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Haemodynamic effects of macrocyclic and linear gadolinium chelates in rats: role of calcium and transmetallation

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Several studies were undertaken to compare four magnetic resonance imaging (MRI) contrast media (CM) as regards acute haemodynamic effects in rats and to investigate the mechanisms involved. (1) Normotensive rats received a rapid bolus intravenous injection of 0.5 mmol kg^{-1} of each CM. The effects of Gd-DOTA, Gd-HP-DO3A, Gd-DTPA and Gd-DTPA-BMA on blood pressure (BP) were compared. (2) The haemodynamic effects of Gd-DTPA (0.5 mmol kg^{-1}) were compared to those of isovolumic and isoosmolar Zn-DTPA and glucose solutions. (3) The haemodynamic profiles of Gd-DTPA and Gd-DTPA-BMA were recorded with and without addition of ionized calcium. (4) The mechanism of Gd-HP-DO3A-induced transient rise in BP was investigated by evaluating the effects of phentolamine or diltiazem pretreatment. For (1) the greatest drop in BP occurred following Gd-DTPA (a linear chelate) injection (–18 ± 2% vs baseline, $P < 0.01$). Gd-DTPA-BMA, another lineate chelate, also induced a slight but significant reduction in BP (–8 ± 2% at 45 s, $P < 0.05$). Gd-DOTA, a macrocyclic CM, had virtually no haemodynamic effects. For (2) the Gd-DTPA-induced drop in BP was greater than that of the osmolality-matched glucose control and lower than that of osmolality-matched Zn-DTPA. For (3) a transmetallation phenomenon versus free ionized calcium is possible in the case of both linear CM (Gd-DTPA and Gd-DTPA-BMA) since Ca^{2+} significantly reduced the CM-induced decrease in BP. For (4) a transient rise in BP was observed following Gd-HP-DO3A, another macrocyclic chelate, associated with a concomitant increase in stroke volume. This effect was antagonized neither by phentolamine nor by diltiazem. The decrease in BP following injection of Gd-DTPA or Gd-DTPA-BMA may not only be osmolality-related since (a) Gd-DOTA solution, whose osmolality is greater than that of Gd-DTPA-BMA, had a lesser effect, and (b) this hypotensive effect was corrected by addition of ionized calcium. The transient Gd-HP-DO3A-induced rise in BP is probably the consequence of a positive inotropic effect.

Keywords: calcuim, gadolinium, magnetic resonance imaging contrast media, transmetallation

Introduction

Magnetic resonance imaging (MRI) has become the modality of choice for the diagnosis of central nervous system disease. The rapid development of this technique has prompted the need for a new class of drug which would be administered to a patient in order to enhance the image contrast between normal and diseased tissue (Lauffer 1987, Runge & Wells 1995). Because of its high number of unpaired electrons and its especially long electron relaxation time, gadolinium is of major interest as a contrast medium for MRI. However, as other rare earth metals, gadolinium interacts with a large number of physiologic systems such as reticuloendothelial system, noncompetitively inhibits Ca^{2+} binding, decreases the velocity of Ca^{2+} uptake and inhibits the activity of some enzymes (Ca^{2+}-activated Mg^{2+}-ATPase, some dehydrogenases and aldolase)

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(Krasnow 1972, Bourne & Trifaro 1982, Evans 1990). Chelation of gadolinium ions by appropriate polyamino-polycarboxylic ligands strongly contributed to minimizing such biochemical reactions and subsequent toxicity. Gadolinium chelates are currently used as MRI contrast media (CM) for evaluation of both intracranial and extracranial lesions (Rinck 1993).

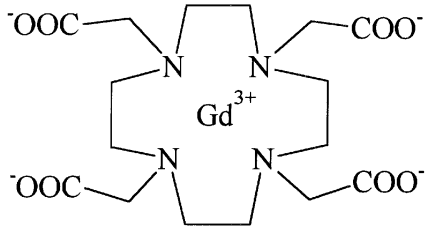
However, the biocompatibility of gadolinium complexes depends on the equilibrium:

$$[Gd^{3+}.Ligand] \rightleftharpoons Gd^{3+} + Ligand$$

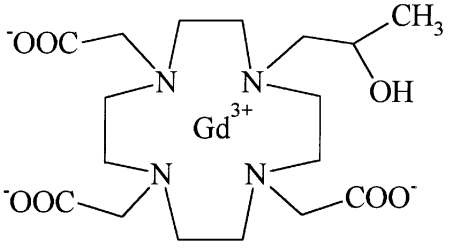
and free gadolinium or free ligand may induce biochemical reactions and thus have potentially deleterious effects. The kinetic rate which characterizes this equilibrium and particularly its dissociation rate is of major importance (Tweedle *et al.* 1995).

To date, two categories of gadolinium complexes are commonly used: (a) 'macrocyclic', which can be either 'ionic' (i.e. Gd-DOTA) or 'nonionic' (or 'neutral' (Tweedle 1991)) (i.e. Gd-HP-DO3A) and (b) 'linear', which can also be either 'ionic' (i.e. Gd-DTPA) or 'nonionic' (i.e. Gd-DTPA-BMA) (Figures 1 and 2). 'Ionic' complexes contain a carboxylic group, the associated cation being methylglucamine. This in turn, leads to an increase in the number of osmotically-active particles and thus in the osmolality of the solution.

Recently, it has been suggested that 'nonionic' low-osmolar MRI CM injected as a fast bolus of a high dose may induce less transient haemodynamic disturbances, due to a lower 'osmotoxicity' (Oksendal & Hals 1993), thus pointing out osmolality of CM solutions as a further deleterious parameter, besides the stability of the complex. Various rat (Mühler *et al.* 1992a, Harpur *et al.* 1993, Li *et al.* 1993, Masui *et al.* 1995), dog (Bokenes *et al.* 1997), and *in vitro* (isolated rat heart) (Akre *et al.* 1997) studies have compared CM in this respect. They all concluded to a beneficial role for a low osmolality. However, no clinical advantage for 'nonionic' has been published so far (Balériaux *et al.* 1993, Hieronim *et al.* 1995). To our knowledge, all currently commercially-available agents were never studied in parallel with respect to their haemodynamic properties. The aim of the present studies was, thus, to examine the relative role of complex stability, molecular structure, pharmaceutical formulation and osmolality in the cardiovascular biocompatibility of all four available MRI CM, using a classical haemodynamic approach.

