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USE OF NITROXIDES AS MRI CONTRAST AGENTS TO STUDY *IN VIVO* CARBON TETRACHORIDE INDUCED HEPATOTOXICITY IN RATS

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CCl_4 and related compounds, such as halothane, are metabolized by the liver to form free radical intermediates, which are thought to be implicated in the hepatotoxic response. Two to three hours following CCl_4 exposure (i.p.) there is a localized edematous region surrounding the portal vein which is observable by proton MRI *in vivo*. Enhancement of the CCl_4-induced edematous region was possible using Gd-DTPA, a paramagnetic contrast agent. However, with the use of a nitroxide contrast agent (3-PCA) there was no enhancement, but rather a significant diminution of the CCl_4-induced edematous response. These results suggest that the nitroxide contrast agents, which are themselves free radicals, act as free radical scavengers and therefore reduce the formation of the CCl_4-induced hepatic 'damage' observed in proton MR images.

KEY WORDS: Carbon tetrachloride (CCl_4), hepatotoxicity, *in vivo*, magnetic resonance imaging (MRI), contrast agents, 3-carboxy-pyrrolidine-N-oxyl (3-PCA), gadolinium diethylene triamine pentaacetic acid (Gd-DTPA).

INTRODUCTION

Understanding the toxicity and metabolic consequences of halogenated aliphatic hydrocarbons or halocarbons is of considerable interest due to their extensive use as anesthetics and their widespread applications in industry. The mechanism of hepatotoxicity of many halocarbons, such as carbon tetrachloride (CCl_4) or halothane ($CF_3CHClBr$), is thought to be a free radical mediated process leading to the peroxidative decomposition of intracellular membrane structures and eventual hepatocellular necrosis. The altered permeability of the plasma membrane leads to loss of intracellular K^+, proteins including cytoplasmic enzymes and coenzymes, and an intracellular accumulation of Ca^{++}, Na^+ and H_2O[1]. With the use of the EPR (electron paramagnetic resonance) spin trapping technique we have been able to detect four radical species from the metabolism of CCl_4 in rat liver *in vitro*; the trichloromethyl radical ($\cdot CCl_3$), the carbon dioxide radical anion ($\cdot CO_2^-$), a carbon-centered lipid derived radical with a methylene group ($\cdot CH_2R$), and an oxygen-centered lipid derived radical ($\cdot OR'$)[2-4].

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We are searching for an alternate observable to follow pathological events in systems where free radicals are produced *in vivo* in live animals. Since MRI (magnetic resonance imaging) is an appropriate non-invasive observable, we have studied the rat liver free radical metabolism of halocarbons *in vivo*. Previous studies in our laboratories have shown that localized edema is detected in rat liver, in the region surrounding the hepatic portal vein, by proton MRI as a result of CCl_4-induced hepatocellular 'damage'[5]. In addition, we have also demonstrated that administration of α-phenyl N-*tert*-butyl nitrone (PBN), a free radical spin trap, 30 min prior to CCl_4 administration, effectively inhibits the CCl_4-induced hepatic edema observed in the magnetic resonance (MR) images[6]. These results indicated that PBN prevented the formation of damaging radical species from the metabolism of CCl_4 by possibly acting as a free radical scavenger[6]. To test this hypothesis, we also studied the effect of a free radical nitroxide contrast agent (3-carboxy-pyrrolidine-N-oxyl or 3-PCA) on CCl_4-induced hepatotoxicity. Ordinarily, nitroxide contrast agents are used in MRI to enhance tissue damage, but due to the presence of an unpaired electron, on the >N-O moiety, nitroxides can also act as free radical scavengers. Comparatively, the effect of a transition metal contrast agent, gadolinium diethylene triamine pentaacetic acid (Gd-DTPA), was also studied.

EXPERIMENTAL

Fasted (24 h) male Wistar rats (Charles River, Canada) weighing 180–200 g ($n = 6$) were given CCl_4 i.p. (160 μl/200 g rat in saline with 5% Emulphor) and anesthetized with sodium pentobarbital for approx. 3–4 hours. Control rats ($n = 4$) received an equivalent volume of saline. For the contrast agent experiments, either 3-PCA (i.v.; 0.3 mM) or Gd-DTPA (i.v.; 0.02 mM) was administered 60–120 min after CCl_4 ($n = 4$ for either CCl_4 and PCA or CCl_4 and Gd-DTPA treated rats).

