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Gd(ABE-DTTA), a Novel Contrast Agent, at the MRI-Effective Dose Shows Absence of Deleterious Physiological Effects in Dogs

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Key Words
Magnetic resonance imaging · Gadolinium-based contrast agent · Ischemic heart disease

Abstract
The physiological effects of a novel MRI contrast agent, Gd(ABE-DTTA), were investigated in dogs, monitoring parameters in blood samples. Each animal (n = 8 in the short-term, n = 4 in the long-term group) underwent isoflurane anesthesia followed by the generation of myocardial infarction and received a contrast agent at the MRI effective dose. Blood samples were collected 24 and 48 h, and 7, 14, 28, 35, 49 and 56 days after contrast agent administration. No significant changes exceeding the normal range were detected in any of the investigated parameters except in alanine aminotransferase (ALT). ALT enzyme activity increased in the short-term group 24 and 48 h after agent administration as expected from the effect of isoflurane anesthesia. Between days 7 and 56 no elevation in ALT was observed. In dogs no substantial short- or long-term effect was observed on the investigated, physiological parameters after Gd(ABE-DTTA) administration at the MRI effective dose.

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Introduction

Contrast-enhanced cardiac MRI (ceMRI) has been used since 1986 to detect ischemic or infarcted myocardial tissue [1]. This method has been shown to be a superior approach to contrast-enhanced MRI determination of size of the insulted tissue in combination with the assessment of function recovery. CeMRI can be carried out based on the accumulation of contrast agent into irreversibly injured areas of the myocardium [2]. Gadolinium(Gd)-based contrast agents in cardiac MRI were also introduced into clinical practice [3].

Gd, as a free ion, is extremely toxic, but chelation with suitable organic ligands decreases its toxicity. Thus investigation has focused on the development of stable paramagnetic ion complexes. Both the free metal ion and some ligands exhibit substantial toxicity in the unbound state. Complexed, however, they may create a thermodynamically and kinetically stable compound which is

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much less toxic and are acceptable for clinical use. Organic chelators that bear negatively charged carboxylate groups have the ability to form strong complexes with Gd^{3+}, forming non-toxic contrast agents for proton MRI. To date, Gd(DTPA) is the most widely and routinely used agent at many clinical MRI facilities. Search for new ligands for Gd^{3+} complexation is an ongoing process. Newly developed agents are typically compared to Gd(DTPA) as a benchmark. Toxicity, lipophilicity, stability, relaxivity are key issues to consider when designing and synthesizing new contrast agents.

For several years now we have been developing a family of contrast agents for use in the context of ischemic heart disease [4–12]. From these, Gd(ABE-DTTA) seems the most appropriate candidate for future clinical use.

Gd(ABE-DTTA) differentiates ischemic or infarcted myocardium from unaffected, remote myocardium [13, 14]. It contains two short fatty acyl chains (fig. 1) and displays high MRI relaxivity, 16.24 s^{-1} $(mmol/l)^{-1}$ (at 1.5 Tesla), thus it is about four times more effective than Gd(DTPA), and its relaxivity (i.e., efficiency) remains high even at increased magnetic field strengths [9, 14]. It is lipophilic, completely soluble in water up to a 25 mmol/l concentration, allowing the preparation of an injectable dose [9]. Its tissue kinetics indicate both intra- and extravascular agent characteristics. The slow washout kinetics of our agent compared to that of other agents is very likely due to its butyryl side chain, because when this chain is changed to the shorter propyl chain, the agent's tissue lifetime becomes shorter. When we change the chains to the long myristoyl ones, the resulting agent (Gd(BME-DTTA)) has an increased tissue lifetime and it becomes even more effective when persistency is required, but at the not so surprising price of lower water solubility, making it less practical [9]. Thus Gd(ABE-DTTA) became our optimized agent having taken into consideration all aspects of its tissue and MRI properties compared to those of other agents of its family [9].

Due to its sufficiently long lifetime in tissue it enables the detection of ischemic events in the heart for the entire duration of ischemia [13]. We have shown that, as does Gd(DTPA), Gd(ABE-DTTA) also displays delayed accumulation into infarct areas and it induces a considerable relaxation rate enhancement ($\Delta R1$) which is still observable at the end of the first week after administration [14]. Thus infarction evolution can be followed for 7–10 days without repeat administration (fig. 2). Its water solubility makes the agent clinically applicable [9].

