Manganese-calcium interactions with contrast media for cardiac MRI: A study of manganese chloride supplemented with calcium gluconate in isolated guinea pig hearts.

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ABSTRACT.

Objectives. Manganese ions (Mn^{2+}) enter cardiomyocytes via calcium (Ca^{2+}) channels and enhance relaxation intracellularly. To prevent negative inotropy new Mn^{2+} releasing contrast agents have been supplemented with high Ca^{2+}. The study aim was to investigate how this affects cardiac function and MR efficacy.

Materials and methods. $MnCl_2$ based contrast agents, *Manganese* and *Manganese-Calcium* (Ca^{2+} to Mn^{2+} 10:1), were infused during 4 repeated washin-washout sequences in perfused guinea pig hearts. [Mn^{2+}] were 10, 50, 100 and 500 μM.

Results. During washin *Manganese* depressed left ventricular developed pressure (LVDP) by 4, 9, 17 and 53 % whereas *Manganese-Calcium* increased LVDP by 13, 18, 25 and 56 %. After experiments tissue Mn contents (nmol/g dry wt.) were: *Control* < 40, *Manganese* 3720 and *Manganese-Calcium* 1620. T_1 was reduced by 85-92 % in Mn^{2+} enriched hearts.

Conclusions. High Ca^{2+} supplements to Mn^{2+} releasing contrast agents may be counterproductive by inducing a strong positive inotropic response and by reducing the MR efficacy.

KEY WORDS: MRI, Manganese, Calcium, Heart, Safety, Efficacy

INTRODUCTION.

Magnetic resonance imaging (MRI) is in rapid development towards providing a comprehensive evaluation in ischemic heart disease.[1] In this context contrast media releasing paramagnetic manganese (Mn) ions (Mn^{2+}) after intravenous (iv) administration are held as promising for assessment of myocardial function and viability.[2] Experiments have shown that important mechanisms [3-9] behind Mn^{2+} based MRI (Mn-MRI) are:

- Mn^{2+} uptake via slow calcium (Ca^{2+}) channels in the cell membrane (sarcolemma);
- intracellular (ic) Mn^{2+} retention for hours; and,
- strong ic T_1 relaxation obtained by ic Mn^{2+} protein adducts.

A returning notion has been that release of Mn^{2+} may induce an acute heart failure [10-12] due to blockade of Ca^{2+} channels. Against this view speaks the fact that transient depression of contractility is less likely to occur in vivo.[13-16] Still, in order to prevent postulated cardiac side-effects different strategies are chosen for formulation or administration of Mn^{2+} containing contrast media. The first is to use a slow-release Mn^{2+} chelate like MnDPDP (Mn-dipyridoxyl-diphosphate). MnDPDP (Teslascan™, Amersham Health, Oslo, Norway) has over a decade been safely employed in humans for MRI of liver and pancreas [15,16] but is not yet tried for cardiac MRI in patients. The second strategy is to add a "cardioprotective" Ca^{2+} salt to a "MR-effective" Mn^{2+} salt. This approach has recently been described for new contrast media developed for MRI of heart and other organs.[12,17,18] And the third strategy, widely explored in animal research,[5,6,8,9,14] is to avoid cardiodepression by controlled infusion of a nonsupplemented rapidly dissolving Mn^{2+} salt like $MnCl_2$.

The principle of adding Ca^{2+} to a Mn^{2+} based contrast medium may appear logic, but an improved safety may in theory be at the expense of efficacy. Thus elevation of extracellular

(ec) Ca^{2+} may impair the influx of Mn^{2+} into cardiac cells.[4,9] Whether this may be relevant for recently formulated [12,17] contrast media has not been reported on. The aim of the present study was therefore to assess the physiology and the efficacy of a new contrast medium based on a recommended Ca^{2+} to Mn^{2+} ratio of 10 : 1.[18] For the experiments we have used isolated perfused guinea pig hearts and recorded changes in cardiac physiology during repeated washin-washout of ascending concentrations of test substances. At end of experiments Mn metal content and T_1 relaxation parameters were measured in excised myocardial specimens.

MATERIALS AND METHODS.

In vitro relaxography.