Nuclear magnetic resonance (NMR) measurements were made with a Spectroscopy Imaging Systems (SISCO) 2.0 Tesla/31 cm bore imaging spectrometer. Respiratory gating was used to trigger acquisition of phase-encoding steps in the imaging sequence[5]. Multiple ^{1}H-NMR image slices were taken in the transverse plane (TE 25 ms; TR 1 s). Image slices were 3 mm thick, field of view was $8 \times 8\ cm^2$ with 256 phase-encoding steps, 2 acquisitions per step, and 512 frequency encoding points. An in-house computer program was used to measure localized (6 mm dia. areas in the vicinity of the hepatic portal vein) proton NMR signal intensities (measured in arbitrary units) from the 'damaged' or corresponding normal regions of the livers in the images.

RESULTS

Two to three hours following CCl_4 exposure (i.p.) a localized edematous region is readily observed in the *in vivo* transverse (or axial) proton MR image shown in Figure 1B, which is not present in the liver image of a saline-treated rat shown in Figure 1A. This result has been observed previously on numerous occasions and is more fully discussed elsewhere[5–7]. Further enhancement of the CCl_4-induced edematous region was possible with use of Gd-DTPA as shown in the transverse image in Figures 1C. The CCl_4-induced 'damage', observed by proton MRI with

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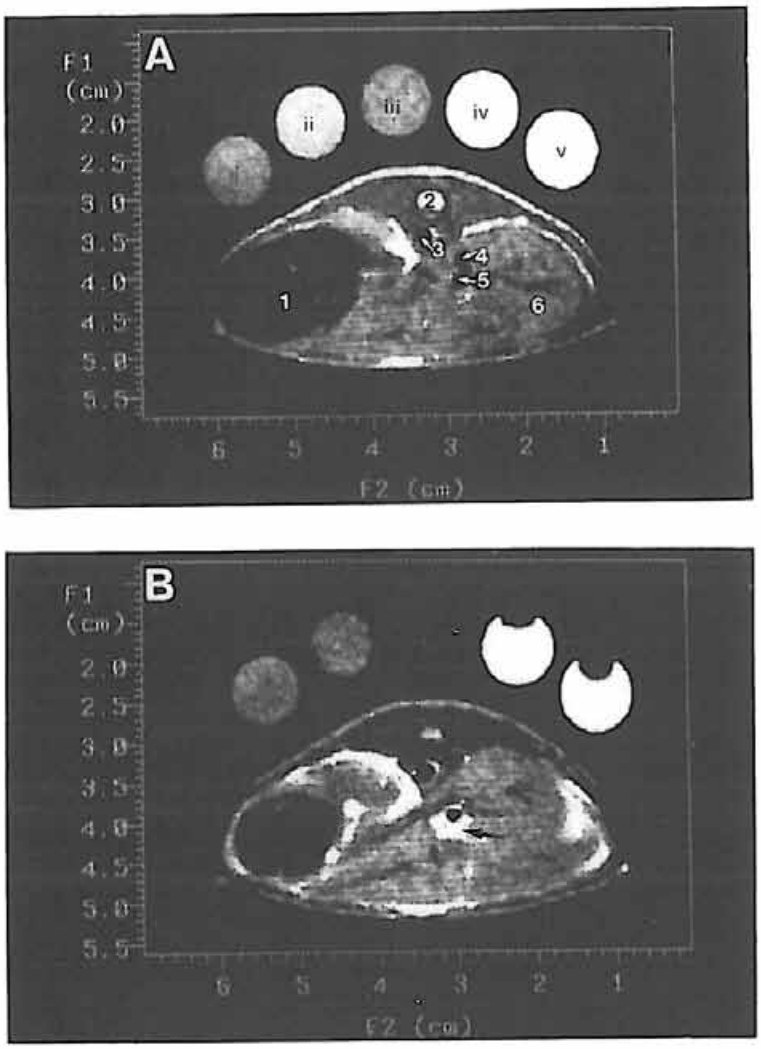