Our goal in the present study has been to investigate whether any deleterious physiological effect may be caused by Gd(ABE-DTTA) given at the MRI effective dose. We have focused on a set of parameters measurable in blood samples and which are typically monitored when studying the physiological effects of contrast media and agents [15–17].

Materials and Methods

Contrast Agent Preparation

The gadolinium complex of N-(2-butyryl-oxyethyl)-N′-(2-ethyloxyethyl)-N,N′-bis[N′,N′-bis(carboxymethyl)acetamido]-1,2-ethanediamine (Gd(ABE-DTTA)) was synthesized, and sample preparation and relaxivity measurements were carried out as described by Saab-Ismail et al. [9]. Each animal received the contrast agent at the dose of 0.05 mmol/kg, which is the MRI effective dose.

Canine Model

Our experimental protocol was approved by the University of Alabama at Birmingham IACUC and we fully complied with 'Guidelines for the Care and Use for Laboratory Animals' (NIH). The animals were fed commercial dog food and had free access to water. Food was withheld 12 h prior to the collection of blood samples as these samples were taken under general anesthesia. A control blood sample was taken from each dog before undergoing isoflurane anesthesia followed by the generation of myocardial infarction using a 180-min balloon occlusion followed by reperfusion. On average a total of 6-hour isoflurane anesthesia was required. The contrast agent was administered 48 h after reperfusion.

Short-Term Follow-Up

Eight male hounds weighing 18–20 kg participated in our short-term study. Blood samples were taken for pre-agent, pre-anesthesia control and 24 and 48 h after contrast agent administration.

Long-Term Follow-Up

Four male hounds weighing 18–20 kg were monitored for 8 weeks after agent administration to investigate the long-term effects of Gd(ABE-DTTA). Blood samples were taken from the animals in this group for pre-agent, pre-anesthesia control, and 7, 14, 28, 35, 49 and 56 days after agent administration.

Anesthesia-Only Control Group

An additional group of 4 male hounds was investigated to monitor the short-term effect of anesthesia alone, without contrast agent in our reperfused-infarct animal model. Blood samples were collected 24 and 48 h after isoflurane anesthesia.

Blood Samples

For each sample an aliquot of blood was collected in a microtainer with EDTA, and the remaining portion was placed in a tube containing no anticoagulant for the separation of serum. Five milliliters of blood and serum were used to determine the hematological values and clinical chemistry profiles.

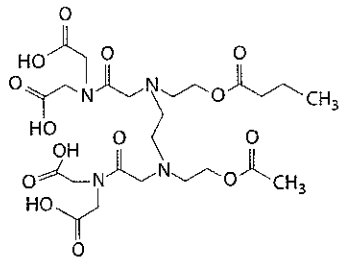

Fig. 1. Structure of the ligand containing two short fatty acyl chains.

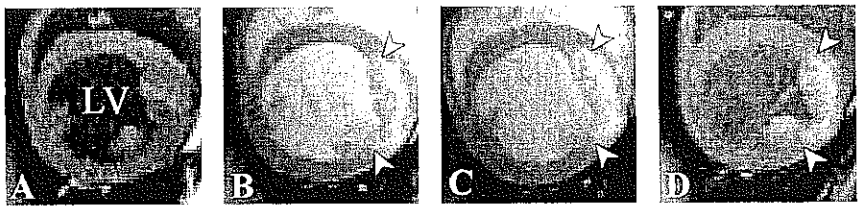

Fig. 2. Contrast agent accumulation at different time points after administration is shown using contrast-enhanced cardiac MRI. The white arrow heads indicate the region of myocardial infarction and LV denotes the left ventricle of the heart. **A** Control short axis cardiac image (before Gd(ABE-DTTA) administration). No contrast can be observed between infarcted and non-infarcted areas. **B** 24 h after contrast agent administration. Accumulation of Gd(ABE-DTTA) in infarcted tissue (bright anteroseptal myocardial region) is apparent. **C** 48 h after contrast agent administration. **D** 7 days after Gd(ABE-DTTA) administration. Apparent signal enhancement (bright area in the myocardium) was observed in the non-viable cardiac tissue delineating a 1-week-old myocardial infarction while the healthy myocardium shows no signal enhancement (dark myocardial regions).

