac outflow tract. MRM was chosen for morphological analysis because it provided a non-destructive way to obtain three-dimensional information, it was faster than conventional serial histology, and it avoided dehydration and cutting artifacts associated with producing three-dimensional reconstructions from serial histology. Every embryo in each litter was prepared for MRM examination by perfusion fixation and infusion of the magnetic resonance contrast agent BSA-DTPA-Gd. This contrast agent binds gadolinium to two large molecules (DTPA and bovine serum albumin) to retain the gadolinium in the vascular spaces for the duration of the imaging experiment.

The image data were analyzed using volume-rendering software to identify the heart chambers, the outflow tract, and the major blood vessels of the thorax. This was accomplished easily because the contrast agent caused significant enhancement of the magnetic resonance signal in all vascular spaces. Anterior views, lateral views, and axial views were available because of the three-dimensional data (Fig. 2). Optimal views were identified after acquiring the data, an option that is not available with histological sectioning. Furthermore, irrelevant anatomic structures were edited away in order to grant unobscured views of the structures under investigation. Thus, the cardinal veins were segmented away in order to afford clear views of the heart chambers, the outflow tract, and the aortic arches (Fig. 3). These vessels were visualized as three-dimensional structures with all their connections in place, an option not available in thin sections. When desired, thin slabs of data were selected in various planes to provide greater detail. However, this higher detail was usually obtained at the expense of contextual information.

Presenting three-dimensional image data as animations allowed concurrent viewing of detailed information and contextual information. Simple QuickTime® animations were generated to allow the investigators to slice through three-dimensional blocks of image data, pausing when desired to view detail, or moving quickly through the data to gain an appreciation for relationships between structures. Another benefit of animated sequences was achieved by gradually dissolving away confounding structures as the data set was rotated. Viewing the abnormal right ventricle and conotruncus as an isolated rotating structure allowed for detailed observation of its shape but did not show its extent or relationships to the rest of the heart or outflow tract. On the other hand, showing the entire heart and outflow tract as they rotated obscured the