FIGURE 1 (A) Transverse ^1H magnetic resonance (MR) image of a control rat (saline) obtained in the liver region with the use of respiratory gating. Distinctive features include (1) the stomach, (2) the spinal cord, (3) the aorta, (4) the vena cava, (5) the hepatic portal vein, and (6) the liver. Intensity reference solutions included: (i) water; (ii) 50 mM EDTA, pH 7.9; (iii) 0.07 M phosphate buffer, pH 7.4; (iv) 0.03 mM 3-PCA; and (v) 0.06 mM 3-PCA. (B) An analogous ^1H-MR image to that of the control in (a) was obtained 2–3 h after CCl_4 treatment (i.p.). Note that there is a region of high proton signal intensity in the liver to the right of the stomach, and surrounding the hepatic portal vein (arrow). (C) Transverse ^1H-MR image of a rat liver treated with CCl_4 and Gd-DTPA (1 h after administration of CCl_4). (D) Transverse ^1H-MR image of a rat liver treated with CCl_4 and PCA (1 h after administration of CCl_4).

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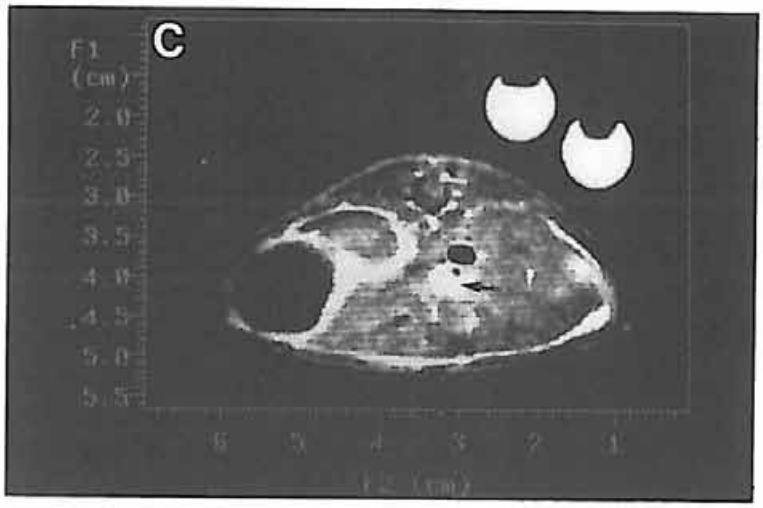

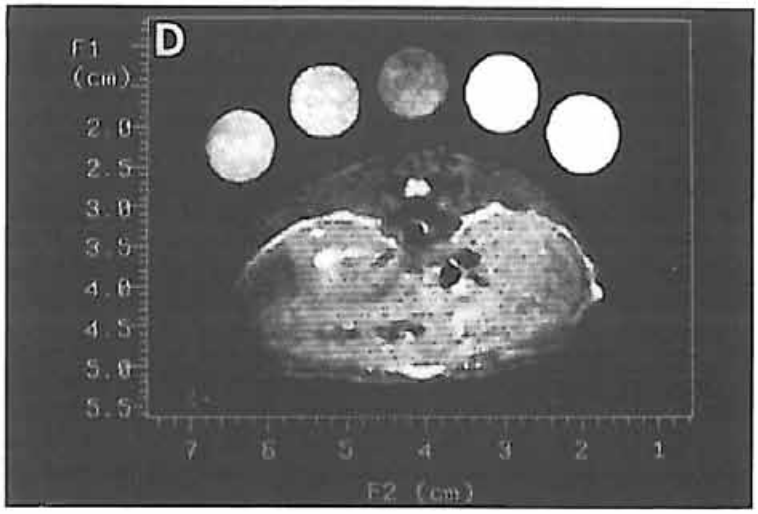

Gd-DTPA contrast enhancement, is localized in a region surrounding the hepatic portal vein in the central portion of the liver, which is shown in the transverse and coronal (or horizontal) images in Figure 2. Confirmation of the localized CCl_4-induced 'tissue damage' was obtained previously from EM analysis of liver sections taken from the excised livers following MRI[6]. The high intensity proton region observed was also previously characterized by using a volume selective spectroscopy (VOSY) technique[5,7]. An increase in $[H_2O]$, in the high intensity proton signal region ($4 \times 4 \times 4\,mm^3$ voxel) of CCl_4-treated rat livers was measured by the VOSY technique, in comparison to saline-treated livers[5,7]. The results from a previous study using PBN, a free radical spin trap, administered 30 min prior to CCl_4 administration, indicated that there was a reduction in the localized