Table 1. Short-term study of physiological blood parameters after administration of 0.05 mmol/kg Gd(ABE-DTTA) (n = 8; mean ± SEM)

Physiological parameter	Normal range[a]	Before treatment baseline value	Time after Gd(ABE-DTTA) administration, days		Significance
			1	2	
Total protein, g/dl	5.5–7.7	5.8 ± 0.2	5.9 ± 0.1	6.1 ± 0.1	*
Globulin, g/dl	2.3–5.2	2.2 ± 0.1	2.5 ± 0.1	2.7 ± 0.1	**
Albumin, g/dl	2.5–4.4	3.6 ± 0.11	3.4 ± 0.06	3.4 ± 0.07	*
Amylase, U/l	200–1,200	555 ± 26.6	541 ± 40.7	514 ± 32.5	*
ALT, U/l	10–100	41 ± 5.0	117 ± 17.6	108 ± 14.4	***
ALP, U/l	20–150	73 ± 4.8	113 ± 12.4	118 ± 9.0	**
Total bilirubin, mg/dl	0.1–0.6	0.5 ± 0.02	0.4 ± 0.05	0.4 ± 0.05	*
BUN, mg/dl	7–25	12 ± 0.6	8 ± 1.6	9 ± 1.0	*
Creatinine, mg/dl	0.3–1.4	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	*
Na^+, mmol/l	135–155	136 ± 1.0	133 ± 1.8	131 ± 1.1	*
K^+, mmol/l	3.5–5.5	5.0 ± 0.1	4.9 ± 0.1	5.2 ± 0.1	*
Ca^{2+}, mg/dl	8.6–11.8	11.1 ± 0.1	11.7 ± 1.8	11.9 ± 1.8	*
Phosphate, mg/dl	2.5–7.7	7.8 ± 0.6	8.6 ± 0.4	8.2 ± 0.2	*
WBC, $\times 10^3/mm^3$	6–17	8.4 ± 1.0	10.4 ± 0.7	10.6 ± 1.1	*
Lymhocytes, %	10–40	34.4 ± 1.6	24.4 ± 2.2	29.9 ± 4.0	*
Monocytes, %	2–10	4.2 ± 0.3	2.9 ± 0.2	3.6 ± 0.9	*
Granulocytes, %	50–80	61.4 ± 1.9	72.7 ± 2.4	68.3 ± 3.4	*
RBC, $\times 10^3/mm^3$	5.5–8.5	5.8 ± 0.2	5.3 ± 0.1	6.0 ± 0.4	*
MCV, fl	65–80	75.9 ± 1.5	72.8 ± 1.6	73.5 ± 1.3	*
MCHC, g/dl	28–40	31.6 ± 1.0	33.1 ± 0.7	30.7 ± 1.3	*
MCH, pg	19.5–24.5	23.8 ± 0.3	24.0 ± 0.2	22.5 ± 0.9	*
Hematocrit, %	35–55	44.4 ± 2.7	38.2 ± 0.6	43.9 ± 2.8	*
Hgb, g/dl	10–18	13.9 ± 0.5	12.7 ± 0.3	13.0 ± 0.4	*
Platelet, $\times 10^3/mm^3$	120–600	170 ± 23.6	129 ± 15.4	180 ± 29.3	*

* Nonsignificant ($p > 0.05$) effect in variable with treatment on every time point compared to the before treatment baseline.

** Significant ($p < 0.05$) change in variable 24 and 48 h after treatment, but not exceeding normal range.

*** Significant ($p < 0.05$) change in variable 24 and 48 h after treatment exceeding normal range.

[a] Obtained from the International Species Information System (www.isis.org) and Vetlab Information (www.vetlab.co.uk).