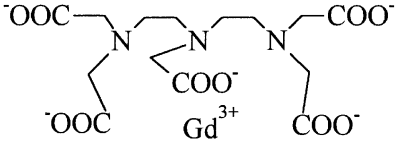


Gd-DOTA

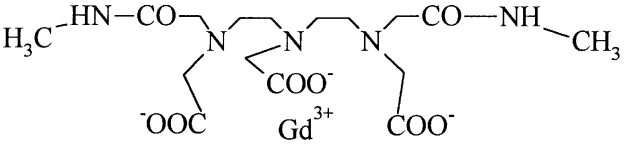


Gd-HP-DO3A

Figure 1. Structures of macrocyclic gadolinium complexes, Gd-DOTA (meglumine gadoterate) and Gd-HP-DO3A (gadoteridol).



Gd-DTPA



Gd-DTPA-BMA

Figure 2. Structures of linear gadolinium complexes, Gd-DTPA (meglumine gadopentetate) and Gd-DTPA-BMA (gadodiamide).

Materials and methods

Animal preparation

All the studies were carried out by using normotensive 7–8 week-old male Sprague Dawley rats (OFA SD Caw) from the Iffa Credo breeding centre (Les Oncins, L'Arbesle, France) weighing 280–320 g, fed on standard chow and allowed *ad libitum* access to drinking water. The animals were anaesthetized with 60 mg kg^{-1} sodium pentobarbital (Laboratoires Sanofi, Libourne, France) injected intraperitoneally and tracheotomized. The animals were allowed to breath spontaneously, except in the studies evaluating the stroke volume (SV) changes where they were mechanically ventilated using a constant volume ventilator (Harvard Biosciences, Les Ulis, France) at a rate of 80 strokes min^{-1} with a volume of 2.5 ml. Each experimental group consisted of 10 rats except in the studies where cardiac output was measured ($N = 5$ rats).

All experiments were carried out in compliance with the EEC directive (86/609/EEC) on animal welfare.

Haemodynamic measurements

The carotid artery (common trunk) and jugular vein were cannulated with respectively G19 and G 22-type catheters (Vygon, Ecouen, France) for the measurement of blood pressure (BP) and the injection of the test solutions, respectively. Following surgery, the preparation was allowed to stabilize for a period of at least 15 min. In one study, BP was recorded from the cannulated femoral artery.

Blood pressure was obtained from a Spectramed Statham P23XL transducer (Gould Electronique, Ballainvilliers, France) connected to the catheter and continuously registered and displayed on respectively a Gould ES 1000 polygraph and Gould V 1000 monitor.

Test solutions

Contrast media were tested exclusively as their commercially-available, sterile and pyrogen-free aqueous solutions. The concentration of all the contrast media was 500 mmol l^{-1}. The following CM solutions were tested: Gd-DOTA (meglumine gadoterate), Gd-DTPA (dimeglumine gadopentetate), Gd-DTPA-BMA (gadodiamide) and Gd-HP-DO3A (gadoteridol). The main physicochemical parameters are given in Table 1.

The control solutions were isotonic saline (Laboratoire Lavoisier, Paris, France), hyperosmolar glucose (Prolabo, Paris, France) (1960 mOsm kg^{-1}), and two hyperosmolar Zn-DTPA solutions (2060 mOsm kg^{-1}). These two Zn-DTPA control solutions (both at 400 mmol l^{-1}) were: solution A containing 0.7 mmol l^{-1} of free DTPA and solution B containing $7.5\ 10^{-4}$ at.g l^{-1} of free Zn^{2+}.

To investigate the mechanisms involved in the CM-induced effects, some studies required injections of phentolamine (Sigma, Saint-Quentin-Fallaviers, France), diltiazem (Sigma), and others the addition of calcium (in the form of $CaCl_2.4\ H_2O$) (Prolabo, Paris, France) at the concentration of 18 mmol l^{-1}.

Experimental protocols

All CM and control solutions were administered as a 3-s bolus injection via the jugular vein catheter.

Study 1: the acute effects of all the four CM at the same dose of 0.5 mmol kg^{-1} (i.e. 1.0 ml kg^{-1}), and isotonic saline (1.0 ml kg^{-1}) on carotid artery and femoral artery BP were compared. The injected dose corresponds to 5 times the clinical dose.

Study 2: Gd-DTPA (0.5 mmol kg^{-1}) was compared with isovolumic control solutions of Zn-DTPA (solutions A and B) (0.4 mmol kg^{-1}), hyperosmolar glucose (isoosmolar to Gd-DTPA), isotonic saline.

Study 3: the effects of the addition of ionized free calcium on BP changes were investigated by adding $CaCl_2.4\ H_2O$ (18 mmol l^{-1}) to the solutions of Gd-DTPA and Gd-DTPA-BMA. The solutions were prepared extemporaneously. These CM were injected at the dose of 0.5 mmol kg^{-1} and thus the associated calcium dose was 18 μmol kg^{-1}. This dose was selected from Mühler *et al.* 1992b.

Table 1. Principal characteristics of the tested contrast media solutions

Chemical name	Commercial name	Batch number	Osmolality (mosm/kg, 37 °C)	Ionicity	Chelate	Formulation
Gd-DOTA (Meglumine gadoterate)	Dotarem®	94 GD 007	1350	Yes	Macrocyclic	–
Gd-HP-DO3A (Gadoteridol)	ProHance®	4c67568	630	No	Macrocyclic	$Ca[Ca\ HP\text{-}DO3A]_2$ 0.1%–0.5 mmol l^{-1}
Gd-DTPA (Meglumine gadopentetate)	Magnevist®	34034	1960	Yes	Linear	Free DTPA 0.2%–1 mmol l^{-1}
Gd-DTPA-BMA (Gadodiamide)	Omniscan®	301154	789	No	Linear	Na[Ca DTPA-BMA] 5.0%–25 mmol l^{-1}

Data from package inserts.