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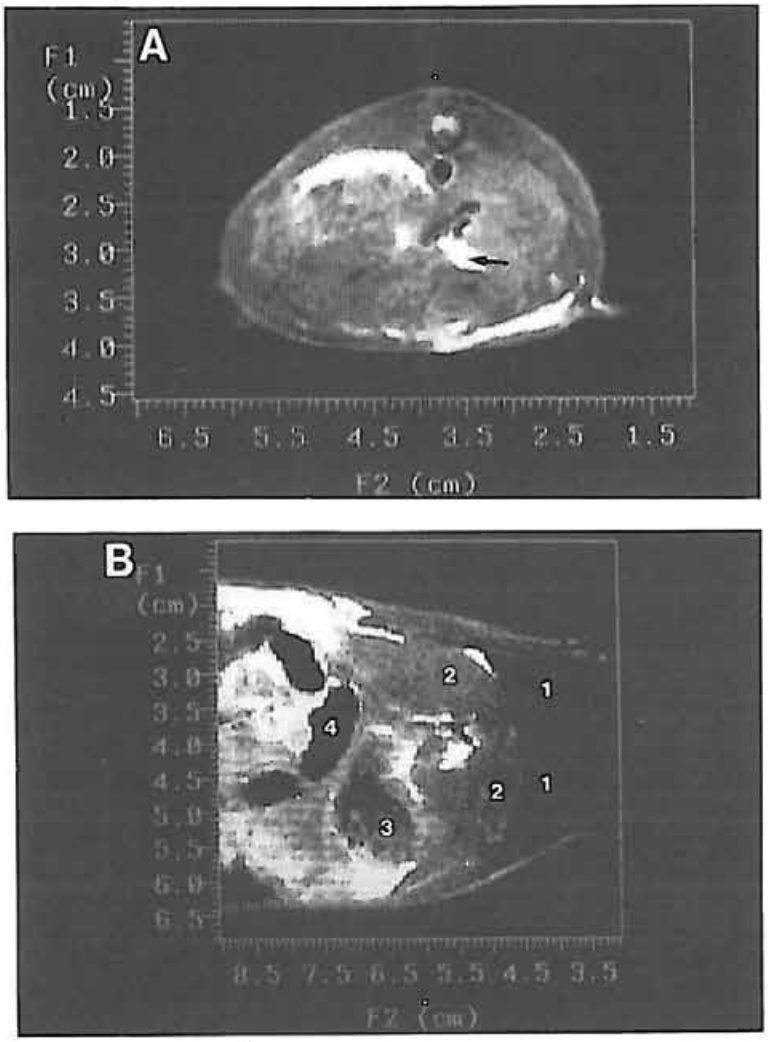

FIGURE 2 (A) Transverse (axial) ^{1}H-MR image of a rat liver treated with CCl_4 and Gd-DTPA. Note the region of high proton NMR signal intensity (arrow). (B) Coronal (horizontal) ^{1}H-MR image of the same rat treated with CCl_4 and Gd-DTPA. The edematous region is centrally located adjacent to the hepatic portal vein. Anatomical features in the image include the (1) lungs, (2) liver, (3) stomach, and (4) intestines.

