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MRI and Histopathologic Study of a Novel Cholesterol-Fed Rabbit Model of Xanthogranuloma

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Abstract

Purpose—To develop a rabbit model of xanthogranuloma based on supplementation of dietary cholesterol. The aim of this study was to analyze the xanthogranulomatous lesions using magnetic resonance imaging (MRI) and histological examination.

Materials and Methods—Rabbits were fed a low-level cholesterol (CH) diet ($n = 10$) or normal chow ($n = 5$) for 24 months. In vivo brain imaging was performed on a 3T MR system using fast imaging employing steady state acquisition, susceptibility-weighted imaging, spoiled gradient recalled, T_1 -weighted inversion recovery imaging and T_1 relaxometry, PD-weighted and T_2 -weighted spin-echo imaging and T_2 relaxometry, iterative decomposition of water and fat with echo asymmetry and least-squares estimation, ultrashort TE MRI (UTE-MRI), and T_2^* relaxometry. MR images were evaluated using a Likert scale for lesion presence and quantitative analysis of lesion size, ventricular volume, and T_1 , T_2 , and T_2^* values of lesions was performed. After imaging, brain specimens were examined using histological methods.

Results—In vivo MRI revealed that 6 of 10 CH-fed rabbits developed lesions in the choroid plexus. Region-of-interest analysis showed that for CH-fed rabbits the mean lesion volume was $8.5 \pm 2.6 \text{ mm}^3$ and the volume of the lateral ventricle was significantly increased compared to controls ($P < 0.01$). The lesions showed significantly shorter mean T_2 values ($35 \pm 12 \text{ msec}$, $P < 0.001$), longer mean T_1 values ($1581 \pm 146 \text{ msec}$, $P < 0.05$), and shorter T_2^* values ($22 \pm 13 \text{ msec}$,

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$P < 0.001$) compared to adjacent brain structures. The ultrashort T_2^* components were visible using UTE-MRI. Histopathologic evaluation of lesions demonstrated features of human xanthogranuloma.

Conclusion—Rabbits fed a low-level CH diet develop sizable intraventricular masses that have similar histopathological features as human xanthogranuloma. Multiparametric MRI techniques were able to provide information about the complex composition of these lesions.

Cholesterol has been implicated in several age-related diseases including atherosclerosis, aortic valve disease,¹ stroke, and Alzheimer's disease.² The cholesterol-fed (CH-fed) rabbit has been used as an animal model for the study of atherosclerosis since 1913, when Anitchkow first reported that CH feeding promoted atherosclerotic plaque formation.³ Recently, the CH-fed rabbit has also been proposed as an animal model of Alzheimer's disease. Sparks et al have shown that a relatively high-level (1–2% w/w), short-term (10–28 weeks) CH diet promotes increased immunoreactivity for amyloid beta protein ($A\beta$) in the brains of NZW rabbits.^{4,5} More recently, we showed that a lower level diet of CH (0.125–0.25%, w/w) for 27 months consistently induced both intracellular $A\beta$ accumulation and the formation of a specific type of extracellular $A\beta$ -positive plaque in the brain.⁶ During further study of this animal model, we found mass lesions within the ventricles, and in the present study we hypothesize that these lesions are similar to xanthogranulomas found in humans.

Xanthogranulomas found in the central nervous system are not rare. These lesions were first reported by Blumer in 1900,⁷ and since then have been encountered incidentally at postmortem autopsy in 1.6 to 7% of examined cases.⁸ Development of xanthogranuloma is occasionally associated with diabetes mellitus, familial hyperproteinemia, juvenile xanthogranuloma, colloid cysts, or histiocytosis X.^{9–11} In addition, xanthogranuloma development has been linked to hyperlipidemia, familial hypercholesterolemia, and arteriosclerosis.^{12,13} The pathogenesis of xanthogranuloma is poorly understood, and whether xanthogranuloma are granulomatous, neoplastic, inflammatory, or metabolic in origin is still a matter of debate. The pathologic features of xanthogranuloma are complex, including foamy cells, CH crystal clefts, foreign body giant cells, chronic inflammatory cellular reaction, and hemosiderin deposits.^{12,13} Although small and benign in most cases, larger xanthogranuloma can be problematic. Reports concerning symptomatic xanthogranuloma usually involve the choroid plexus of the third ventricle and the lateral ventricle, obstructing the flow of cerebrospinal fluid due to mass effect.¹⁴ In some cases, the lesions protrude into adjacent brain parenchyma, producing neurological deficits.^{14,15}

