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Gallbladder Bile: An Experimental Study in Dogs Using MR Imaging and Proton MR Spectroscopy[1]

Magnetic resonance (MR) imaging, proton MR spectroscopy, and biochemical analysis were performed to investigate MR signal intensity (SI) differences between concentrated and dilute gallbladder bile of seven fasting and five sincalide-treated dogs. MR images revealed high SI from bile of fasting dogs and low to medium SI in sincalide-treated dogs when spin-echo (SE) pulse sequences with repetition rates of 0.5 and 2.0 sec were used. Proton MR spectra were similar for fasting and sincalide-treated dogs. In fasting dogs, water content in the bile was slightly lower, and cholesterol, phospholipid, and bile acid concentrations were higher. More than 90% of proton signals in all Fourier transform free induction decay spectra emanated from water molecules, and no lipid proton resonances were detected in Fourier transform SE spectra after τ delays of 7 msec. These results indicate that the differences in SI are caused by alterations in relaxation times of water protons, possibly resulting from the interactions of water protons and macromolecules.

Index terms: Bile • Gallbladder, function • Gallbladder, magnetic resonance studies, 762.1299 • Magnetic resonance, spectroscopy

Radiology 1985; 157:453-455

MAGNETIC resonance (MR) images have revealed differences in signal intensity (SI) of gallbladder bile in fasting and nonfasting human subjects (1). The concentrated bile in fasting subjects emits a strong signal regardless of repetition time (TR) or echo time (TE). The nonconcentrated bile in nonfasting subjects emits a weak to medium-strength signal with TR of 0.5 or 1.0 sec and demonstrates a relative increase in SI with prolongation of TR or TE. This difference in SI has been thought to be caused by alterations in bile composition, mainly by increases in cholesterol content that occur with bile concentration. To address this possibility and to investigate the exact cause of SI differences, we conducted a study designed to correlate MR imaging and proton MR spectroscopy (MRS) of gallbladder bile with biochemical assays.

MATERIALS AND METHODS

The gallbladder bile of 12 adult mongrel dogs was examined in this study. To concentrate the bile in the gallbladder, all the dogs were made to fast for 16–24 hours before examination. Five dogs underwent MR imaging in vivo before needle aspiration of their gallbladder bile; aspirated bile was then submitted for proton MRS analysis and biochemical assay, including cholesterol, phospholipid, bile acid, and water content. In the remaining seven dogs, which subsequently underwent laparotomies in surgical teaching laboratories, the aspirated bile was examined by MRS alone.

Of the five dogs studied with both MR imaging and MRS, three were examined immediately after several hours of fasting. Two were examined 1 hour after treatment with an intravenous cholecystokinetic agent (sincalide; Squibb), allowing time between injection and imaging for the gallbladder to contract and refill with dilute hepatic bile. Of the seven dogs studied with MRS alone, four underwent gallbladder bile aspiration immediately after fasting and three, 1 hour after intravenous sincalide injection.

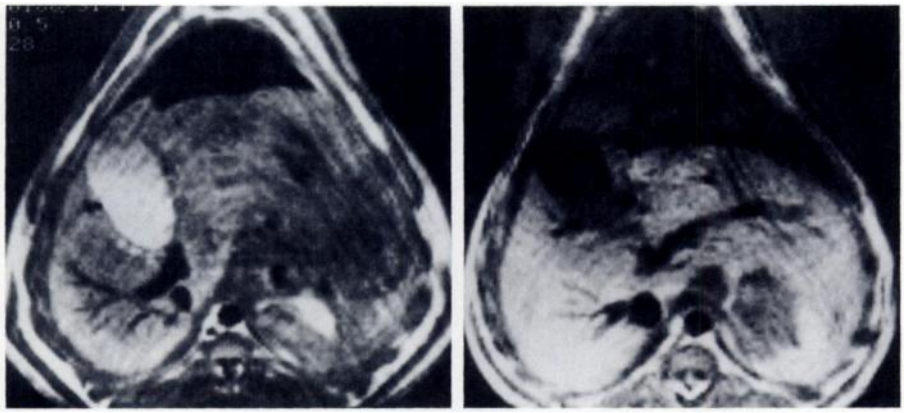
MR images were obtained with a Diasonics MT/S imaging system (Milpitas, Calif.), with a superconducting magnet operating at 0.35 T. Spin-echo (SE) technique was used with short (0.5 sec) and long (2.0 sec) TRs, and TEs of 28 and 56 msec. High-resolution free induction decay (FID) spectra were obtained for each bile specimen on a 240-MHz superconducting magnet spectrometer (Varian; Palo Alto, Calif.). The relative proton fractions for water and cholesteric/lipid methylene (CH_2) regions were determined from a peak integration technique.