CCl_4-induced 'tissue damage' observed by ^{1}H-MRI[6]. In this study, there was also a substantial decrease in the high intensity ^{1}H-MRI signal from the CCl_4-induced edema observed 5–15 min after treatment with 3-PCA, a free radical nitroxide contrast agent (Figure 1D). Proton NMR signal intensities from CCl_4-induced edematous regions and similar regions in control and CCl_4/3-PCA treated rat liver images were measured using an in-house computer program (see Figure 3). Localized proton NMR signal intensity values measured from CCl_4-treated rat liver images were found to be 74.88 ± 2.92 (S.D.; $n = 6$) in comparison to proton

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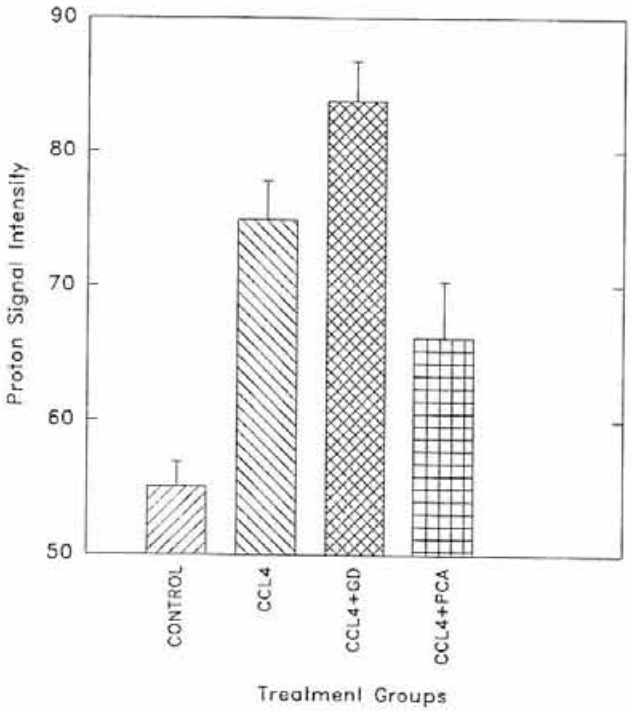

FIGURE 3 Bar graph depicting localized (6 mm dia. area in the vicinity of the hepatic portal vein) proton NMR signal intensities in rat liver as a function of treatment. The treatment groups are as follows: (a) control (saline-treated; $n = 4$), (b) CCl_4 (CCl_4-treated only; $n = 6$), (c) CCl_4 + GD (CCl_4 and Gd-DTPA treated; $n = 4$), and (d) CCl_4 + PCA (CCl_4 and 3-PCA treated, $n = 4$). Error bars represent standard deviations.

NMR signal intensity values of 55.05 ± 1.87 ($n = 4$) measured from similar regions in control rat liver images, i.e. a 26% increase in proton NMR signal intensity. With the use of Gd-DTPA the localized proton NMR signal intensities from CCl_4-treated rat liver images were found to be 83.74 ± 3.01 ($n = 4$), i.e. an 11% increase from CCl_4 administration alone. In contrast, localized proton NMR signal intensities measured from CCl_4 and 3-PCA treated rat liver images were found to be only 66.12 ± 4.26, i.e. a 12% decrease from CCl_4 treatment alone or a 21% decrease from CCl_4 and Gd-DTPA treatment. Localized proton NMR signal intensities measured from either control 3-PCA or Gd-DTPA treated rat liver images (not shown in Figure 3) were found to be 60.46 ± 3.91 and 64.68 ± 1.72, respectively.

CONCLUSIONS

Although enhancement of the CCl_4-induced edematous region was possible with the use of a transition metal (Gd-DTPA) contrast agent, there was no enhancement with the use of a nitroxide (3-PCA) contrast agent, but rather a significant