Xanthogranulomas in the brain do not present with typical imaging features,¹⁸ which makes differential diagnosis of xanthogranuloma from other ventricular lesions difficult. The majority of reported xanthogranuloma cases were initially incorrectly identified as craniopharyngiomas or pituitary adenomas.^{19,20} Moreover, as the diagnosis mostly depends on surgical intervention, the natural history of xanthogranuloma is unclear.¹⁶

Magnetic resonance imaging (MRI) and computed tomography (CT) have both been explored as ways to non-invasively identify xanthogranuloma in individuals. Signal characteristics of xanthogranuloma on MRI vary depending on the mixture of foamy macrophage accumulation, fluid, CH crystal, fibrous proliferation, and blood products.¹⁷

The lipid deposits of a xanthogranuloma exhibit T_1 -weighted high and T_2 -weighted low signal intensities; however, foamy cell accumulation, focal hemorrhages, and hemosiderin deposits contained in lesions can result in a heterogeneous appearance and may be essential in the development of xanthogranulomas.¹⁸

In this study we developed a rabbit model of xanthogranuloma based on supplementation of dietary CH. This model may allow the natural history of this disease to be more thoroughly studied, as well as exploration of imaging tools that can help differentiate this disease from other diseases of the lateral ventricle. The purpose of this study was to analyze the complex features of the xanthogranulomatous lesions using both MRI and histopathology.

Materials and Methods

Animal Model

All animals for this study were cared for in accordance with the guidelines from the Canadian Council on Animal Care. Adolescent male New Zealand white rabbits ($n = 15$, 4–5 months old, 2500–3000 g, Charles River Laboratories, Wilmington, MA) were randomly assigned to experimental and control groups.

Ten rabbits were initially fed 100 g/day of CH-supplemented (0.25% w/w) rabbit chow. Thereafter, diet was titrated as needed (between 0.125% and 0.25%) to maintain a serum CH level of ~400 mg/dL for 24 months (Table 1). Five age-matched control rabbits were fed normal chow. Serum CH levels were determined for each animal. Blood was drawn from the central ear artery following acepromazine administration (0.10 mL/kg intramuscular) to promote arterial dilation and analgesia. Total serum CH was measured using a colorimetric enzyme-based kit (Wako Diagnostics, Richmond, VA).

MRI Protocol

Animals were sedated via an intramuscular injection of stock anesthetic (ketamine 23.4 mg/kg, xylazine 1.3 mg/kg, and glycopyrrolate 0.0075 mg/kg) followed by intravenous administration of a 10-fold dilution (in saline) of this stock at a rate of 3 to 12 mL/h. Anesthetized rabbits were imaged with a clinical 3T MRI scanner (GE Discovery MR750, GE Healthcare, Waukesha, WI) using a custom-made, 2-channel, phased array surface radiofrequency coil.^{22,23}

Three-dimensional fast imaging employing steady state acquisition (FIESTA) was performed in the axial plane using the following parameters: FOV $5.1 \times 5.1 \text{ cm}^2$; image matrix 256×256 ; slice thickness 0.2 mm; repetition time / echo time (TR/TE) 20/10 msec; flip angle (FA) 20° ; bandwidth (BW) $\pm 10.42 \text{ kHz}$; phase cycling number/recon: 4/sum-of-squares; number of scans averaged 1; scan time 69 minutes.

Three-dimensional susceptibility-weighted imaging, using a 3D multiecho gradient echo sequence to enhance the T_2^* effect (SWAN) was performed in the axial plane using the following parameters: field of view (FOV) $5.1 \times 5.1 \text{ cm}^2$; image matrix 256×256 ; slice thickness 0.2 mm; TR/TE 83.1/29.9 msec; FA 30° ; BW $\pm 31.25 \text{ kHz}$; five echoes with first echo delay 2 msec; number of scans averaged 1; scan time 72 minutes.

Three-dimensional fast spoiled gradient recalled (SPGR) T_1 -weighted sequence was performed in the axial plane using the following parameters: FOV $5.1 \times 5.1 \text{ cm}^2$; image matrix 170×170 ; slice thickness 0.3 mm; TR/TE 8.6/3.09 msec; FA 20° ; BW $\pm 15.6 \text{ kHz}$; number of scans averaged 12; scan time 4.6 minutes.

T_1 -weighted images and T_1 relaxometry were acquired with an inversion recovery (IR) fast spin-echo imaging sequence. Imaging parameters were: FOV $5.1 \times 5.1 \text{ cm}^2$; image matrix 128×128 ; slice thickness 0.4 mm; TR/TE 3000/11.3 msec; BW $\pm 6.41 \text{ kHz}$; echo train length 2; eight inversion times 50, 150, 300 450, 600, 750, 900, and 1400; 1 repetition, and a scan time of 3.3 minutes per TI.