To measure relaxation times more accurately than would be possible by image analysis, the proton spin-lattice (T1) and spin-spin (T2) relaxation times were measured in vitro at 37°C using a Varian XL-100 high-resolution spectrometer, operating at 100 MHz (2.35 T) and connected to a Nicolet 1080 data system (Madison, Wis.). All T1 values were obtained using standard inversion-recovery experiments (2) ($180° - \tau - 90°$) with 14 increments of a τ delay of 7 msec between 180° and 90° radiofrequency pulses, from a nonlinear, least squares fit of the observed Fourier transform water proton peak intensities. Corresponding T2 values were determined from Carr-Purcell-Meiboom-Gill experiments (2) ($90° - \tau - 180° - \tau -$ echo), with 14 increments of a τ delay of 7 msec. The Fourier transform water peak intensities were fitted in the T2 least squares program.

RESULTS

MR images obtained using TR values of both 0.5 and 2.0 sec exhibited high SI from gallbladder bile in the three fasting dogs

[1] From the Department of Radiology, University of California, San Francisco. Received January 10, 1985; accepted and revision requested February 22, 1985; revision received April 5, 1985.



1. 2.

Figures 1 and 2. **(1)** Transverse MR image (SE, TR = 0.5 sec; TE = 28 msec) through the gallbladder shows high SI emanating from the bile of this fasting dog. The high SI at this short TR value reflects the shortening of T1. **(2)** Transverse MR image (SE, TR = 0.5 sec; TE = 28 msec) through the gallbladder of a sincalide-treated dog reveals low SI from dilute bile.

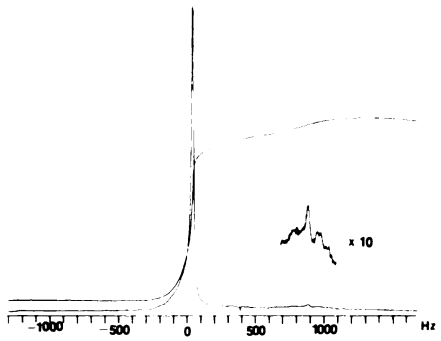


Figure 3. Proton MR (FID) spectrum obtained at 240 MHz of gallbladder bile from a fasting dog. The spectrum is plotted on a relative scale, with the water peak at 0 Hz and the lipid peak at approximately 900 Hz. The insert, labeled "× 10" is a magnified view of the lipid peak, which includes contributions from aliphatic protons. From a peak area integration routine, the major portion of the signal emanates from water protons.

Table 1
T1 and T2 Relaxation Times for Canine Bile Specimens (n = 5)

Dog/Test Group	T1 H_2O (sec)	T2 H_2O (sec)
1/after fasting	0.89 ± .01	0.16 ± .01
2/after fasting	0.84 ± .01	0.16 ± .01
3/after fasting	0.77 ± .01	0.15 ± .01
4/after sincalide	1.35 ± .01	0.35 ± .01
5/after sincalide	2.40 ± .01	0.36 ± .01

Note.—These five dogs were studied by MR, MRS, and biochemical analysis.

Table 2
Biochemical Analysis of Canine Bile Specimens (n = 5)

Dog/Test Group	Cholesterol (mg %)	Phospholipid (g %)	Bile Acid (g %)	H_2O Content (%)
1/after fasting	111	5.03	4.2	80.2
2/after fasting	228	3.09	3.2	83.9
3/after fasting	92	3.51	3.5	83.2
4/after sincalide	65	1.95	2.5	86.7
5/after sincalide	108	2.89	2.7	85.5

Note.—These five dogs were studied by MR, MRS, and biochemical analysis.