Study 4: the aim of this study was to investigate the mechanism of the transient rise in BP following injection of Gd-HP-DO3A (0.5 mmol kg^{-1}):

(a) The effects of a phentolamine pretreatment (0.3 mg kg^{-1} intraperitoneally, 30 min prior to the CM) on the Gd-HP-DO3A-induced transient increase in BP were investigated.
(b) The effects of a diltiazem infusion (1.0 mg kg^{-1}, 0.1 ml min^{-1}, starting 10 min before CM injection). Two groups were evaluated: (i) Gd-HP-DO3A injected during a saline infusion, (ii) Gd-HP-DO3A injected during a diltiazem infusion. In this study, Gd-HP DO3A or saline were injected as a bolus into the tail vein (and not the jugular vein as above).
(c) The cardiac output following injection of Gd-HP-DO3A (0.5 mmol kg^{-1}) was measured by placing an ultrasound transit-time flowprobe around the ascending aorta just above the coronary arteries, in a group of 5 rats. Stroke volume (SV) was calculated according to the formula $SV = CO/HR$, where CO is the cardiac output and HR the heart rate. Data were recorded using a T 206XM transit time flowmeter (Transonic Systems, Ithaca, NY, USA), and calculations were carried out by means of the HEM software (Notocord, Croissy-sur-Seine, France).

Statistical analysis

Measured values are expressed as the mean ± the standard error of the mean (SEM). For each study, the homogeneity of the groups in terms of preinjection values was checked, by means of an analysis of variance (ANOVA). The percentage change vs. baseline at various specified time points after injection of the test solutions were compared with preinjection values by using the paired Student's *t*-test adjusted by the number of measurements (Bonferroni's adjustment). The haemodynamic effects of the test solutions were compared, using repeated measure ANOVA to determine the presence of significant differences. When the test showed significant differences, the haemodynamic profiles of the test solutions were compared two by two by using repeated-measure ANOVA and calculating the interaction time × product factor (Milliken, 1990).

Statistical analysis was conducted using the Number Cruncher Statistical System program version 5.0 (Kaysville, UT, USA) on an IBM personal system computer. Significance was accepted when $P < 0.05$.

Results

Study 1

Baseline BP was 119 ± 4 mm Hg (Gd-DTPA), 127 ± 5 mm Hg (Gd-DTPA-BMA), 129 ± 6 mm Hg (Gd-DOTA), 114 ± 5 mm Hg (Gd-HP-DO3A) and 118 ± 6 mm Hg (isotonic saline). ANOVA revealed no significant difference among these groups ($P = 0.24$).

(i) Gd-DTPA induced an important and transient drop in BP (maximum at 15 s post-injection). The haemodynamic profile of this CM differed from that of all other CM and the isotonic saline ($P < 0.01$) (Figure 3).
(ii) Gd-DTPA-BMA induced a slight but significant drop in BP ($P < 0.05$ vs. baseline) 45 s following injection. The haemodynamic profile of this agent significantly differed from that of Gd-DOTA, isotonic saline and Gd-DTPA.
(iii) Gd-DOTA induced no significant changes in BP and did not significantly differ from isotonic saline over the measurement period ($P = 0.94$).
(iv) Gd-HP-DO3A induced a transient rise in BP immediately following injection (+ 8 ± 2% vs. baseline at 10 s, $P < 0.05$) and no subsequent drop in BP.

When recorded at the femoral artery level, BP changes were similar to those measured at the carotid artery level, except Gd-HP-DO3A which induced a greater rise at 10 s following injection (+ 19 ± 4% vs. baseline) (Table 2).

Study 2

Baseline BP was 119 ± 4 mm Hg (Gd-DTPA), 118 ± 6 mm Hg (saline), 123 ± 3 mm Hg (hypertonic glucose), 121 ± 3 mm Hg (Zn-DTPA, sol. A) and 104 ± 7 mm Hg (Zn-DTPA, sol. B). ANOVA revealed no significant difference between these groups ($P = 0.07$).

Table 2. Effect of contrast media solutions (intravenous bolus, 0.5 mmol kg^{-1}) on femoral artery blood pressure in anaesthetized rats

Test solution	Baseline femoral mean blood pressure (mm Hg)	Max.effect (% vs. baseline)	Time to peak effect (s)	Statistical comparison vs. baseline
Gd-DTPA	106 ± 5	–17 ± 2	10	< 0.01
Gd-DTPA-BMA	118 ± 5	–11 ± 3	60	< 0.01
Gd-DOTA	116 ± 7	+3 ± 4	60	NS
Gd-HP-DO3A	113 ± 6	+19 ± 4	10	< 0.01