Baseline information about proton relaxation was obtained with the two $MnCl_2$ based test substances *Manganese* and *Manganese-Calcium* (composition - see below) dissolved in pure N_2-equilibrated water and with relaxography (T_1 and T_2) carried out at 37°C and 20 MHz (see below). The below equations are for longitudinal (1) and transversal (2) relaxation.

$$M_z(t) = M_0 * \left(1 - 2 * \alpha * e^{-t/T_1}\right) \qquad \textbf{(Equation 1)}$$

$$M_{xy}(t) = M_0 * \left(e^{-t/T_2}\right) \qquad \textbf{(Equation 2)}$$

$M_z(t)$ is the instantaneous longitudinal magnetization, $M_{xy}(t)$ is the instantaneous transversal magnetization, M_0 is its Boltzmann equilibrium value, α is a correction factor for deviation from a perfect 180° pulse, T_1 is the longitudinal relaxation time, T_2 is the transversal relaxation time and t is the time constant between the 180° and 90° pulses for T_1 and the echo time for T_2. Relaxation rate constants $R_1=1/T_1$ and $R_2=1/T_2$ were calculated. Single component exponential regression analysis (Levenberg-Marquardt method) [19] of T_1 and T_2 showed that the test substances were dissolved in a homogenous solution, i.e. in a single compartment.

As seen in Figure 1a-b there were, as would be expected, no significant differences between *Manganese* and *Manganese-Calcium.* Molar relaxivity (r) values were: with *Manganese* 7.1 (s mM)$^{-1}$ for r_1 and 36.2 (s mM)$^{-1}$ for r_2; and with *Manganese-Calcium* 7.4 (s mM)$^{-1}$ for r_1 and 32.1 (s mM)$^{-1}$ for r_2. Relaxivity values were much similar to those recently obtained [9] with $MnCl_2$ at the same temperature and field strength (r_1 6.9 (s mM)$^{-1}$ and r_2 29.9 (s mM)$^{-1}$).

Experimental model.

Experiments were performed according to ethical guidelines for animal experiments. Male guinea pigs (340-435 g body wt.) were anesthetized by intraperitoneal injection of sodium thiopentone (100 mg/kg body wt.) and heparinized (Heparin 300 IU iv). Hearts were rapidly excised, weighed (1.6–1.7 g) and connected to a thermostated (37°C) Langendorff apparatus.[20] Temperature (37.0 ± 0.2°C) was measured in the right ventricle. Constant flow perfusion was provided by a peristaltic pump. Coronary flow rate (CFR) was 15.0 ml/min except for brief (5 min) exposures to test substances. These were infused into the aortic cannula at a flow rate of 0.5 ml/min and raised CFR to 15.5 ml/min. The perfusate was an oxygenated and pH (7.4) adjusted (95% O_2 and 5% CO_2) Krebs-Henseleit bicarbonate buffer (KHbb) [21] containing (mM): 118.5 NaCl, 25.0 $NaHCO_3$, 5.9 KCl, 1.2 $MgCl_2$, 1.5 $CaCl_2$, and 11.0 glucose. Hearts received washin perfusions with test substance followed by washout perfusions. At end of experiments ventricular specimens (~ 1.0 g) were quickly frozen and stored in liquid N_2 or were sampled fresh for proton relaxography.

Experimental design and groups.

All hearts were subjected to an initial 10 min control perfusion period. They were then subjected to five repeated perfusion sequences each consisting of a 5 min washin phase followed by a 10 min washout phase. In the first sequence all hearts received KHbb as the

inactive vehicle. In the following four washin phases (exposures 1-4) hearts were perfused with ascending concentrations of test substances or of KHbb. Three experimental groups (n=5) were included (N=15):

- *Control*, receiving KHbb during all washin periods.
- *Manganese*, receiving KHbb with $MnCl_2$ plus ascorbic acid during washin.
- *Manganese-Calcium*, receiving KHbb with $MnCl_2$ and Ca-gluconate in a 1:10 Mn^{2+} to Ca^{2+} ratio plus ascorbic acid during washin.

The test substances were made by dissolving the active ingredients in water and thereafter lyophilizing them as respectively *Manganese* and *Manganese-Calcium*. These were later dissolved in stock solutions containing: either 15 mM Mn^{2+} and 30 mM ascorbic acid (*Manganese*); or 15 mM Mn^{2+}, 150 mM Ca^{2+} and 30 mM ascorbic acid (*Manganese-Calcium*). Prior to the experiments dilutions were made in KHbb to yield these ascending [Mn^{2+}] in the perfusate: 10, 50, 100 and 500 μM.

Physiological parameters and analysis.

A water-filled latex balloon (0.15 ml) was placed in the left ventricle (LV) and connected to a transducer (SensoNor, Horten, Norway) for monitoring of: LV pressure (LVP), LV developed (systolic – diastolic) pressure (LVDP); first LVP derivative (LV dp/dt); and heart rate (HR). Signals were amplified, AD-converted and processed by computer (Quadbridge and MacLab units, AD Instruments Ltd, London, UK). Pressure-rate product (LVDP x HR) was calculated.

Biochemical parameters and analysis.

Frozen ventricular tissue was freeze-dried, powdered and analysed for Mn metal content by an inductive coupled plasma (ICP) technique, and for content of phosphocreatine (PCr) and

adenosine-triphosphate (ATP) by high performance liquid chromatography. Tissue contents were expressed in nmol/g dry wt. for Mn metal and in μmol/g dry wt. for PCr and ATP.