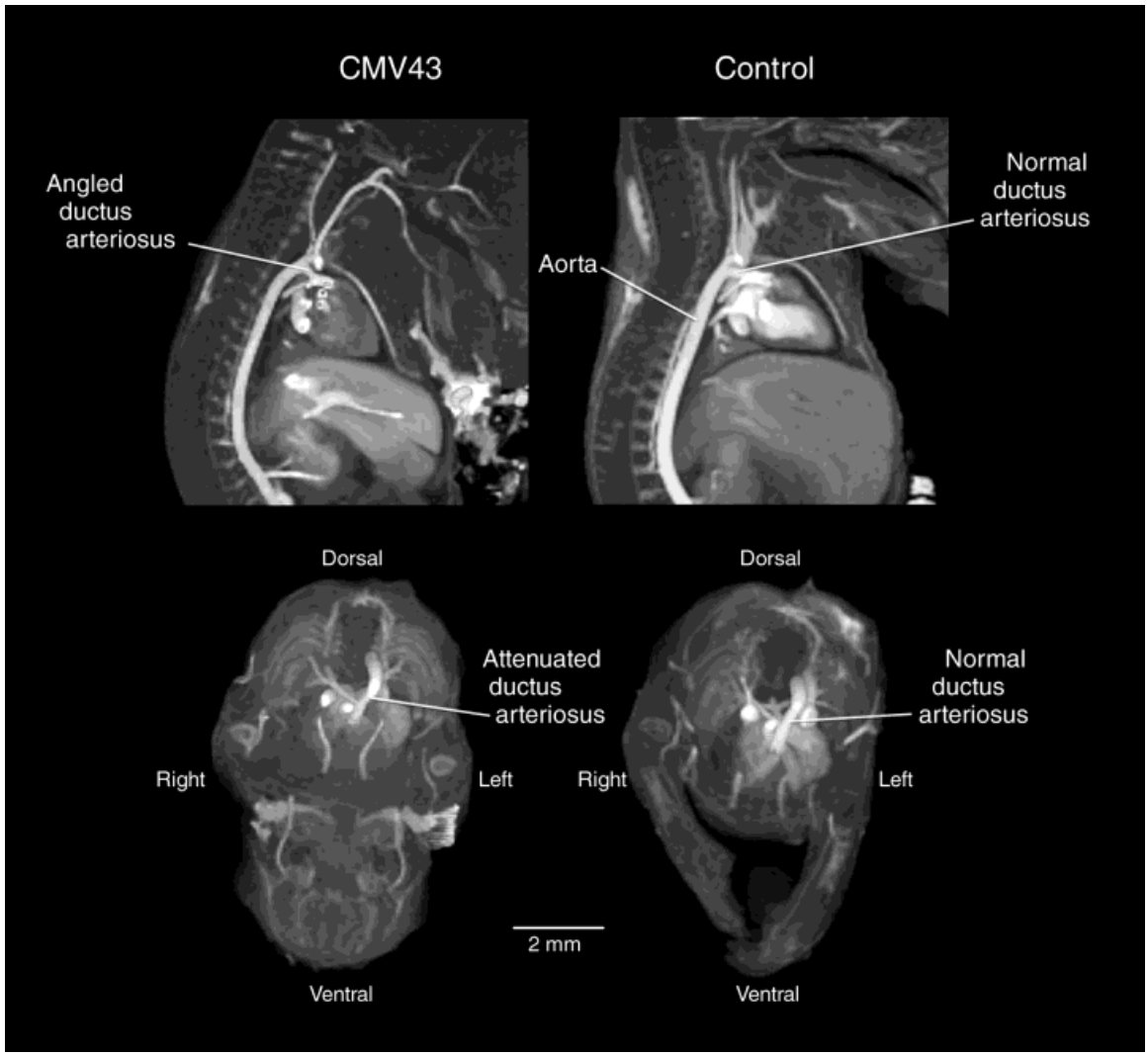

Fig. 2. Three-dimensional MRM data are volume-rendered to reveal right-lateral, and superior views of 14.5-day mouse embryo thorax. **Left:** Embryos over-expressing the Cx43 gap-junction gene (CMV43). **Right:** Control littermates. Editing sub-volumes of the data removes atria, venous vessels, ventricles, or cranial vessels to reveal the structures under investigation: Ductus arteriosus, right ventricle, and pulmonary artery.

malformations. Creating an animated dissolve of the left ventricle and major vessels as they rotated allowed the investigators to pause and study the three-dimensional detail of the right ventricular and conotruncal space and still appreciate their relationships to the ventricular walls and major vessels.

In the CMV43 transgenic mice, MRM revealed enlargement or dilation of the right ventricle and conotruncus with evidence of thinning of the right ventricular and conotruncal walls. The ductus arteriosus was typically attenuated and angled cranially just distal to the right and left pulmonary arteries (Fig. 2). This is in contrast to the normal straight course the ductus takes toward the descending aorta. The left ventricle appeared normal in size in control and CMV43 embryos.

MRM analysis of homozygous Cx43 knockout embryos showed double pouches extending from the outflow tract just proximal to the pulmonic valve (Fig. 3). There were also some irregularities in the right ventricular wall, although MRM showed no evidence of right ventricular enlargement or wall thickening. In some homozygous Cx43 knockout embryos, a single bulge was observed in the conotruncus. This was accompanied by right ventricular enlargement. MRM revealed an enlarged right ventricle and widened conotruncus in some of the heterozygous Cx43 knockout embryos. These embryos also showed some thinning of the ventricular and conotruncal walls. These heterozygous knockout embryo phenotypes were strikingly similar to the phenotypes of the CMV43 transgenic embryos.