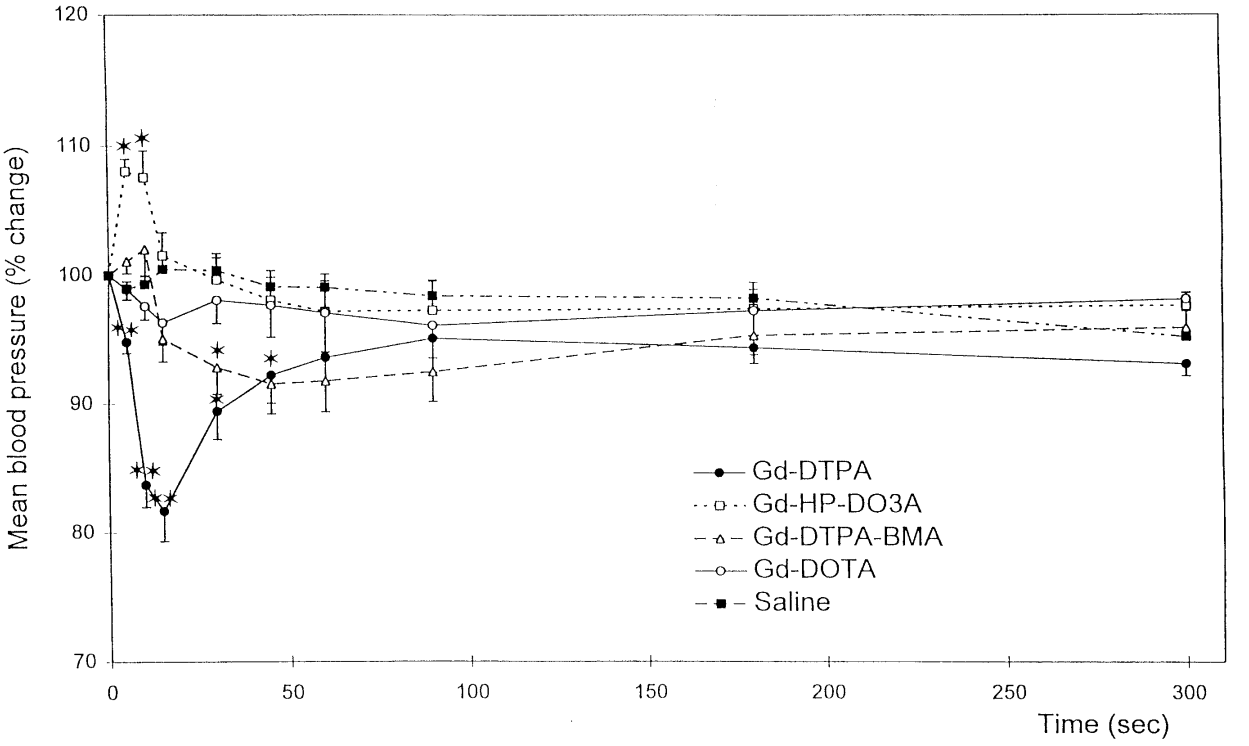


Figure 3. Acute effects of commercially-available solutions of four Gd^{3+} chelates on blood pressure in rats. All contrast media were injected at 0.5 mmol kg^{-1} by intravenous route. Each group consisted of 10 male Sprague Dawley rats. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with baseline.

The transient effect of the Gd-DTPA solution on BP was greater than that of the osmolality-matched glucose control ($P < 0.01$) and isotonic saline ($P < 0.01$). Both Zn-DTPA hypertonic control solutions induced an important drop in BP (maximum effect 15 s following injection), more marked than with of Gd-DTPA, hypertonic glucose and saline ($P < 0.001$). These two solutions induced similar effects on BP (at 15 s: sol. A: –35 ± 5%, sol. B: –41 ± 7% vs. baseline) (Figure 4).

Study 3

The dose of 18 μmol kg^{-1} of calcium inhibited the transient drop in BP induced by 0.5 mmol kg^{-1} Gd-DTPA ($P < 0.001$) (Figure 5). This dose was therefore selected to interact with Gd-DTPA-BMA (0.5 mmol kg^{-1}).

Baseline BP were homogeneous ($P = 0.88$). Gd-DTPA-BMA alone induced a slight but significant decline in BP vs. baseline and saline ($P < 0.01$). When the same dose of Gd-DTPA-BMA was injected in conjunction with 18 μmol kg^{-1} Ca^{2+}, an early and transient rise in BP (= 9.8 ± 1.1% vs. baseline at 5 s postinjection, $P < 0.01$) was noted. The subsequent decline in BP was significantly lower than with the solution of Gd-DTPA-BMA without added ionized calcium (at 1 min postinjection: –2.5 ± 0.6% vs. –7.6 ± 1.9% of preinjection values, $P < 0.05$). The $CaCl_2$ control solution only induced transitory BP changes immediately after injection, similar to those observed with all other treatments (Figure 6). The effects of this control solution on BP did not differ from those of saline.

Study 4

Effect of pretreatment by phentolamine on Gd-HP-DO3A-induced early rise in BP. Phentolamine, *per se*, induced a drop in BP (–17 ± 2 mm Hg, $P < 0.05$) whereas isotonic saline had no effect (–1 ± 1 mm Hg). Baseline BP values, before Gd-HP-DO3A injection were 108 ± 6 mm Hg in the phentolamine-treated group and 125 ± 5 mm Hg in the saline-treated group. Comparison of these two mean BPs did not reach the significance level ($P = 0.07$).

Phentolamine did not prevent the occurrence of the Gd-HP-DO3A-induced early rise in BP (in the saline-treated group: +14 ± 2%, in the phentolamine-treated group: +15 ± 1% vs. baseline, NS).