Relaxography of tissue specimens.

T_1 measurements. Immediately after end of perfusion ventricular specimens were excised, blotted and placed in a 10 mm NMR tube. Measurements at 37°C of T_1 were completed within 40 min using a 20 MHz Bruker Minispec spectrometer (Bruker AG, Rheinstetten, Germany). T_1 was measured by the inversion recovery (IR) method collecting at most 22 data points with inversion times (TI) spanning from 5 to 8425 ms logaritmically chosen. Data were fitted to a two component nonlinear least square regression curve [19], with one short and one long relaxation time component ($T_{1\text{-}1}$ and $T_{1\text{-}2}$). Apparent values for relaxation rate constants ($R_{1\text{-}1}$ and $R_{1\text{-}2}$) and population fractions (p_{01} and p_{02}) were calculated.

Analysis of T_1. The regression equation for T_1 (below) is:

$$M_z(t) = M_0 * \left\{ p_{01} * \left(1 - 2 * \alpha * e^{-t/T_{1-1}}\right) + p_{02} * \left(1 - 2 * \alpha * e^{-t/T_{1-2}}\right) \right\} \quad \textbf{(Equation 3)}$$

Eq. 3 contains four variables: M_0, p_{01}, $T_{1\text{-}1}$ and $T_{1\text{-}2}$. α is set to 1.0. The sum of p_{01} and p_{02} equals one (or 100%).

Relaxivity:

Relaxivity (r) is another parameter that describes the effectiveness of the contrast agent to reduce T_1 as a function of the concentration.[22,23]

$$R_i = \left(\frac{1}{T_i}\right)_{obs} = \left(\frac{1}{T_i}\right) + \left(\frac{1}{T_i}\right)_p \qquad i = 1,2 \qquad \textbf{(Equation 4)}$$

$(1/T_i)_{obs}$ is the observed relaxation rate constant (R_i) in the presence of a paramagnetic species, and $(1/T_i)$ is the (diamagnetic) relaxation rate constant in the absence of a paramagnetic species. $(1/T_i)_p$ represents the additional paramagnetic contribution.

In the absence of solute-solute interactions, the solvent relaxation rate constants are linearly dependent on the concentration of the paramagnetic contrast agent ([CA]), which is the case for the standards (above):

$$R_{i,obs} = R_i + r_i * [CA] \qquad \textbf{(Equation 5)}$$

r_i is the relaxivity defined as the slope of this dependence in units of $(s\,mM)^{-1}$ for in vitro experiments [24] and $(s\ nmol/g\ dry\ wt.)^{-1}$ for ex vivo heart experiments.

Two-site water exchange (2SX) analyses. In order to determine intrinsic relaxation values T_1 data were analysed according to a 2SX model as described in detail by Labadie et al.[24] and Landis et al.[25] for use with ec contrast agents and as recently employed by us [9] after washin-washout experiments with $MnCl_2$. In the former studies a stable gadolinium (Gd^{3+}) chelate was trapped in the ec space of various cell types. In the latter setting with ic Mn^{2+} the 2SX model was used to examine five intrinsic parameters, here named according to the terminology applied by Labadie et al.[23] and Landis et al.[24]: R_{1ec}, the ec relaxation rate constant; r_{1ic}, the ic relaxivity; R_{1ic0}, the ic relaxation rate constant at [Mn]=0 nmol/g dry wt.; τ_{ic}, the ic water lifetime; and, $p_{ic,}$ the ic population fraction. Our apparent $R_{1\text{-}1}$, $R_{1\text{-}2}$ and p_{02} data were accommodated by a simplex minimalization routine [19] which was modified to allow for locking of selected parameters and keeping them at constant values, by varying the five intrinsic 2SX model parameters.

Statistics.

Results are expressed as mean values in the text, as the mean ± SEM in figures and as the mean ± SD in tables. Comparison between groups was made by use of one-way ANOVA and subsequently by Fischer´s Protected Least Significant Difference (PLSD) for the physiology data and the Tukey post-hoc test for the relaxation data. Differences are noted as significant for $p < 0.05$.

RESULTS.

Physiology of hearts during Mn^{2+} washin and washout.

General information. No changes in HR or LVDP were observed in the three groups during the initial washin of vehicle only. This showed that a rise in CFR from 15.0 to 15.5 ml/min had no effect on cardiac function. Also, no significant changes occurred in LVDP or HR during the 4 repeated washin periods in the *Control* group (results not presented).