Proton density-weighted images, T_2 -weighted images and T_2 relaxometry were acquired using a multislice spin-echo sequence, with the following parameters: FOV $5.1 \times 5.1 \text{ cm}^2$; image matrix 128×128 ; slice thickness 0.4 mm; TR 2000 msec; BW $\pm 6.41 \text{ kHz}$; six echo times of 17, 25, 35, 45, 55, and 70 msec; 1 repetition, and a scan time of 4.7 minutes per TE.

Iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL) imaging was performed, producing four kinds of images: water, fat, recombined water, and fat (in-phase), and water minus fat (out-of-phase) images. We applied IDEAL gradient-echo imaging with the following parameters: FOV $5.1 \times 5.1 \text{ cm}^2$; image matrix 170×170 ; slice thickness 0.6 mm; TR/TE 10.5/3.4 msec; FA 30° ; BW $\pm 15.63 \text{ kHz}$; number of scans averaged 12; scan time 28 minutes.

Ultrashort TE MRI (UTE-MRI) and T_2^* relaxometry were performed using a half-pulse 2D projection reconstruction radial sequence with a minimum TE capability of $8 \mu\text{s}$. Additional parameters included FOV $6.0 \times 6.0 \text{ cm}^2$, slice thickness 0.6mm with interslice gap 0.8 mm, FA 30° , 515 half projections, readout of 128 points with a $\pm 125 \text{ kHz}$ sampling bandwidth, number of scans averaged 2. Four sets of images with TE of 0.008, 5, 10, and 20 msec were produced with a repetition time of 200 msec. Short- T_2 -selective UTE images were obtained through the subtraction of images with TE 5 msec from the images with TE 0.008 msec.

Histology

TISSUE COLLECTION—Following MRI, three CH-fed animals and two control animals were sacrificed with an overdose of ketamine (2mL intravenous bolus) and pressure-perfused via the left ventricle with a minimum of 1.5 L of heparinized (1 U/ml) Hanks' balanced salt solution. During collection of the brains we determined the lesion number, size, and locations. Serial coronal sections were cut throughout the brain with a vibrotome (Leica, Nussloch, Germany) at a thickness of $40 \mu\text{m}$ and collected in PBS.

HEMATOXYLIN AND EOSIN (H&E) STAINING—Brain sections were washed briefly in distilled water, stained in Harris hematoxylin solution for 8 minutes, and differentiated in 1% acid alcohol for 30 seconds. After washing in 0.2% ammonia for 5 minutes, sections were counterstained in eosin-phloxine solution for 30 seconds; dehydrated through 95% alcohol, two changes of absolute alcohol, and 5 minutes each. Sections were then dehydrated and cleared through 95% ethyl alcohol, absolute alcohol, and xylene, and mounted with resinous medium.

OIL-RED-O STAINING FOR LIPID—Brain sections were fixed in ice-cold 10% formalin for 10 minutes and rinsed in distilled water. After washing in absolute propylene glycol three times, sections were stained for lipids in prewarmed Oil-red-O working solution (0.5 gram of oil-red-O in 100 mL of absolute propylene glycol) for 8–10 minutes in a 45°C oven. Sections were then immersed in 85% propylene glycol solution for 2–5 minutes and counterstained with Gill's hematoxylin for 30 seconds. Sections were mounted with glycerin jelly for microscopic observations.

PRUSSIAN BLUE STAINING FOR IRON—Iron deposition was evaluated in brain sections using a modified Prussian blue protocol.⁶ Sections were washed with distilled water three times and immersed in Perl's solution (1 part 7% aqueous potassium ferrocyanide and 1 part of 3% hydrochloric acid) at 37°C for 1 hour, followed by three washes with distilled water for 5 minutes. Sections were then dehydrated with 70–100% alcohol, cleared with xylene, and coverslipped for microscopic examination.

SILVER STAINING—Brain sections were placed in prewarmed (40°C) 10% silver nitrate solution and stained for 20 minutes. After washing in distilled water, slides were placed in an ammonium silver solution (adding concentrated ammonium hydroxide to 10% silver nitrate solution until the precipitate formed was clear) in a 40°C oven for 20 minutes, then directly placed in a developer working solution (20.0mL 37–40% formaldehyde, 0.5 gm citric acid, 2 drops nitric acid, and 100mL distilled water). Sections were dipped in 1% ammonium hydroxide solution for 1 minute to stop the silver reaction, washed three times, and placed in 5% sodium thiosulfate solution for 5 minutes. Sections were then dehydrated and cleared through alcohol and xylene, and mounted with xylene based mounting medium.