(Fig. 1). Low SI was noted from bile of the two sincalide-treated dogs on the images with 0.5-sec TR (Fig. 2). A slight increase in SI was detected with prolongation of TR to 2.0 sec and of TE to 56 msec in both the fasting and sincalide-treated dogs.

The proton MR spectra (FID) for bile specimens from the seven fasting and five sincalide-treated dogs were qualitatively identical and were similar to the spectrum in Figure 3. Common features seen in all spectra included a large water proton signal (0 Hz) and smaller lipid methylene and methyl resonances (approximately 900 Hz). The integration of the peak area in Figure 3 shows the dominance of the water resonance. In all cases, more than 90% of proton signals in the Fourier transform FID spectra emanated from water molecules. More importantly, MRS analysis revealed no lipid proton resonances in Fourier transform SE spectra with τ delays as short as 7 msec.

T1 and T2 relaxation times (determined by spectrometer) for bile specimens from the five dogs studied by MR imaging, MRS, and biochemical assay are summarized in Table 1. Both T1 and T2 relaxation times of water protons were shortened in bile from fasting dogs, compared with values from bile of sincalide-treated dogs. We expect similar trends to occur for the corresponding relaxation times at 0.35 T.

Biochemical analysis of bile from the same five dogs is summarized in Table 2. Cholesterol, bile acid, and phospholipid concentrations were greater in fasting dogs than sincalide-treated dogs. Water content was slightly less for fasting dogs than sincalide-treated dogs. While biochemical analysis revealed the expected relationships of cholesterol, bile acid, and phospholipid contents in the concentrated and nonconcentrated bile samples, spectroscopic experiments revealed no consistent relationship between biochemically determined lipid concentrations and integrated MRS signal contribution from lipid protons. We believe this was caused by the large majority of water protons in the MRS spectra, which dominated all the integration determinations (Fig. 3).

DISCUSSION

A recent study (1) of human volunteers and patients reported the finding of high SI from gallbladder bile of fasting subjects and low SI from bile of nonfasting subjects. Low SI was also seen in bile of fasting patients with documented chronic cholecystitis (1). Our study was designed to correlate the relative SI of gallbladder bile with proton MR spectra and biochemical composition. We hoped to learn whether the previously reported shortening of T1 relaxation times of concentrated gallbladder bile was caused by a decrease in total water content, an increase in cholesterol or phospholipid concentration, or an alteration in interactions between water and macromolecules in bile.

Our results indicate a decreased water content in gallbladder bile of fasting dogs compared with that of nonfasting dogs. In addition, the high-resolution proton MR spectra of bile

from fasting and nonfasting dogs were similar. In all the SE experiments, Fourier transform spectra after τ delays of 7 msec revealed no lipid proton resonances. Therefore, the lipid protons had very short T2 values, which would not contribute to observed signal intensity in conventional MR systems.

These findings indicate first that the relative increase in SI of concentrated gallbladder bile on MR images regardless of TR value is caused primarily by water proton T1 and T2 shortening and not by the greater contribution of lipid protons to the total signal. The lack of correlation between aliphatic contribution to total MR signal and measured concentrations of cholesterol and phospholipids may be caused by restricted mobility of these molecules when aggregated into micellar structures.

Early work in MRS and tissue characterization revealed striking differences in relaxation properties of free water and of water in biologic systems (3–5). Although a complete analysis of water relaxation times in these systems can be quite complex, the basic observations are that the T1 and, in particular, the T2 relaxation times of water protons are decreased for water bound to (adsorbed or in short contact with) relatively immobile nucleotides, proteins, and other macromolecular structures found in vivo. This behavior is consistent with the picture of decreased, average water proton motion upon interaction with the cholesterol, phospholipid, and bile acid fractions in bile. This decrease in motion is reflected in the observed decrease in T1 and T2 of water in the concentrated bile samples.

We believe that, in the absence of detectable SE lipid proton resonances, the increase in SI from gallbladder bile in fasting subjects reflecting T1 shortening is caused by an increase in the fraction of water molecules bound to macromolecules in concentrated bile. ■

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