MRM has also been used to assess morphological changes to the heart and aortic arches induced by altering the blood flow in embryonic chick hearts. Blood flow was altered in chick embryos by partial

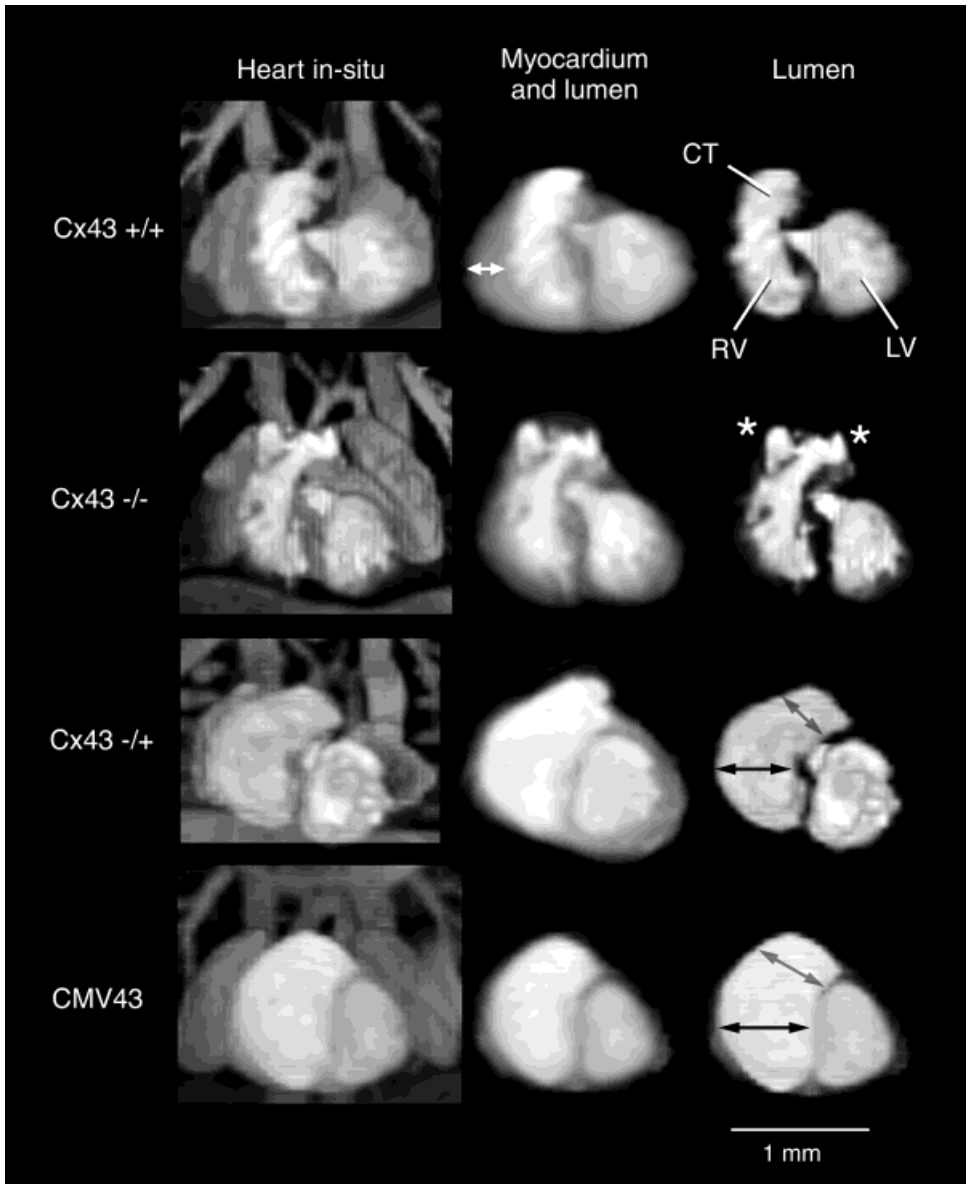

Fig. 3. The right and left ventricles and the conotruncus of 14.5-day mouse embryos are segmented (isolated) from the three-dimensional MRM data set to allow unobscured visualization of the outflow tract in embryos with altered expression of the connexin 43 gap junction gene. BSA-DTPA-Gd was used as a vascular contrast agent to enhance the magnetic resonance signal in the heart chambers. The middle column shows normal right ventricular wall thickness (Cx43 +/+) compared to the thinned ventricular wall in the heterozygous knockout embryo (Cx43 −/+) and the homozygous transgenic embryo (CMV43). The right column shows a normal conotruncal lumen (Cx43 +/+) compared to the double pouches in the lumen of the knockout embryo (Cx43 −/−), and the widened outflow tracts in heterozygous knockout embryos (Cx43 −/+) and homozygous transgenic embryos (CMV43). CT = conotruncus; RV = right ventricle; LV = left ventricle.

left atrial ligation with nylon suture at stage 21. The chick embryos were reincubated until stage 24 at which time they were perfused with fixative and BSA-DTPA-Gd contrast material via the cardinal vein. MRM generated three-dimensional data sets that were used to evaluate the primitive ventricle and aortic arches. Qualitative assessment of the aortic arches revealed diminished and incomplete formation of the left fourth aortic arches in embryos with left atrial ligation and normal fourth aortic arches in the control embryos (Fig. 4a). This assessment was made quickly and easily because the three-dimensional data sets could be oriented for optimal viewing. The relative sizes of the arches, their orientations, and their connections could all be evaluated from a single rotational animation.

Quantitative assessment of ventricular size was also available from these data sets. The number of voxels contained in the ventricle was counted automatically in the volume rendering software. A "seed point" was manually selected within the ventricle, the range of MRM signal values unique to the ventricle were entered (the contrast agent provided clear boundaries for these threshold values), and the software counted all voxels contained within the threshold limits that were also connected to the seed point. This number of voxels (approximately 8,000), when multiplied by the volume for each voxel (7.95E-5 μl), provided the total ventricular volume (0.636 μl) (Fig. 4b).

MRM also has the capability of generating images with various "staining" characteristics. A single embryo can be imaged several times with different pulse sequences that each emphasizes distinct anatomical or chemical properties in the subject (Fig. 5). This is accomplished with pulse sequences that alter the timing and strength of the radiofrequency pulses used to ex-