IMMUNOHISTOCHEMISTRY—Immunostaining was performed using a mouse monoclonal antibody against rabbit macrophage marker, RAM11 (1: 1000 dilution; Dako, Carpinteria, CA) and endothelial cell marker, CD31 (1:100 dilution; Abcam, Cambridge, MA). Sections were incubated for 30 minutes in TBS containing 3% hydrogen peroxide and 30 minutes in blocking reagent (15% goat serum in TBS), followed by incubation with the primary antibody for 24 hours at 4°C. Immunoreactivity was detected using horse antimouse biotinylated secondary antibodies for 30 minutes followed by processing using an ABC kit (Vector Labs, Burlingame, CA). Signal was detected using 0.05% 3-3-diaminobenzidine (Sigma, St. Louis, MO) and 0.005% hydrogen peroxide (pH 7.2). Adjacent sections incubated in the absence of primary antibody served as negative controls. After processing, the tissue sections were mounted onto gelatin-coated slides and airdried. Sections were examined by light microscopy using a Zeiss Axioskop 2 microscope equipped with an AxioCam digital camera (Carl Zeiss Canada, Toronto).

MRI and Histopathology Analysis

The FIESTA and SWAN images were reviewed by four observers: one board-certified radiologist (M.M.Z.) with over 20 years experience, two researchers (Y.C. and A.M.H.) with 10 years and 11 years, respectively, experience in MR image analysis, and a researcher (K.M.P.) with 1 year experience. All investigators were blind to the animal diet and histological findings, and had no knowledge of the type and frequency of lesion in the

images. The reviewers scored their confidence in lesion detection in the ventricular areas (third ventricle and lateral ventricle) using a 5-point Likert scale: 1, lesion definitely not present; 2, lesion probably not present; 3, uncertain; 4, lesion probably present; 5, lesion definitely present. The number of lesions with detection scores of 4 or 5 were tabulated across all rabbits and the size of these lesions was rated using a semiquantitative scale from 1 to 5: 1, very small lesions; 2, small lesions; 3, medium-size lesions; 4, large lesions; 5, large lesions causing enlargement of lateral ventricle or protruding into the adjacent cerebral parenchyma.

Further quantitative analysis of the MRI characteristics of lesions was performed independently by three investigators (Y.C., A.M.H., and K.M.P.). Region-of-interest (ROI)-based volumetry on 10 CH-red rabbits and five controls was performed on the FIESTA and SWAN images by manually outlining the entire lesion region and lateral ventricle in each MR slice. Both lesion volume and ventricular volume were calculated in cubic millimeters by multiplying the number of pixels within the traced ROIs by the volume of each voxel. T_1 maps were obtained from a voxel-wise nonlinear least squares regression fitting of the image signal intensity as a function of inversion time (eight TI values: 50, 150, 300, 450, 600, 750, 900, and 1400 msec) to a single exponential inversion recovery model. T_2 maps were obtained from a voxel-wise linear regression fitting of the log signal intensity as a function of echo time (six spin echo TE values: 17, 25, 35, 45, 55, and 70 msec) to a single exponential decay model. T_2^* maps were obtained from a voxel-wise linear regression fitting of the normalized log image signal intensity as a function of echo time (5, 10, and 20 msec) to a single exponential decay model. All fitting was performed using custom software developed in MatLab (MathWorks, Natick, MA). ROIs of five lesions in two rabbits and their adjacent cortical/subcortical areas were manually drawn on the original images and used to define regions from which mean T_1 , T_2 , and T_2^* values were extracted from the relaxation maps.

The histology and immunohistochemistry staining was initially examined by an individual with more than a decade histology experience (Y.C.) and his observations were confirmed by an expert pathologist who was blind to the results of MRI evaluation. Five histological components: CH clefts, foamy histiocytes, macrophages, capillary vasculature, and hemosiderin deposits were assessed in the lesions using H&E and Bielschowsky's silver staining, Oil-red-O staining, Ram11 immunostaining, CD31 immunostaining, and Prussian blue staining, respectively. Microscopic images of the stained slides were obtained with a Zeiss Axioskop 2 microscope, equipped with digital camera AxioCam and AxioVision Rel (v. 4.8) software for image processing (Carl Zeiss Canada, Toronto).