Table 2. Long-term study of physiological blood parameters after administration of 0.05 mmol/kg Gd(ABE-DTTA) (n = 4; mean ± SEM)

Physiological parameter	Normal range[a]	Before treatment baseline value	Time after Gd(ABE-DTTA) administration, days						Significance
			7	14	28	35	49	56	
Total protein, g/dl	5.5–7.7	5.3 ± 0.1	5.7 ± 0.1	5.9 ± 0.2	5.8 ± 0.1	5.6 ± 0.04	5.7 ± 0.1	5.5 ± 0.04	*
Globulin, g/dl	2.3–5.2	2.0 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1	*
Albumin, g/dl	2.5–4.4	3.3 ± 0.1	3.4 ± 0.04	3.5 ± 0.1	3.6 ± 0.1	3.5 ± 0.04	3.5 ± 0.04	3.5 ± 0.04	*
Amylase, U/l	200–1,200	560 ± 6.3	551 ± 22.6	849 ± 205	675 ± 46.0	656 ± 21.9	804 ± 147	638 ± 53.7	*
ALT, U/l	10–100	45 ± 7.4	41 ± 7.4	28 ± 3.2	35 ± 3.2	31 ± 1.8	32 ± 1.1	33 ± 1.4	*
ALP, U/l	20–150	92.6 ± 3.0	95 ± 4.9	107 ± 8.1	88 ± 2.4	88.0 ± 4.9	82.8 ± 5.5	77 ± 3.9	*
Total bilirubin, mg/dl	0.1–0.6	0.4 ± 0.04	0.4 ± 0.04	0.4 ± 0.04	0.5 ± 0.04	0.5 ± 0.04	0.5 ± 0.04	0.5 ± 0.0	*
BUN, mg/dl	7–25	13.3 ± 0.2	14.8 ± 1.5	16.8 ± 1.0	13.3 ± 0.4	13.8 ± 1.4	13.0 ± 0.6	13 ± 1.9	*
Creatinine, mg/dl	0.3–1.4	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.8 ± 0.04	0.7 ± 0.1	*
Na^+, mmol/l	135–155	136.1 ± 0.2	137 ± 0.9	132 ± 1.8	133 ± 1.0	133 ± 1.1	135 ± 0.9	136 ± 0.5	*
K^+, mmol/l	3.5–5.5	4.9 ± 0.1	5.3 ± 0.2	5.1 ± 0.1	5.2 ± 0.1	4.9 ± 0.1	4.8 ± 0.4	5.3 ± 0.1	*
Ca^{2+}, mg/dl	8.6–11.8	11.3 ± 0.5	11.5 ± 0.2	14.7 ± 1.0	11.1 ± 0.1	10.9 ± 0.1	11.7 ± 0.6	10.9 ± 0.2	*
Phosphate, mg/dl	2.5–7.7	7.6 ± 0.7	7.4 ± 0.3	8.3 ± 0.5	6.7 ± 0.4	6.4 ± 0.2	6.4 ± 0.1	6.2 ± 0.2	*
WBC, $\times 10^3/mm^3$	6–17	9.4 ± 1.0	11.3 ± 0.5	10.5 ± 0.4	10.3 ± 0.4	9.3 ± 1.0	10.3 ± 0.7	10.1 ± 1.0	*
Lymhocytes, %	10–40	34.6 ± 2.4	36.5 ± 1.4	31.0 ± 3.5	36.6 ± 2.6	39.0 ± 4.2	37.0 ± 1.5	33.5 ± 2.6	*
Monocytes, %	2–10	4.0 ± 0.3	4.0 ± 0.4	3.6 ± 0.4	3.7 ± 0.2	3.6 ± 0.0	3.8 ± 0.1	3.7 ± 0.2	*
Granulocytes, %	50–80	61.3 ± 2.6	59.6 ± 1.4	65.5 ± 3.5	59.7 ± 2.7	51.6 ± 8.5	59.8 ± 0.2	63 ± 2.7	*
RBC, $\times 10^3/mm^3$	5.5–8.5	5.4 ± 0.2	5.5 ± 0.2	5.7 ± 0.2	5.8 ± 0.1	5.6 ± 0.1	6.1 ± 0.1	5.7 ± 0.2	*
MCV, fl	65–80	73.8 ± 1.2	70.9 ± 1.7	74.4 ± 0.7	74.2 ± 0.4	74.6 ± 0.4	72.3 ± 0.4	73 ± 0.7	*
MCHC, g/dl	28–40	32.8 ± 0.8	33.9 ± 0.7	31.2 ± 0.6	31 ± 0.4	31.1 ± 0.2	31.0 ± 0.3	31 ± 0.7	*
MCH, pg	19.5–24.5	24.1 ± 0.5	23.6 ± 0.2	23.1 ± 0.3	23 ± 0.2	23.1 ± 0.1	22.4 ± 0.1	22.3 ± 0.4	*
Hematocrit, %	35–55	39.9 ± 2.4	38.8 ± 2.2	42.7 ± 1.6	43 ± 1.1	41.9 ± 0.6	44.2 ± 1.1	41.6 ± 1.1	*
Hgb, g/dl	10–18	13.0 ± 0.5	13.6 ± 0.2	13.3 ± 0.5	13.4 ± 0.3	13.0 ± 0.1	13.7 ± 0.3	12.8 ± 0.5	*
Platelets, $\times 10^3/mm^3$	120–600	172.3 ± 20.7	284 ± 38.9	168 ± 24.0	179 ± 17.3	192 ± 26.9	172 ± 21.2	184 ± 18.0	*