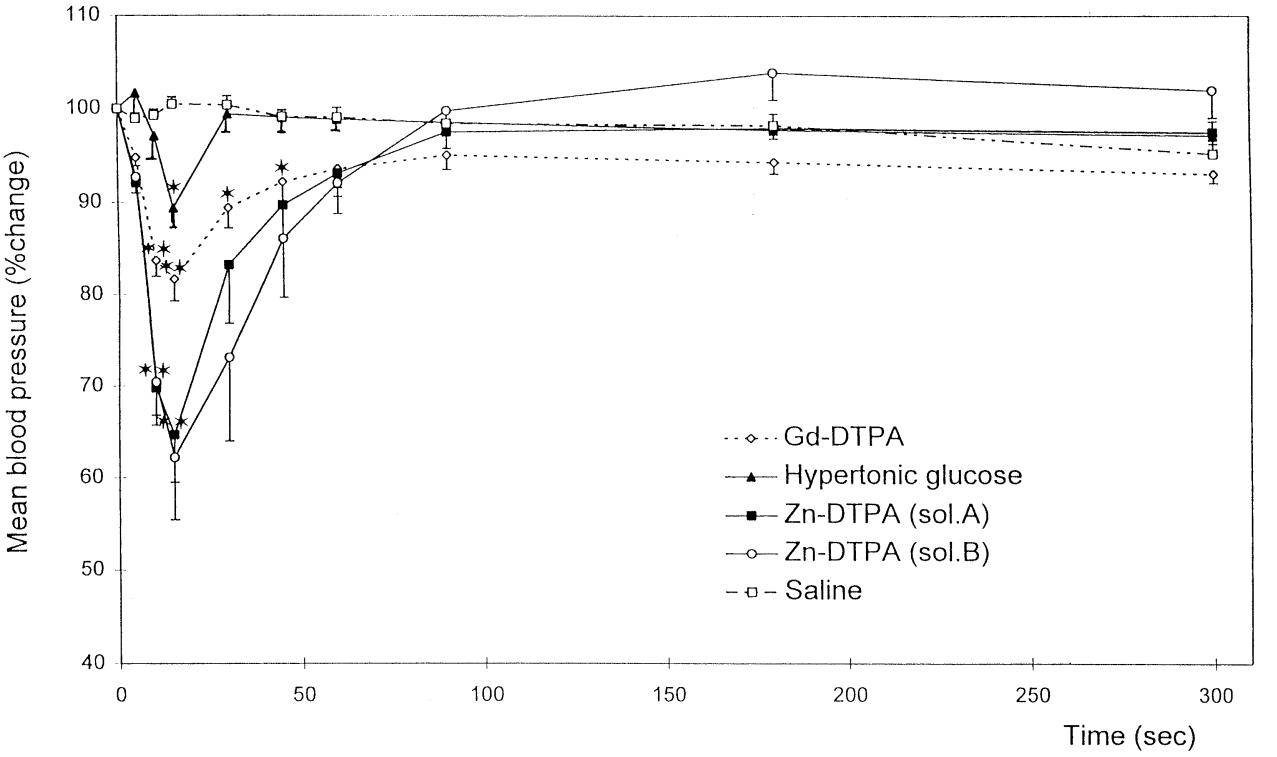


Figure 4. Comparison of acute effects of intravenously-injected Gd-DTPA (0.5 mmol kg^{-1}) and iso-osmolar control solutions (i.e. glucose solution and two Zn-DTPA solutions, A and B), and isotonic saline, on blood pressure in rats. Zn-DTPA solution A contains 0.7 mmol l^{-1} of free DTPA and solution B contains 7.5 10^{-4} at.g l^{-1} of free Zn^{2+}. Each group consisted of 10 male Sprague Dawley rats. $*P < 0.05$, $**P < 0.01$ compared with baseline.

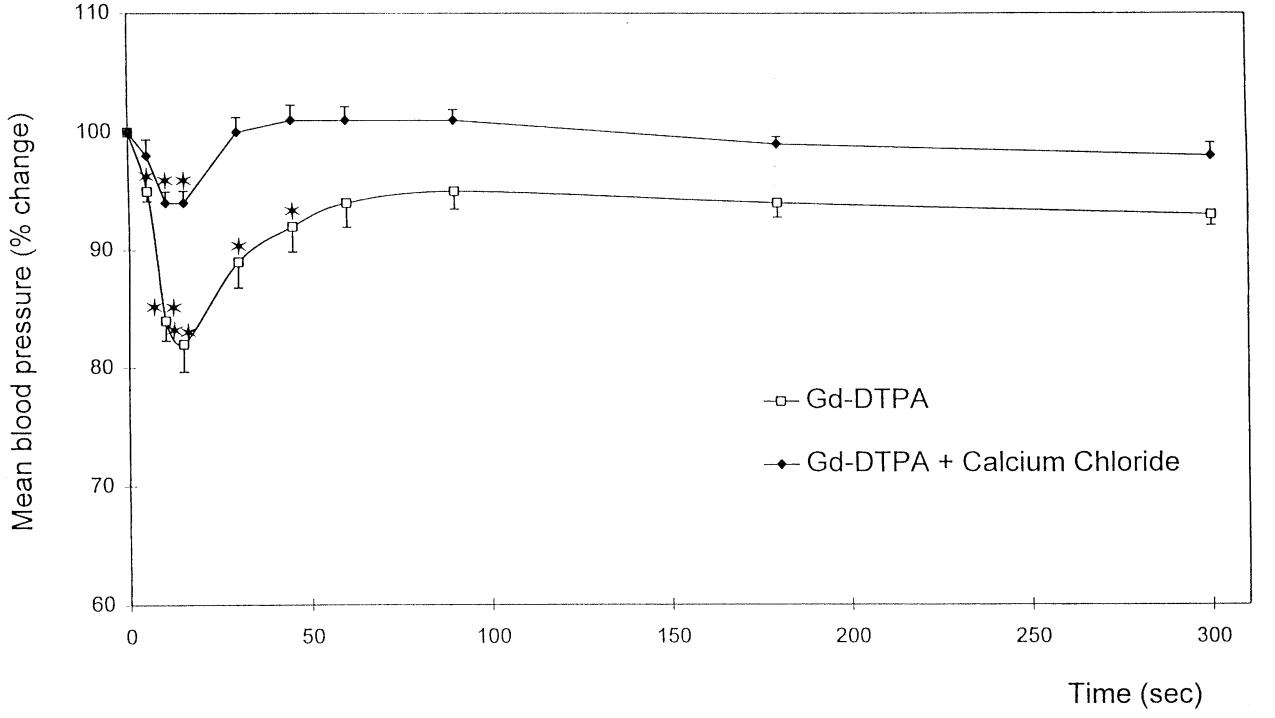


Figure 5. Acute blood pressure changes following intravenous injection of commercially-available solution of Gd-DTPA (0.5 mmol kg^{-1}) with or without addition of 18 μmol kg^{-1} free ionized calcium, in rats. Each group consisted of 10 male Sprague Dawley rats. $*P < 0.05$, $**P < 0.01$ compared with baseline.