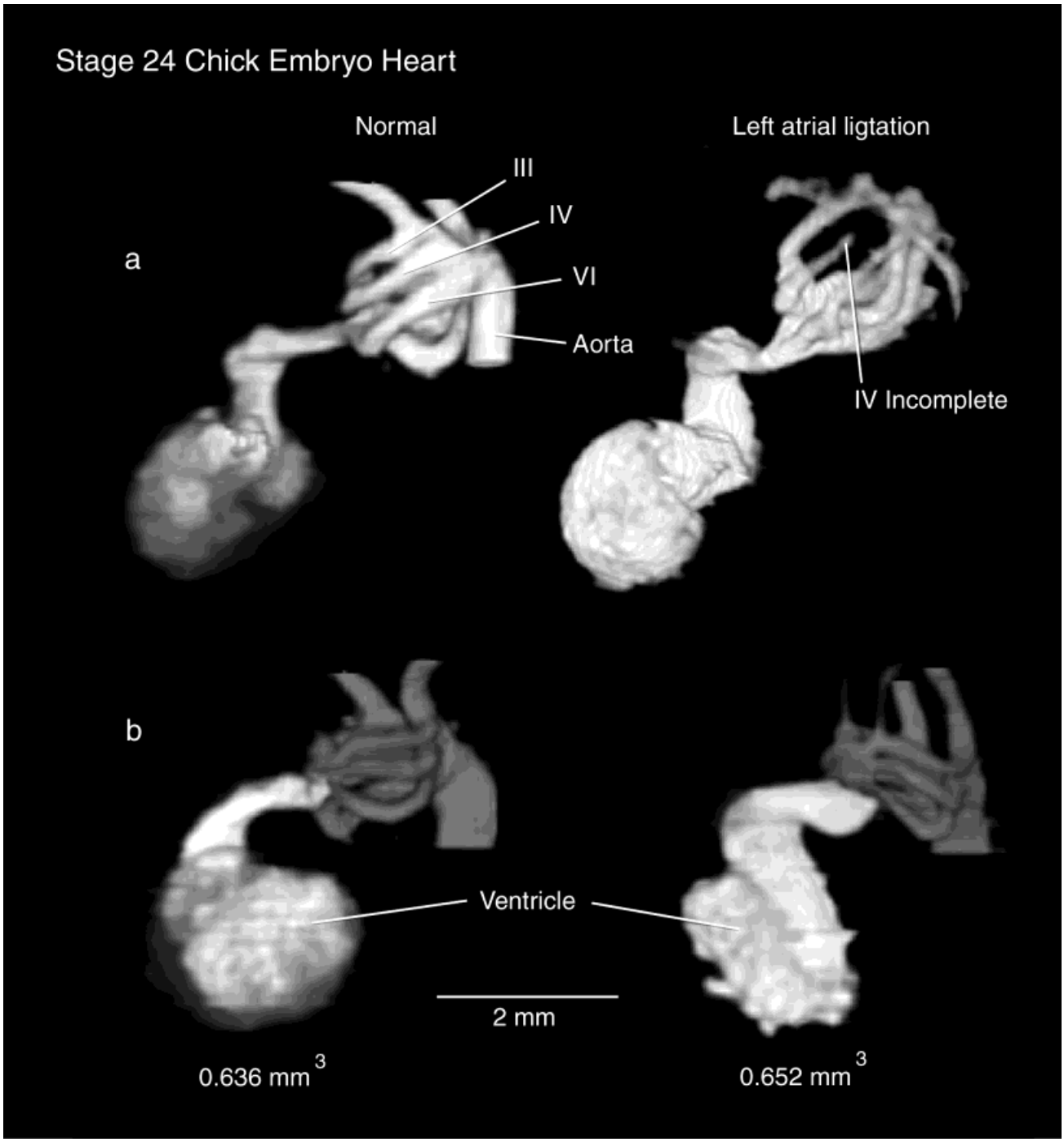

Fig. 4. **a:** MRM reveals the incomplete development of the left fourth aortic arch in a stage 24 chick embryo that underwent partial left atrial ligation to alter the normal hemodynamics. **b:** This technique also allows quantitative evaluation of ventricular volume in normal and experimental embryos.

cite the water protons in the subject and the magnetic gradient pulses that are used to encode the magnetic resonant signals. For example, diffusion-weighted pulse sequences emphasize contrast in tissues that vary in the anisotropy of their microstructure. Thus, tissues with highly aligned fibers (nerves) will provide contrast to tissues with randomized fiber orientation (dermis), or to structures with large water-filled regions (blood vessels, cranial ventricles). Pulse sequences must be selected to match the imaging conditions of the experiment and the tissues being distinguished or compared.