* Nonsignificant ($p > 0.05$) effect in variable with treatment on every time point compared to the before treatment baseline value.
[a] Obtained from the International Species Information System (www.isis.org) and Vetlab Information (www.vetlab.co.uk).

The white blood cell count, red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and the platelet count were measured. Lymphocyte, monocyte and granulocyte counts were automatically determined and expressed as a percentage of the total white blood cell count. Serum chemistry values, including the concentration of the electrolytes such as sodium, potassium, calcium and phosphate as well as including total protein, globulin, albumin and amylase were also obtained. The serum enzyme activity of alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total bilirubin concentration, blood urea nitrogen and creatinine levels were measured. Each blood sample was read on a Vetscan HMT machine (ABAXIS Inc., Union City, Calif., USA).

Data Analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical analysis was carried out using SigmaStat version 3.0 (SPSS, Inc). One-way ANOVA was used to compare the groups if data were normally distributed with equal variance. For data that were not normally distributed with equal variance, the non-parametric test, Kruskal-Wallis one-way ANOVA on ranks was used instead. If these tests indicated that a parameter was significantly different among experimental groups, an all pairwise multiple comparison procedure, Holm-Sidak or Dunn, was carried out to determine which of the groups were significantly different from others for that particular parameter.

Results and Discussion

The levels of the monitored parameters obtained at the different time points are summarized in tables 1–3 for the short-, long-term and anesthesia-only control studies, respectively.

Plasma Proteins and Amylase

There was no detectable change exceeding the normal range in the levels of plasma proteins representing the hepatic synthetic capacity. Total protein, globulin and, most importantly, albumin remained close to their con-

Table 3. Short-term study of physiological blood parameters after isoflurane anesthesia only without contrast agent (n = 4; mean ± SEM)