Intra- and Interobserver Reliability Analysis

Each observer rated the MRI a second time and in a different random order more than 1 week after the first assessment. To determine the degree of intra- and interobserver reliability for the dichotomous measure (lesion presence in the brains), Cohen's kappa coefficient was calculated. For the ordinal measure (lesion size grade), reliability was assessed using linear-weighted Cohen's kappa. For ROI-based measurements, the intra- and interobserver reliability was assessed using the intraclass correlation coefficient (ICC).

Cohen's kappa and two-way ICCs were calculated for each pair of observers and 95% confidence intervals were generated. Reliability for both kappa and ICCs was rated as moderate agreement for values ranging from 0.41 to 0.59, as substantial agreement for values between 0.60–0.79, and as excellent agreement for values above 0.8.^{19,20} Interobserver reliability analysis was performed using the second set of each observer.

Statistical Analysis

Quantitative measurements from all four observers were averaged to generate final measurements. χ^2 testing was used to investigate the incidence rate of the lesion in the CH-fed group and control group. Student's *t*-test was used to compare the volume of the lateral ventricle between CH-fed group and control group. One-way analysis of variance (ANOVA) was performed to assess the mean T_1 , T_2 , and T_2^* values between lesions and adjacent cortex and subcortical regions with post-hoc analysis (Neumann-Keuls test). A nominal *P*-value of less than 0.05 was considered significant for all tests.

RESULTS

In Vivo MRI Revealed Mass Lesions in Choroid Plexus of Rabbits Fed a Long-Term, Low-Level CH-Diet

In vivo MRI revealed that CH-diet rabbits developed mass lesions in their brains. The lesions were present in multiple regions and were mainly confined to the choroid plexus of the lateral ventricle and ventral fornix at the roof of the third ventricle. In 6 of 10 CH-fed rabbits, all observers judged lesions to be present and a total of 13 lesions (lesions with detection scores of 4 or 5) were identified in the CH-fed rabbits compared to no lesions in the control animals (Three observers did not detect any lesion in the control rabbits, while one observer found one lesion in one control rabbit). Two observers identified that two large-size lesions were accompanied by remarkable enlargement of the ventricular system (Fig. 1A,B; Supplementary Fig. 1). In one case, the lesion was present in the left dorsal lateral ventricle, protruding into the medial cerebral parenchyma, and the dorsal portion of the left lateral ventricle was dilated. The semiquantitative analysis of lesion size showed a mean score of 3.3 (medium to large size). Quantitative analysis showed that the mean lesion volume in the lateral ventricle was $8.5 \pm 2.6 \text{ mm}^3$, occupying on average 18.5 percent of the lateral ventricular space. Compared with control rabbits, the mean volume of lateral ventricle of CH-fed rabbits was significantly increased from 28.1 ± 6.1 (control) to 45.9 ± 10.0 (CH-fed) mm^3 (~1.6-fold; $P < 0.01$; Fig. 1D).

Qualitative evaluation of mass lesions in FIESTA images and SWAN images revealed lesions that were generally hypointense compared with adjacent brain parenchyma and had foci that were completely devoid of signal (Fig. 1; Supplementary Fig. 2). Compared to adjacent brain parenchyma, the lesions also showed slightly hypointense signal in PD-weighted images, mixed isointense signal with markedly hypointense foci in T_1 -weighted images, and generally hypointense signal in T_2 -weighted images. (Fig. 2; Supplementary Figs. 3 and 4).

Consistent with the T_1 - and T_2 -weighted imaging, quantitative analysis of lesions on T_1 and T_2 maps revealed significantly longer mean T_1 values (1581 ± 146 msec) compared to cortex (1417 ± 56 msec) on T_1 maps (Fig. 2C, left graph; $P < 0.05$) and significantly shorter mean T_2 values (35 ± 12 msec) compared to cortex (71 ± 6 msec) and subcortex (63 ± 4 msec) on T_2 maps (Fig. 2C, right graph; $P < 0.001$).

We performed IDEAL imaging on two CH-fed rabbits. Lesions in water-only IDEAL images exhibited as isointense signal mixed with foci of hypointensity. The fat-only IDEAL images showed bright signal from subcutaneous fat and subarachnoid lipids, but did not reveal positive signal within the lesions (Supplementary Fig. 5).

UTE-MRI indicated that the xanthogranulomatous lesions contained ultrashort T_2^* components, which presented as positive signal intensity in the short- T_2 -selective UTE images. Consistently, color-coded T_2^* maps showed foci of ultrashort T_2^* values (shorter than 1 msec), with mean lesion T_2^* values (22 ± 13 msec) significant shorter than the mean T_2^* values of adjacent brain structures (cortex: 67 ± 8 msec; subcortical structures: 57 ± 6 msec) ($P < 0.001$, Fig. 3; Supplementary Fig. 6).