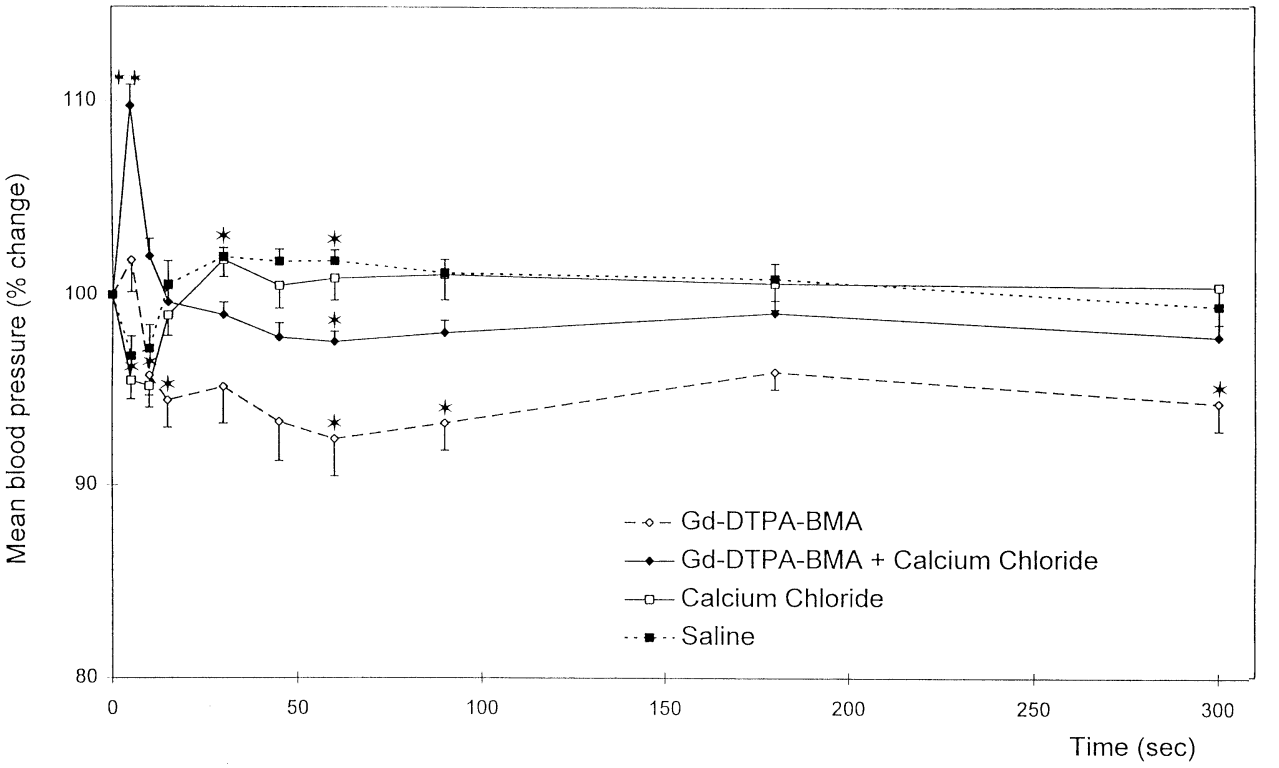


Figure 6. Acute blood pressure changes following intravenous injection of commercially-available solution of Gd-DTPA-BMA (0.5 mmol kg^{-1}) with or without addition of 18 μmol kg^{-1} free ionized calcium, in male Sprague Dawley rats. Control groups received either isovolumic saline, or an isovolumic injection of calcium chloride (corresponding to 18 μmol kg^{-1} Ca^{2+}). Each group consisted of 10 male Sprague Dawley rats. $*P < 0.05$, $**P < 0.01$ compared with baseline.

Effect of an infusion of diltiazem on the Gd-HP-DO3A-induced early rise in BP. In diltiazem-infused rats, baseline BP values were 114 ± 6 mm Hg prior to infusion and 94 ± 7 mm Hg prior to injection of Gd-HP-DO3A (mean change in BP = –21 ± 13 mm Hg, $P < 0.01$). In saline-infused rats, baseline BP values were 117 ± 6 mm Hg prior to infusion and 127 ± 15 mm Hg prior to injection of Gd-HP-DO3A (mean change in BP = +10 ± 4 mm Hg, $P = 0.03$).

Diltiazem infusion did not prevent the occurrence of the Gd-HP-DO3A-induced transient rise in BP (in the saline-infused group: +9 ± 2% vs. baseline, $P < 0.05$, in the diltiazem-infused group : +10 ± 3% vs. baseline, $P < 0.05$).

Effect of Gd-HP-DO3A on cardiac output and stroke volume. Gd-HP-DO3A induced a transient increase in CO and SV (significant vs. baseline at 5 s). Changes in HR were minor (Table 3).

Discussion

The current studies compared the haemodynamic effects of all the four available MRI-CM, and aimed at investigating the mechanisms involved. The experimental model selected here is classical and widely used (Waynforth, 1980). It has been suggested that neck surgery before carotid artery cannulation may interact with nerve fibres that arise from the aortic arch baroreceptors, resulting in an increase in BP measured at the level of the carotid artery (Pang & Scott 1980). We therefore carried out one study in rats in which BP was recorded from the femoral artery (Table 2). CM had similar effects to those reported in the case of carotid artery cannulation. This gives us grounds to believe that the method used here is valid.

CM differ not only by the chemical structure of their macrocyclic or linear framework, but also by their pharmaceutical formulation which, in some cases, may include either a large amount of free ligand, or calcium or sodium salts of calcium complexes. Such excipients are intended to ensure the absence of free Gd^{3+} in the pharmaceutical solutions over their shelf lives. The formulation may affect the acute toxicity and the distribution profile of the CM, as clearly shown in the case of Gd-DTPA-BMA (Cacheris *et al.* 1990; Tweedle *et al.*

Table 3. Effect of Gd-HP-DO3A (intravenous bolus 0.5 mmol kg^{-1}) on cardiac output, stroke volume and heart rate in anaesthetized rats