Physiological parameter	Normal range[a]	Before treatment baseline value	Time after isoflurane anesthesia, days		Significance
			1	2	
Total protein, g/dl	5.5–7.7	6.0 ± 0.2	5.5 ± 0.2	5.6 ± 0.2	*
Globulin, g/dl	2.3–5.2	2.2 ± 0.2	2.3 ± 0.1	2.5 ± 0.1	*
Albumin, g/dl	2.5–4.4	3.8 ± 0.10	3.2 ± 0.21	3.3 ± 0.10	*
Amylase, U/l	200–1,200	578 ± 78	462 ± 59	522 ± 53	*
ALT, U/l	10–100	29 ± 1.3	161 ± 27.3	120 ± 15.8	***
ALP, U/l	20–150	75 ± 6.3	102 ± 4.6	79 ± 10.1	*
Total bilirubin, mg/dl	0.1–0.6	0.4 ± 0.05	0.3 ± 0.03	0.3 ± 0.04	*
BUN, mg/dl	7–25	10 ± 1.2	13 ± 2.7	10 ± 1.6	*
Creatinine, mg/dl	0.3–1.4	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.2	*
Na^+, mmol/l	135–155	138 ± 3.1	139 ± 2.4	133 ± 0.9	*
K^+, mmol/l	3.5–5.5	5.1 ± 0.2	5.0 ± 0.1	5.0 ± 0.1	*
Ca^{2+}, mg/dl	8.6–11.8	11.3 ± 0.2	10.3 ± 0.3	10.01 ± 0.2	*
Phosphate, mg/dl	2.5–7.7	6.2 ± 0.5	7.2 ± 0.4	5.6 ± 0.7	*
WBC $\times 10^3/mm^3$	6–17	7.5 ± 0.6	12.2 ± 1.1	9.7 ± 1.2	*
Lymphocytes, %	10–40	37.9 ± 2.3	29.6 ± 5.1	26.3 ± 3.3	*
Monocytes, %	2–10	4.6 ± 0.3	4.2 ± 0.5	3.3 ± 0.3	*
Granulocytes, %	50–80	57.5 ± 2.5	66.3 ± 5.2	70.4 ± 3.5	*
RBC $\times 10^3/mm^3$	5.5–8.5	6.5 ± 0.5	5.2 ± 0.6	5.4 ± 0.4	*
MCV, fl	65–80	77.1 ± 3.0	78.1 ± 2.5	75.3 ± 1.1	*
MCHC, g/dl	28–40	30.2 ± 1.9	30.3 ± 1.9	33.4 ± 1.5	*
MCH, pg	19.5–24.5	23.1 ± 0.5	23.5 ± 0.7	25.0 ± 0.7	*
Hematocrit, %	35–55	50.3 ± 5.1	41.1 ± 5.8	40.9 ± 3.4	*
Hgb, g/dl*	10–18	15.0 ± 0.9	12.2 ± 1.3	13.6 ± 0.8	*
Platelets, $\times 10^3/mm^3$	120–600	178 ± 40	122 ± 13	119 ± 19	*

* Nonsignificant ($p > 0.05$) effect in variable with treatment on every time point compared to the before treatment baseline value.

*** Significant ($p < 0.05$) change in variable 24 and 48 h after treatment exceeding normal range.

[a] Obtained from the International Species Information System (www.isis.org) and Vetlab Information (www.vetlab.co.uk).

trol values. The amylase level was not significantly elevated in any of the dog groups.

Electrolytes

No significant changes were observed in electrolyte levels in either the long-term or the short-term follow-up groups or in the anesthesia-only group.

Red Blood Cells

No significant deviation from normal was observed in red blood cell values (red blood cell count, hemoglobin concentration, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration). Based on the normal total red blood cell count, hematocrit and hemoglobin levels, agent-induced hemolysis and anemia could be also excluded.

White Blood Cells

Gadolinium-based contrast agents may be deposited in tissue during agent metabolism. These deposits may generate sterile inflammation in the body and they can be histologically identified in the liver and kidney [18]. Nevertheless, there was no significant white blood cell elevation in either agent-treated animal group. Judged by the white blood cell counts, no inflammation including hepatic and/or renal inflammation was present.

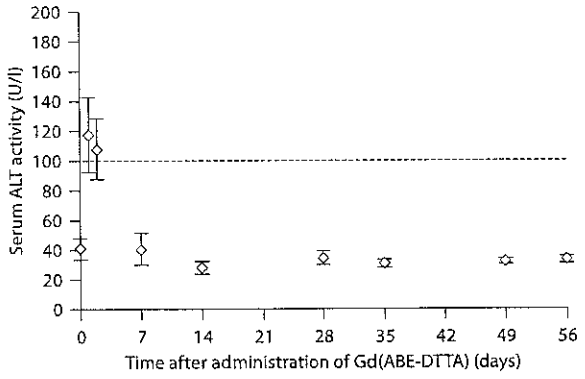

Fig. 3. Mean (±SEM) serum alanine aminotransferase (ALT) activity is shown in 4 dogs as a function of time following Gd(ABE-DTTA) administration at the MRI-effective dose (0.05 mmol/kg). A slight transient ALT elevation was detected during the first 2 days but activity returned to the baseline level by the end of the first week and remained normal throughout the 8-week follow-up. The upper limit of the normal range is 100 U/l, represented by the dotted line on the graph. Results of statistical analysis are shown in tables 1 and 2.