Statistical evaluation of intra- and interobserver variation using Cohen's kappa and ICCs demonstrated good to excellent correlation in a majority of the parameters evaluated (Supplementary Table 1). Cohen's kappa was used to assess the categorical measurement (presence of lesion and lesion size grade) and ICCs were used to assess the quantitative ROI-based measurement. The intraobserver kappa and ICCs were excellent (kappa 0.82 to 1.0 and ICCs 0.84 to 0.95). The interobserver kappa values ranged from 0.78 to 0.84, indicating good to excellent interobserver correlation. For the ROI-based measurement, the interobserver reliability was moderate to excellent (ICCs: 0.56 to 0.82).

Gross and Histological Characteristics of the Lesion

Pathological autopsy of the brains showed that, although the number of lesions varied among animals, their location usually centered on the choroid plexus at the roof of the third ventricle and the lateral ventricle. Small lesions were typically tumorous swellings that were confined to the area of the choroid plexus. More severe lesions invaded adjacent structures of the brain parenchyma, especially hippocampus and dorsal diencephalons. In contrast, none of the five control animals on the normal diet demonstrated cerebral xanthogranuloma.

Histological and immunohistochemical examination of the lesions from three CH-fed rabbits indicated that they contained foamy cells (histiocytes), multinucleated giant cells as shown in the H&E staining, iron-rich hemosiderin deposits, crystalline CH clefts, and capillary vasculature (Fig. 4). Foam cells in and around the xanthogranuloma were macrophage-derived (RAM11-positive) and their cytoplasm was strongly positive for lipids (Oil-red-O-positive).

DISCUSSION

Human xanthogranulomas are often found in the third ventricle and the choroid plexus of the lateral ventricle.^{18,21} Their histologic features typically include CH clefts, macrophages

(xanthoma cells), chronic inflammatory cellular reaction, and hemosiderin deposits.¹³ The pathogenesis of xanthogranuloma is obscure and may be the manifestation of different pathological processes. Most investigators believe the disease to be a granulomatous disorder rather than a neoplastic process.^{8,22} Lipid dysregulation has been implicated as a risk factor for xanthomatosis.^{11,22,23} Case reports have shown that extensive intracranial xanthomas are present in patients with hyperlipidemia and hypercholesterolemia.^{23,24} Fulminant atherosclerosis, accompanied by xanthomatous accumulation of CH in skin and tendons, is the hallmark of homozygous familial hypercholesterolemia.²⁴ The high levels of free CH and CH esters in xanthogranuloma indicate that these lesions result from active lipid storage caused by a metabolic derangement of xanthoma cells.²² In this study, most rabbits maintained on hypercholesterolemia for 24 months developed granulomatous lesions in the choroid plexus. Pathologic examination of these lesions revealed the typical characteristics of xanthogranuloma in humans, including characteristic foamy macrophages (histiocytes), giant cells, CH clefts, hemosiderin deposits, and capillary vasculature. These results indicate that hyperlipemia/ hypercholesterolemia is a sufficient predisposing factor to the formation of cerebral xanthogranuloma in rabbits.

Human intracranial xanthogranuloma exhibits a heterogeneous appearance on MRI scans. These lesions have been reported to be hyperintense or isointensity on T_1 -weighted MRI and heterogeneous on T_2 -weighted MRI.^{17,21,25,26} The varied appearance of xanthogranuloma on MR scans is probably due to the complexity of the lesion's composition. In this work we applied multiple MRI techniques to explore the MRI features of the lesions in rabbits. In FIESTA and SWAN images the lesions were generally hypointense in signal intensity. The FIESTA and SWAN methods used in this study are known to be sensitive to iron-induced local magnetic field inhomogeneity, and have been applied for tracking iron-loaded cells and detecting iron deposition in the brains.^{6,27,28} We found in ex vivo analysis that the lesions contained iron, and while further work will be needed to confirm this, we believe the signal loss on FIESTA and SWAN images to be due to significant hemosiderin deposition. Quantitative analysis showed that the lesions have short T_2 and T_2^* values and low-to-intermediate T_1 values compared with normal brain parenchyma. Overall, many of the MRI features of the xanthogranuloma in our rabbits were similar to those found in humans,²⁶ except that in our study the MRI signal of the lesions was more heterogeneous and interspersed with areas of marked hypointensity in both T_1 - and T_2 -weighted images. This might be attributed to the extent of extracellular depositions of crystalline CH and hemosiderin, both of which might create foci of signal voids in susceptibility-weighted, T_2 -weighted, and T_2^* -weighted MRI images.