Time(s)	0	5	10	15	20	25	30	60	120	150	300
Cardiac output (ml min^{-1})	43 ± 8	47 ± 8 $P < 0.05$	48 ± 9	47 ± 9	46 ± 9	45 ± 9	45 ± 9	43 ± 7	36 ± 7	35 ± 7	41 ± 8
% Change vs. baseline	–	+12 ± 3	+12 ± 4	+9 ± 3	+7 ± 3	+5 ± 3	+5 ± 4	+1 ± 6	–10 ± 12	–13 ± 12	–0.5 ± 5
Stroke volume (ml)	0.17 ± 0.05	0.19 ± 0.05 $P < 0.05$	0.19 ± 0.06	0.19 ± 0.06	0.18 ± 0.06	0.18 ± 0.06	0.18 ± 0.06	0.16 ± 0.04	0.14 ± 0.03	0.13 ± 0.03	0.17 ± 0.05
% Change vs. baseline	–	+16 ± 4	+14 ± 2	+13 ± 2	+9 ± 2	+8 ± 2	+8 ± 2	+5 ± 6	–8 ± 12	–9 ± 13	+2 ± 7
Heart rate (b min^{-1})	296 ± 36	284 ± 27	281 ± 26	284 ± 29	291 ± 34	290 ± 34	290 ± 34	290 ± 34	285 ± 32	281 ± 32	275 ± 29
% Change vs. baseline	–	–3 ± 4	–4 ± 4	–3 ± 3	–2 ± 1	–2 ± 1	–2 ± 1	–2 ± 2	–3 ± 2	–5 ± 2	–6 ± 3

1995). We therefore chose to compare exclusively commercially-available solutions in order to be as close as possible to clinical situations.

It was the purpose of this study to investigate whether osmolality is the most important determinant in the acute haemodynamic effects of MR agents since this parameter may theoretically become more relevant if a high dose is used, a protocol which has been suggested to be of clinical interest in certain occasions (Runge & Wells 1995).

Contrary to what was concluded from rat studies comparing 'nonionic' CM to high osmolar Gd-DTPA only (Mühler *et al.* 1992a, Masui *et al.* 1995), our data are not in favour of an exclusive role for osmolality. In Study 1, CM do not act as they might be expected to on the basis of their osmolality. Notably, Gd-DTPA-BMA has a significantly more marked effect than Gd-DOTA whereas the osmolality of the latter is higher. The Gd-DTPA-BMA-induced transient reduction in blood pressure is not consistent with some results previously published (Mühler *et al* 1992a, Geschwind *et al.* 1996). Reasons for such a discrepancy are unclear. In our hands, this effect, although slight, was significant, verifiable, reproducible (Study 1 and Study 2), and was not dependent upon the measurement site (carotid vs. femoral artery) (Table 2). Moreover, we focused on BP changes immediately following injection since the major effects occurred at this time. It should be stressed that the anaesthetic agent used in our study was identical to that selected in similar studies (Mühler *et al.* 1992a and b, Masui *et al.* 1995, Geschwind *et al.* 1996).

In Study 2, the depressive effect of Gd-DTPA on BP was significantly greater than that of osmolality-matched glucose control solution, thus suggesting that another parameter is involved. Immediate and transient free calcium chelation by free DTPA either present in the tested commercial solution (i.e. 1.0 mmol l^{-1}) or resulting from *in vivo* dechelation (Kasokat & Urich 1992) may possibly explain this discrepancy.

Both Zn-DTPA control solutions induced a similar transient drop in BP, greater than Gd-DTPA tested at the same osmolality. This suggests that: (a) osmolality, *per se*, had no major role, and (b) the excess in either free DTPA or free Zn^{2+} also had no influence on BP. However, this is not consistent with the above-mentioned suggested role for the free ligand DTPA. The concentration in free DTPA in

the case of the Zn-DTPA sol. A (i.e. 0.7 mmol l^{-1}) was only slightly lower than in the commercial solution of Gd-DTPA. Furthermore, an important hypocalcaemic effect of Zn-DTPA, at doses consistent with that used in the present study, has been reported in rats by Fukuda *et al.* 1986.

Assuming that the extracellular fluid volume in rats is 250 ml kg^{-1} of body weight and that Zn-DTPA is distributed in the extracellular fluid, it is of interest to note that, in our conditions, the early Zn-DTPA concentration (i.e. about 1.6 mmol l^{-1}) was equivalent to that of free Ca^{2+} (i.e. about 1.2 mmol l^{-1}, according to Mühler *et al.* 1992b).

The haemodynamic depressive effect of Zn-DTPA was more marked than that of Gd-DTPA. This is consistent with the thermodynamic stability constants (Log *K*), lower in the case of Zn-DTPA (i.e. 18.70) than in the case of Gd-DTPA (i.e. 22.46) (Cacheris *et al.* 1990). Further, in our hands, Ca-DTPA was more toxic than other DTPA complexes (drop in BP = –36 ± 8% vs baseline at 0.25 mmol kg^{-1}, when tested at equivalent osmolality with Gd-DTPA) (data not shown). Interestingly, Log *K* for Ca-DTPA is the lowest (i.e. 10.75) (Cacheris *et al.* 1990). Taken as a whole, these data suggest that stability is a crucial point in the case of complexes involving the linear DTPA ligand.

Our data indicate that addition of 18 μmol kg^{-1} of Ca^{2+} to 0.5 mmol kg^{-1} of Gd-DTPA attenuates the transient drop in BP induced by the CM alone. This result is consistent with an earlier study by Mühler *et al.* 1992b. Interestingly, addition of calcium ions, at the same dose, also corrected the slight decrease in BP induced by Gd-DTPA-BMA, thus suggesting that a similar phenomenon occurs for both linear Gd^{3+} complexes. Since, in the current study, $CaCl_2$ injected alone at the same dose, had no stimulating effect, a cumulative effect appears unlikely. An early chelation of free plasma Ca^{2+} by linear complexes thus appears likely in our conditions.

The uptake of free ionized calcium ions by myocardial cells during depolarization, through voltage-dependent calcium channels, leads to final interaction with troponin C and cardiac contraction (Langer 1990).