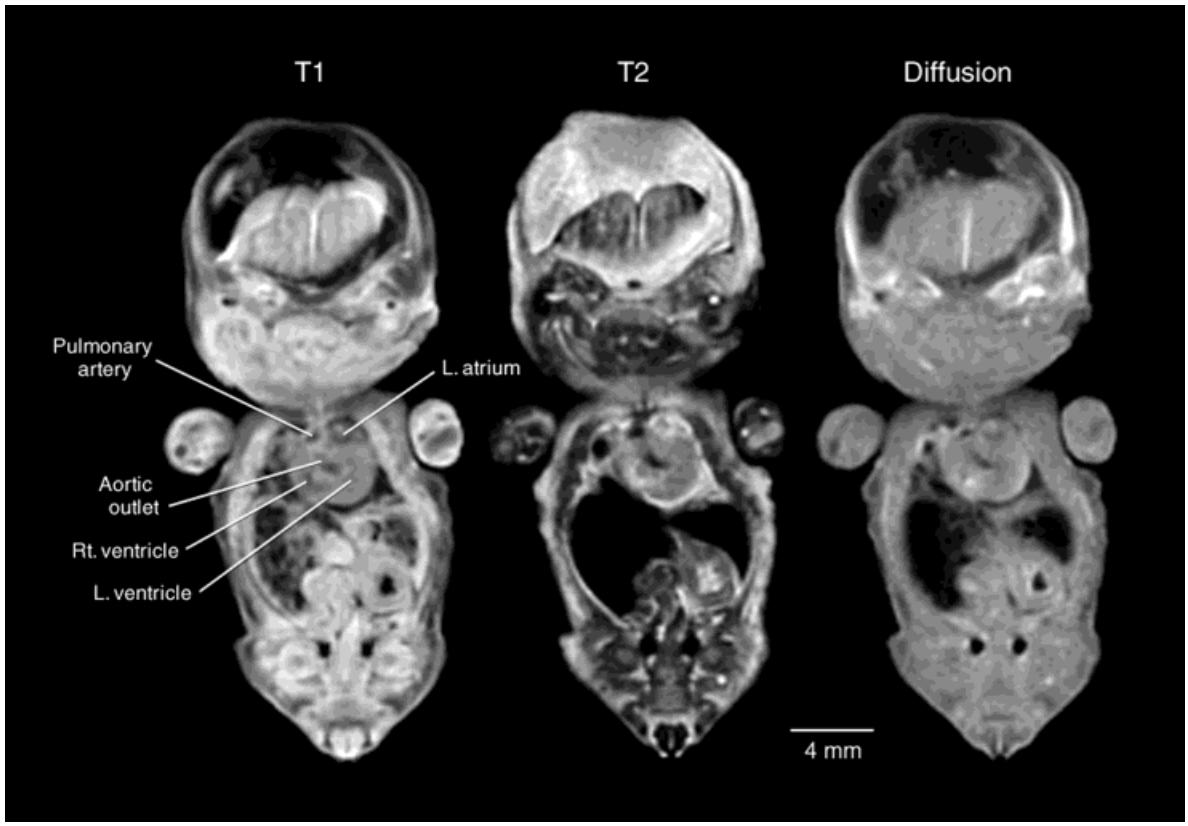

Fig. 5. A Carnegie stage 19 (47 days postovulatory) human embryo specimen reveals distinct anatomical properties when imaged with T1-, T2-, and diffusion-weighted pulse sequences. MRM can obtain these multiple "stains" through consecutive scans because it is a non-destructive imaging process.

SUMMARY

MRM is a fast and non-destructive technique that can produce three-dimensional images to analyze, qualitatively and quantitatively, growth and development in genetically altered animals and embryos. Researchers of development need access to these kinds of tools to provide rapid three-dimensional phenotypic analysis. Careful classification and characterization of phenotypes is essential for ultimately mapping the genes responsible for normal and abnormal development (Chien, 1996). MRM has several distinct strengths when compared to other imaging techniques used to characterize phenotypes in embryos and small animals: (1) it is fast, (2) it acquires three-dimensional data, (3) it can produce multiple "stains" of each specimen, (4) it is non-destructive, and (5) it can image deep within the specimen.

MRM generates non-distorted three-dimensional data that can be manipulated interactively in a fraction of the time required by conventional optical image reconstruction techniques. A three-dimensional data set can be produced from a fixed embryo specimen in 1.75 hours. That three-dimensional data set can then be processed and loaded into visualization software for real-time manipulation in less than 15 minutes. The three-dimensional nature of the data allows the creation of visual models that can be manipulated for fast and easy interpretation of complex data. The image data can be sliced digitally in any orientation including arbitrary cutting planes. Quantitative measurements of three-dimensional structures can be extracted for statistical analysis.