Bruck et al²⁹ published a case report of an intracranial xanthogranuloma showing hyperintensity on the T_1 -weighted images. The high-intensity signal on T_1 -weighted scans may be attributed to accumulated extracellular lipids in the lesion. However, in our case the lesions exhibited isointensity or hypointensity on T_1 -weighted images compared to adjacent cortex. To determine if MRI visible lipid is present in the rabbit xanthogranuloma, we imaged two CH-fed rabbits with IDEAL. IDEAL imaging, which is designed to image lipids with long T_2 and short T_1 , did not detect lipid signal in the lesions. Histological studies on the brain tissue from our CH-fed rabbits revealed that the predominant extracellular lipids in

the lesions are crystalline CH, which is not in a liquid state at physiological temperature.^{30,31} This type of lipid is associated with very short T_2 and hence the MR signal from this lipid characteristically decays very rapidly.^{30,31} This suggests that conventional clinical sequences (subject to minimum TE values of a few milliseconds) may not detect the crystallized cholesterol deposits in the xanthogranulomatous lesions in this model.

Conventional imaging methods can only image species with T_2 values greater than a few milliseconds. To determine if the xanthogranulomatous lesions contain ultrashort T_2 species, we implemented a pulse sequence with a UTE. UTE methods have been useful for demonstrating increased signal from short T_2 components in conditions such as cavernomas, metastases in the brain,³² lung parenchyma,³³ periosteum,³⁴ tendons and menisci,³⁵ hemochromatosis and cirrhosis in the liver³⁶ and articular cartilage.³⁷ In our study of two CH-fed rabbit brains, UTE imaging showed that xanthogranulomatous lesions contained ultrashort T_2 components, which presented as positive signal intensity in the short- T_2 -selective UTE images. While the source of these ultrashort T_2 components is the focus of future work, we feel that they may arise from either the crystalline CH and/ or hemosiderin within in the lesion. Although it is not clear whether this is a unique characteristic of xanthogranuloma, UTE-MRI could be a useful contrast mechanism for differential diagnosis of xanthogranuloma from other ventricular tumors.

As the secretory source of cerebrospinal fluid (CSF), the choroid plexus synthesizes numerous peptides and hormones, absorbs brain metabolic by-products, transfers nutrients from the blood to CSF, and participates in brain immunosurveillance.³⁸ It has become increasingly apparent that the choroid plexus is involved in a wide variety of neurological disorders and may play a major pathogenetic role in nonneoplastic disease, such as Alzheimer's disease (AD).^{38,39} Several features characterize the choroid plexus in AD, such as epithelial cell truncation, deposition of Biondi body in the epithelium, and reduction of thickness of basement membrane and stromal fibrosis.^{40,41} Secretion, synthesis, and transport functions of choroid plexus are impaired, resulting in decreased CSF turnover, increased glycation phenomena, reduced $A\beta$ clearance, and oxidative stress.⁴² MRI volumetric studies in AD have shown that ventricular volumes are increased progressively, going from normal to mild cognitive impairment (MCI) to AD groups, and have demonstrated a relationship between increasing ventricular volume and decreasing levels of $A\beta$ clearance in CSF in preclinical and early AD.⁴³ These studies suggest that enlarging ventricles, being a sign of malfunction in the brain's choroid plexus, may serve as a marker of faulty CSF $A\beta$ clearance mechanisms.^{39,43} The formation of xanthogranuloma in the choroid plexus is a slow pathological progress, disrupting the structure and function of choroid plexus. Besides xanthogranulomatous lesions in the choroid plexus, we have reported the development of a specific type of amyloid plaque in the brains of our CH-fed rabbits.⁶ The hypercholesterolemia-induced xanthogranulomatous lesions that we found in choroid plexus may therefore also be involved in the pathological progress of AD in these animals.

Two major limitations of this study are the limited number of animals we were able to scan with some of the pulse sequences and our inability to register in vivo MRI to histopathology

due to the small size of the lesions. The latter would have allowed us to better determine the histopathologic features that were causing the intralesion variations in MR signal intensity.