A solution of Gd-DTPA, tested at 2.0 mmol l^{-1}, i.e. a concentration consistent with early values *in vivo*, induced *in vitro* a 5.1% reduction of the ionized calcium levels in rat serum (Mühler *et al.* 1992b). According to Mühler *et al.* 1992b, the calcium ions may interact not only with the excess DTPA present in the commercial solution (i.e. 1.0 mmol l^{-1}) (Table 1), but also with charges of the two carboxyl groups salified by meglumine.

Our data thus strongly suggest that a qualitatively similar chelation of serum free Ca^{2+} ions occurs in the case of the nonionic Gd-DTPA-BMA commercial solution, following bolus injection of a large dose. This phenomenon may explain the slight and temporary reduction in BP. It is difficult to explain this effect by the Na[Ca-DTPA-BMA] complex added to the commercial solution (25 mmol l^{-1}), since the DTPA-BMA ligand is already associated with calcium. It should thus be suggested that Ca^{2+} may displace Gd^{3+} *in vivo*. However, DTPA-BMA has a relatively low stability constant with this ion (Log *K* at 25 °C = 7.17 [Cacheris *et al.* 1990]). In fact, as stressed by Tweedle (1995), the kinetic rate of chelate dissociation is a much more important parameter. In our test conditions (i.e. by injecting 1.0 ml kg^{-1}), the injected dose of complexed calcium already present in the commercial solution of Gd-DTPA-BMA is 25 μmol kg^{-1}, i.e. higher than the dose extemporaneously added in Study 3.

Elevation of iron and bilirubin levels has been reported in patients following administration of both linear complexes Gd-DTPA-BMA (Van Wagoner 1991) and Gd-DTPA (Niendorf & Brasch 1993). Other clinical (Hattner & White 1990, VanWagoner *et al.* 1991, Puttagunta *et al.* 1996a), or *in vitro* (Puttagunta *et al.* 1996b) studies are highly suggestive of a greater transmetallation potential for linear than for macrocyclic Gd^{3+} complexes, irrespective of the 'ionicity' of the molecule. Furthermore, Corot *et al.* 1994 have shown that the linear complexes of gadolinium Gd-DTPA and Gd-DTPA-BMA strongly inhibited the catalytic activity of a zinc-dependent metallopeptidase, angiotensin-converting enzyme (ACE), whereas the macrocyclic complex Gd-DOTA was virtually inactive when tested at the same concentration. The inhibition potential of linear gadolinium complexes seems to be related to metal exchange since addition of an excess of zinc to the medium partially suppressed their inhibitory effects on ACE activity (correction was complete for Gd-DTPA-BMA).

The early and transient rise in BP following injection of Gd-HP-DO3A is consistent with data reported by Masui *et al.* 1995. Since a similar volume of saline had no effect, a volumetric positive haemodynamic effect may be ruled out. A pharmacologically-active dose of the alpha-1 adrenoreceptor antagonist phentolamine did not prevent this effect, thus indicating that Gd-HP-DO3A has no stimulating effect on the sympathetic system.

The commercial solution of Gd-HP-DO3A contains 0.5 mmol l^{-1} Ca[Ca-HP-DO3A] (Table 1). To investigate whether this effect on BP is calcium-

dependent, Gd-HP-DO3A was injected as a bolus in the tail vein (second study) of rats infused by the calcium-channel blocker diltiazem. This route of injection was selected to avoid a transient hypervolemia-dependent hypotensive effect following bolus injection of either Gd-HP-DO3A or saline in diltiazem treated rats. Such an effect has been observed in preliminary studies where test solutions were injected into the jugular vein (data not shown). It may be hypothesized that, in diltiazem-treated rats, the reflex cardiac stimulation following the transient increase in volemia in the right heart chambers (Bainbridge 1915) may be hampered, due to the selective blockade of slow calcium channels by this agent (Scott 1983). Diltiazem was selected because of its balanced vascular and cardiac (inotropic) pharmacologic effects (Triggle 1996). In this study, diltiazem did not prevent the rise in BP. We conclude that a transient activation of cardiac calcium channels by Gd-HP-DO3A appears unlikely.

Baseline CO and SV were slightly lower than those classically reported in conscious rats but consistent with the anaesthetic status of the animals (Gross 1994). CO was increased, following injection of Gd-HP-DO3A (Table 3). This effect occurred concomitantly to the rise in BP. Since HR was virtually unchanged, this effect is the consequence of an increase in SV. SV depends upon two factors: the diastolic volume and the amount of myocardial shortening (Rushmer 1970). The increase in SV was not the consequence of a transient reduction in the post-load since BP was increased. An increase in the diastolic distension of the ventricles (Frank-Starling mechanism) also appears unlikely. A transient positive inotropic effect thus appears probable. By using the isolated rat heart model, Akre *et al.* 1997 have found an increase in left ventricular dP/dt_{max} following injection of Gd-HP-DO3A. The amount of ionized calcium available from solutions of Gd-HP-DO3A is low (in our conditions, it corresponds to a bolus injection of 0.5 μmol kg^{-1} Ca^{2+}. This makes the possibility of it playing a positive inotropic role rather unlikely (in humans, inotropic doses of calcium salts are in the range of 30 μmol kg^{-1} [Anonymous 1989]). Furthermore, a similar transient increase in BP is obtained when adding 18 μmol kg^{-1} Ca^{2+} to Gd-DTPA-BMA, a much higher dose of calcium (Fig. 6). The mechanism of the suspected Gd-HP-DO3A-induced positive inotropic effect still remains unclear.

In conclusion, we have found that: (a) the two macrocyclic complexes Gd-DOTA and Gd-HP-DO3A did not induce transient decrease in blood pressure, unlike linear Gd^{3+} chelates, and only differ in that Gd-HP-DO3A induces a transient rise in BP; (b) osmolality is not the only parameter involved in the transient hypotensive effect of Gd-DTPA; (c) a rapid and transient transmetallation phenomenon vs. free ionized calcium may occur *in vivo* in the case of Gd-DTPA-BMA as in the case of Gd-DTPA.

It would be of interest, in future studies on the same rat model, to measure free calcium levels in plasma following injection of commercial solutions of CM.

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