With MRM a specimen can be investigated with multiple pulse sequences to obtain a series of images with different tissues to tissue contrasts. Inherent properties of the tissues will produce various contrasts between structures, but contrast agents can also be added in order to increase or decrease the MR signal in regions of interest. The non-destructive nature of this technique makes it ideal for investigating rare or valuable specimens and live subjects.

It should be noted that MRM as described in this review does not directly record gene or gene product expression. Rather, these techniques record the morphological outcomes of gene expression. More specifically, these techniques record the changes in water relaxation characteristics as they are affected by gene product expression. Thus, MRM can document the morphological consequences of changes in gap junction gene expression, but it does not directly record the presence of the gap junction or its components. On the other hand, MRM is highly sensitive to bulk changes in extracellular and intracellular water distribution. Therefore, it is possible that MRM could document changes in gap junction activity to the extent that gap junctions influence this distribution of water.

An important challenge to using MRM in quantitative analysis of development results from the natural variation and experimental variation present during most experiments. This variation confounds the subtle differences measured in the vascular structures of embryos. Sources of natural variation include strain to strain differences in mouse models, embryo to embryo differences due to uterine position, and embryo to embryo differences between litters. Animal strains must be carefully described before experimental results can be compared. Embryo staging must also be used to accommodate variations in growth due to uterine position. Contrast perfusion techniques must also control for pressure and volume of contrast agent injection to minimize the introduction of experimental variation.

CONCLUSION

The greatest benefits of MRM will be realized when in utero imaging techniques allow non-invasive, longitudinal studies of entire litters of embryos. Challenges to in utero imaging include controlling motion artifacts induced by the mother and embryos, localizing the MRI signal from individual embryos in a way that promotes high signal-to-noise, and developing pulse sequences that provide image contrast relevant to the studies. Important benefits will also be gained with the development of targeted contrast agents. Targeted contrast agents can be localized in space or time by receptor specific binding or by becoming activated in the presence of selected enzymes or biochemical conditions. Recent reports describe targeting MRI contrast agents to tumor cells over-expressing a transferrin receptor (Weissleder et al., 2000) and contrast agents that are activated in the cell enzymatically by β-galactosidase (Louie et al., 2000). Data from in utero imaging and targeted contrast studies will become particularly useful when they can be quantified. Three-dimensional images of embryos must be converted into statistical descriptions with three-dimensional averages and three-dimensional standard deviations. This can then be extended into the fourth dimension by measuring average growth trajectories and standard deviations in the growth trajectories. Identification and communication of altered growth and form will become less subjective and more meaningful with statistical data. MRM is well positioned to provide the kind of data needed for these statistical analyses.

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REFERENCES

Benveniste H, Cofer GP, Piantadosi CA, Davis JN, Johnson GA. 1991. Quantitative proton magnetic resonance imaging in focal cerebral ischemia in rat brain. Stroke 22:259–268.

Blümich, Kuhn W, editor. 1992. Magnetic resonance microscopy. Methods and applications in materials science, agriculture and biomedicine. Weinheim: VCH Verlagsgesellschaft.

Chien K. 1996. Genes and physiology: molecular physiology in genetically engineered animals. J Clin Invest 97:901–909.

Evelhoch J, Gillies R, Karczmar GS, Koutcher JA, Maxwell RJ, Nalcioglu O, Raghunand N, Ronen SM, Ross BD, Swartz HM. 2000. Applications of magnetic resonance in model systems: Cancer therapeutics. Neoplasia 2:152–165.

Flynn TJ. 1987. Teratological research using in vitro systems. I. Mammalian whole embryo culture. Environ Health Perspect 72: 203–210.

Huang G, Wessels A, Smith BR, Linask KK, Ewart JL, Lo CW. 1998. Alteration in connexin 43 gap junction gene dosage impairs conotruncal heart development. Dev Biol 198:32–44.