In conclusion, we demonstrate here that rabbits fed a low-level CH diet to maintain long-term hypercholesterolemia develop sizable intracranial xanthogranuloma in the choroid plexus. Multiparametric MRI techniques in vivo were able to provide information about the complex composition of these lesions. As a result of our prior findings and those of other groups that the hypercholesterolemic rabbit is a model of AD, together with the present findings of xanthogranuloma in these same rabbits, we hypothesize a link between hypercholesterolemia, choroid plexus dysfunction, and AD. Our hypercholesterolemic rabbit model may have importance for studying the complex role that CH plays in age-related diseases such as atherosclerosis, AD, and xanthogranuloma, and for testing the effectiveness of CH-lowering therapies or novel pharmaceuticals on the progression of these diseases.

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FIGURE 1.

In vivo FIESTA and SWAN. In vivo FIESTA images (A) and SWAN images (B) shows the mass xanthogranulomatous lesions in the choroid plexus. The tumorous masses (arrows) in the CH-fed rabbit show as areas of inhomogeneous hypointensity, interspersed with foci of signal voids. The lesions are accompanied with hydrocephalus and enlarged ventricle (A,B). Rabbit fed normal chow did not showed evidence of mass lesions in the brain. The Likert scale analysis indicated a total of 13 lesions (scores of 4 or 5) are present in the CH-fed rabbits (C, circle indicates the rabbits with no lesion found and square indicates the Likert scale of the lesions). Quantitative analysis shows the mean volume of lateral ventricle of CH-fed rabbits was significantly increased (D, ** $P < 0.01$).

FIGURE 2.

In vivo proton Density, T_1 and T_2 -weighted images. The xanthogranulomatous lesions were heterogeneous in appearance on proton density, T_1 , and T_2 -weighted spin echo images (A), being slight hypointense in PD-weighted images (arrows), mixed isointense and foci of hypointensity on T_1 -weighted images (arrows), and inhomogeneous low signal on the T_2 -weighted images (arrows). The lesions showed intermediate-long T_1 relaxation times on T_1 maps (B, arrows) and short T_2 values interspersed with foci of marked reduced T_2 on T_2 maps (B, arrows). Quantitative analysis (C) shows the lesion exhibited short mean T_2 values comparing with adjacent cortex and subcortical structures and long mean T_1 values compared to adjacent cortex (** $P < 0.001$; * $P < 0.05$; The color bar is in units of milliseconds).

FIGURE 3.

In vivo UTE-MRI and T_2^* relaxometry. UTE-MRI and T_2^* relaxometry of CH-fed rabbit show the mass xanthogranulomatous lesions. Short- T_2 -selective UTE images (A) were obtained through the subtraction of a long TE images (TE = 5 msec) from the short TE images (TE = 8 μ s). The xanthogranulomatous lesions exhibit as isointense in the images of TE = 8 μ s (arrows) and inhomogeneous hypointensity in the images of TE = 5.0 msec (arrows). The lesions contain ultrashort T_2 species, which exhibit as the positive signal intensity in the subtracted images (arrows). T_2^* maps indicate that the positive signal in the subtracted images has the marked reduced T_2^* values (D, arrows). Quantitative analysis (C) shows that mean T_2^* value of the lesion is significant shorter than the adjacent brain structures. (** $P < 0.001$; the color bar is in units of milliseconds).

FIGURE 4.

Histopathologic examination of the lesion. Histopathologic examination of the lesion shows the foamy histiocytes, CH clefts, hemosiderin iron deposits, histiocytes capillary vasculature, consistent with the diagnosis of xanthogranuloma. **A,B:** H&E and Bielschowsky's silver staining show foamy histiocytes are surrounded by needlelike CH clefts interspersed among foam cells. **C:** The foamy cells were positive for lipid in Oil-red-O-stain. **D:** Foamy cells stain positively with the Ram11, a marker for macrophages. **E:** CD31 immunostaining shows vasculature scattered among CH clefts and foamy cells. **F:** Prussian blue staining for iron in the hemosiderin shows the lesions are interspersed with iron deposits (scale bar represents 100 μ m).

TABLE 1

Total Serum Cholesterol Levels (mg/dl) of CH-Fed and Control Rabbits Over Time

Diet	Baseline	9 months	12 months	18 months	24 months
CH-fed	30.8 ± 19.5 (n=10)	^a 263.6 ± 82.5 (n=10)	^a 474.7 ± 325.5 (n=10)	^a 631.7 ± 419.8 (n=10)	^a 421.1 ± 189.4 (n=10)
Control	22.1 ± 5.1 (n=5)	28.9 ± 2.0 (n=5)	25.8 ± 8.8 (n=5)	24.0 ± 5.7 (n=5)	7.3 ± 14.6 (n=5)

Values represent mean ± SD.

^a *P* < 0.001 when compared with control animal values at 9, 12, 18, and 24 months.