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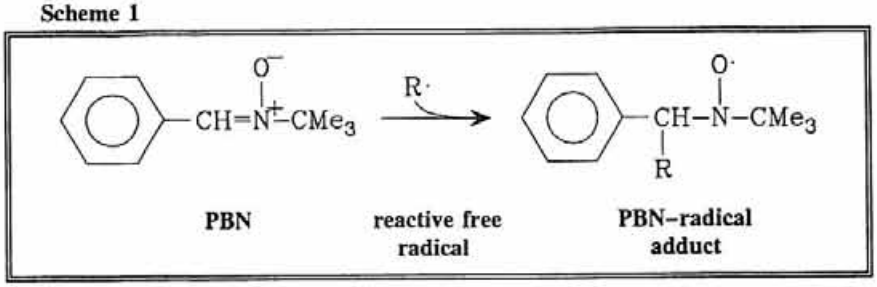
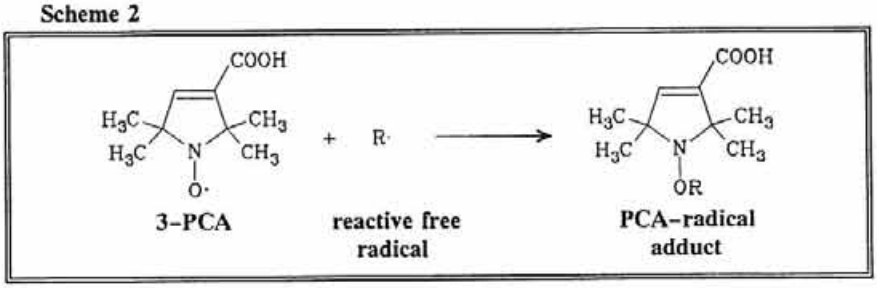
Scheme 1



SCHEME 1 Reaction of PBN with a reactive free radical to form a PBN-radical adduct.

Scheme 2



SCHEME 2 Reaction of 3-PCA with a reactive free radical to form a 3-PCA-radical adduct.

diminution of the CCl_4-induced edematous response. It is our contention that a free radical mediated attack on hepatocellular membranes after acute CCl_4 intoxication results in an increase in cell permeability to extracellular ions and water, leading to an edematous response that can be detected by ^{1}H-MRI. Inhibition of the observed CCl_4-induced 'tissue damage' in rat liver by PBN or 3-PCA supports our hypothesis that free radicals are the major causal factors in acute CCl_4 hepatotoxicity. These results suggest that PBN, a spin trap, and 3-PCA, a nitroxide contrast agent, both act as a free radical scavengers (see Schemes 1 & 2) preventing the CCl_4-induced hepatic 'damage'.

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References

1. R.O. Recknagel, E.A. Glende Jr., J.A. Dolak and R.L. Waller (1989) Mechanisms of carbon tetrachloride toxicity. *Pharm. Ther.*, **43**, 139–154.
2. E.G. Janzen, R.A. Towner and D.L. Haire (1987) Detection of free radicals generated from the *in vitro* metabolism of carbon tetrachloride using improved ESR spin trapping techniques. *Free Rad. Res. Commun.*, **3**, 357–364.
3. E.G. Janzen, R.A. Towner and M. Brauer (1988) Factors influencing the formation of the carbon

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dioxide radical anion ($\cdot CO_2^-$) spin adduct of PBN in the rat liver metabolism of halocarbons. *Free Rad. Res. Commun.*, **4**, 359–369.

4. E.G. Janzen, R.A. Towner, P.H. Krygsman, E.K. Lai, J.L. Poyer, G. Brueggemann and P.B. McCay (1990) Mass spectroscopy and chromatography of the trichloromethyl radical adduct of phenyl *tert*-butyl nitrone. *Free Rad. Res. Commun.*, **9**, 353–360.
5. M. Brauer, R.A. Towner, I. Renaud, E.G. Janzen, D.L. Foxall (1989) *In vivo* proton nuclear magnetic resonance imaging and spectroscopy studies of halocarbon-induced liver damage. *Mag. Res. Med.*, **9**, 229–239.
6. E.G. Janzen, RA. Towner and S. Yamashiro (1990) The effect of phenyl *tert*-butyl nitrone (PBN) on CCl_4-induced rat liver injury detected by proton magnetic resonance imaging (MRI) *in vivo* and electron microscopy (EM). *Free Rad. Res. Commun.*, **2**, 325–335.
7. R.A. Towner, L.A. Reinke, E.G. Janzen and S. Yamashiro (1991) Enhancement of carbon-tetrachloride-induced liver injury by a single dose of ethanol: proton magnetic resonance imaging (MRI) studies *in vivo*. *Biochem. Biophys. Acta*, **1096**, 222–230.

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