Jacobs RE, Fraser SE. 1994. Magnetic resonance microscopy of embryonic cell lineages and movements. Science 256:681–684.

Johnson GA, Bone SN, Thompson MB. 1986. MR microscopy of the developing chick embryo. In: McCarthy S, Haseltine F, editors. Magnetic resonance of the reproductive system. Thorofare, NJ: Slack, Inc., p 137–141.

Johnson GA, Brown JM, Kramer PJ. 1987. Magnetic resonance microscopy of changes in water content in stems of transpiring plants. Proc Natl Acad Sci USA 84:2752–2755.

Johnson GA, Maronpot RR. 1989. Magnetic resonance microscopy of chemically induced liver foci. Toxicol Pathol 17:613–616.

Johnson GA, Benveniste H, Black RD, Hedlund LW, Maronpot RR, Smith BR. 1993. Histology by magnetic resonance microscopy. Magn Reson Q 9:1–30.

Lauterbur P. 1974. Magnetic resonance zeugmatography. Pure Appl Chem 40:149–157.

Lauterbur P. 1975. Magnetic resonance zeugmatography (imaging technique). Proceedings of the 18th Ampere Congress on Magnetic Resonance and Related Phenomena, Nottingham, UK, IUPAP. Amsterdam: North-Holland 1975.

Louie A, Hüber M, Ahren ET, Rothbacher U, Moats R, Jacobs RE, Fraser SE, Meade TJ. 2000. In vivo visualization of gene expression using magnetic resonance imaging. Nature Biotechnol 18:321–325.

Mansfield P, Grannell PK. 1975. Diffraction in microscopy in solids and liquids by NMR. Phys Rev B 12:3618.

Moller H, Chen X, Chawla MS, Cofer GP, Driehuys B, Hedlund LW, Suddarth SA, Johnson GA. 1999. Sensitivity and resolution in 3D NMR microscopy of the lung with hyperpolarized noble gases. Magn Reson Med 41:800–8.

Morehouse H, Levee E, States L, Zimmerman J, Newhouse J, Amis E Jr. 1995. MRI anatomy of the rat kidney at 1.5 T in different states of hydration. MRI 13:81–88.

Picard JJ. 1991. In vitro culture of postimplantation rodent embryos. Reprod Toxicol 5:221–222.

Smith BR, Effmann EL, Johnson GA. 1992. MR microscopy of chick embryo vasculature. J Magn Reson Imag 2:237–40.

Smith BR, Johnson GA, Groman EV, Linney E. 1994. Magnetic resonance microscopy of mouse embryos. Proc Natl Acad Sci USA 91:3530–3.

Smith BR. 1996. Magnetic resonance microscopy with cardiovascular applications. Trends Cardiovasc Med 6:247–254.

Smith BR, Linney E, Huff DS, Johnson GA. 1996. Magnetic resonance microscopy of embryos. Comput Med Imag Graphics 20:483–490.

Smith BR, Shattuck MD, Hedlund LW, Johnson GA. 1998. Time-course imaging of rat embryos in utero with magnetic resonance microscopy. Magn Reson Med 39:673–677.

Smith BR. 2000. Magnetic resonance imaging analysis of embryos. In: Tuan R, Lo CW, editors. Developmental biology protocols, Vol I. Totowa, NJ: Humana Press, p 211–216.

Wehrli F. 1992. Principle of magnetic resonance. In: W. G. B. J. David D. Stark, W. G. B. J. David D. Stark, MRI. St. Louis: Mosby-Year Book.

Weissleder R, Moore A, Mahmood U, Bhorade R, Benveniste H, Chiocca EA, Basilion JP. 2000. In vivo magnetic resonance imaging of transgene expression. Nature Med 6:351–355.

Yavarone MS, Shuey DL, Sadler TW, Lauder JM. 1993. Serotonin uptake in the ectoplacental cone and placenta of the mouse. Placenta 14:149–161.