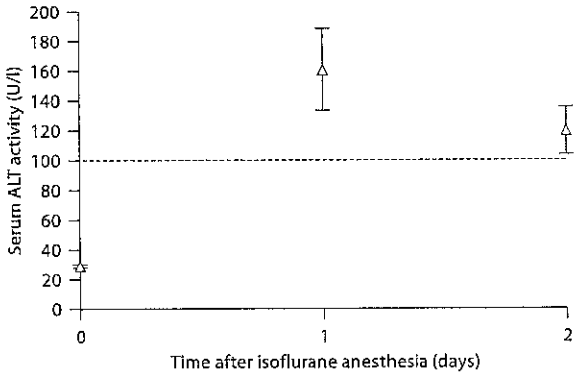

Fig. 4. Mean (±SEM) serum alanine aminotransferase (ALT) activity is shown in dogs (anesthesia-only control group) as a function of time following isoflurane anesthesia. Remarkable ALT elevation was found at the 24- and 48-hour time points. The upper limit of the normal range is 100 U/l (dotted line in the graph). Values of blood chemistry parameters in the anesthesia-only control group are shown in table 3.

Kidney Function

No sign of kidney failure was seen in the entire timeframe of the study. Elevation in blood urea nitrogen and creatinine typically represents azotemia, or in more severe cases uremia, representing kidney involvement. No such elevations were detected, indicating no change in kidney function, showing that our contrast agent is not deleterious for the kidney at the dose administered for MRI.

Representative Liver Function Parameters

We monitored two representative enzymes (ALT, ALP) and the total bilirubin level. Significant transient ALT elevation occurred on days 1 and 2 with a peak at 24 h after contrast agent administration. No increase in liver enzyme activities was detected between days 7 and 56. We have plotted ALT enzyme activity vs. time for the entire time frame of both studies (fig. 3). An initial elevation is evident, but the activity level returned to the normal range by day 7.

The purpose of monitoring liver function enzymes is primarily to assess liver injury [19]. Hepatocyte necrosis in acute hepatitis or toxic or ischemic insult provokes leakage of the transaminase enzymes into the blood stream. ALT lacks tissue specificity however, because it is also expressed in the myocardium. Interestingly, aminotransferases such as ALT were once used to diagnose myocardial infarction [20]. Thus the slight initial elevation that we observed in the ALT level might be, in part, the consequence of the induced myocardial infarction rather than due to agent-induced liver injury.

All dogs participating in the study underwent anesthesia. Transient abnormalities in liver function can be also the consequence of isoflurane inhalation. It has been previously shown that anesthetics could decrease the hepatic blood flow and increase the intracellular calcium concentration and consequently reduce oxygen delivery [21]. However, unlike halothane, isoflurane does not reduce total hepatic arterial blood flow. Nevertheless, it has been reported that isoflurane anesthesia alone can increase the ALT level significantly in dogs between 2 and 7 days after anesthesia [17]. Moreover, serum ALT concentration is also a marker of liver damage caused by anesthesia in humans [22]. Many human studies use this parameter to monitor the severity of liver failure [23, 24]. Therefore the physiological effect of isoflurane anesthesia alone was also tested in our animal model. ALT enzyme activity was remarkably high in the anesthesia-only control group in accordance with the finding of Topal et al. [17] (fig. 4). Thus the significant ALT elevation in the

early time points in our study is probably mainly due to isoflurane anesthesia.

Total bilirubin did not significantly increase in the long- or short-term group. ALP enzyme activity significantly increased 24 and 48 h following Gd(ABE-DTTA) administration but this increase still left the activity values within the normal range.

Conclusion

From this study we can conclude that Gd(ABE-DTTA) has no substantial short- or long-term deleterious effect on the investigated physiological parameters when given at the MRI-effective dose. The only significant change that also exceeded the normal range, the rise in ALT level detected 24 and 48 h after agent administration, can be interpreted as an effect of isoflurane or the myocardial infarction itself or both. Even assuming that this effect is caused by the agent, the mean values are barely above the normal range. They represent 3 times that of the average control and only 1.2 times that of the upper limit of the normal range. This is a small effect compared with the regularly detected 10- or even 100-fold increase in transaminase activity in acute hepatic toxicity in humans [19, 25].

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