Manganese (Figure 2 a-c). Maximal LVDP depressions induced by the four ascending [Mn^{2+}] during washin were 4, 9, 17 and 53 %. Differences from control were significant for the two highest levels. LVDP recovery was rapid and complete during washout. HR decreased significantly by 18 % during with the highest [Mn^{2+}]. LVDP x HR was significantly reduced, by 20 % and 60 %, for the two highest concentrations tested.

Manganese-Calcium (Figure 2 d-f). Washin induced an immediate and significant positive inotropic response for all [Mn^{2+}] tested. Maximal LVDP elevations were by 13, 18, 25 and 56 %. In contrast to the *Manganese* group no significant changes in HR were observed. LVDP x HR was elevated significantly, by 10, 20, 25 and 59%. Recoveries of LVDP and LVDP x HR were rapid and complete during washout.

Biochemical analysis of ventricular tissue at end of experiment.

Tissue Mn content (Table 1). Analysis of freeze-dried and powdered samples revealed these values for Mn metal content (nmol/g dry wt.): *Control* < 40 (detection level); *Manganese* 3720; and *Manganese-Calcium* 1620. Intergroup differences were highly significant. When conversion factors were applied (tissue dry:wet wt. ratio of ~ 1:5, g/ml ratio of 1:1, and sampled ventricular mass ~ 1.0 g), estimates could be made of myocardial Mn^{2+} accumulation vs Mn^{2+} available from the test substances. With *Manganese* and *Manganese-Calcium* fractional accumulation rates were 2.3 % and 1.0 % , respectively.

Tissue PCr and ATP (Table 1). Analysis from tissue samples revealed PCr levels between 31 and 37 μmol/g dry wt. and ATP levels between 17 and 20 μmol/g dry wt. There were no significant intergroup differences.

Relaxography of ventricular tissue at end of experiments.

Tissue T_1 (Table 2). As recently shown by our group [9] T_1 was more adequately described by a biexponential equation, and an "average" T_1 could be dissolved in a short $T_{1\text{-}1}$ and a long $T_{1\text{-}2}$. Values in control hearts were: T_1-average 1031 ms; $T_{1\text{-}1}$ 596 ms; and $T_{1\text{-}2}$ 2017 ms. These values were reduced by 86, 92 and 66 % in the *Manganese* group and by 84, 86 and 49 % in the *Manganese-Calcium* group. Although there was a tendency to lower values for $T_{1\text{-}1}$ and $T_{1\text{-}2}$ with *Manganese*, differences between *Manganese* and *Manganese-Calcium* were not significant. Accumulated apparent values for populations fractions were about 55 % and 45 % for p_{01} and p_{02}, respectively.

2SX analysis (Table 3). The above and apparent relaxation values from all hearts in the study were pooled and employed for 2SX analysis [24,25]. Employing appropriate factors [9] for

conversion of tissue Mn in nmol/g dry wt. to Mn^{2+} in mM an ic relaxivity r_{1ic} of 31 $(s\ mM)^{-1}$ was found. The 2SX analysis revealed lifetimes for ic and ec water of 9.6 s and 5.9 s, and intrinsic population fractions for ic and ec water of 62 % and 38 %, respectively.

DISCUSSION.

Main findings.

Some main findings emerge from the present study:

- *Manganese* and *Manganese-Calcium* both affected cardiac inotropy, the first depressed contractility at the two highest concentrations (100-500 μM) whereas the latter induced a contractile overshoot for all four concentrations (10-500 μM) tested.
- Changes in contractility (*Manganese* and *Manganese-Calcium)* and chronotropy (*Manganese*) were rapidly reversed during the washout of both Mn^{2+} containing agents.
- There was an avid Mn^{2+} uptake in the isolated hearts, but tissue Mn^{2+} accumulation was 2.3 times more effective with *Manganese* than with *Manganese-Calcium*.
- High energy phosphate compounds were at the normal level for guinea pig myocardium [7] signifying that cell metabolism was well maintained in all three groups of hearts.
- Tissue T_1 was biexponential [9] and greatly shortened by both agents. As previously shown in excised rat heart specimens [9] lifetimes of ic and ec water were much longer than described as valid for perfused rat hearts.[26]

Mn^{2+}- Ca^{2+} relationships and cardiac physiology (Figure 3).

The present data are consistent with Mn^{2+} and Ca^{2+} ions competing for cell entry via slow Ca^{2+} channels [3-9] and, potentially also, via the Na^{+}-Ca^{2+} exchanger (NCX) in the sarcolemma.[4] The NCX is bidirectional with a main function in cell Ca^{2+} efflux at onset of diastole but with a secondary function assisting in cell Ca^{2+} influx at onset of systole. Mn^{2+} is known as an

inhibitor of both slow channels and NCX. As indicated in Figure 3, slow channel inhibition is mediated by a higher affinity for Mn^{2+} than for Ca^{2+} to channel binding sites which results in a preference for Mn^{2+} influx. Accordingly, Mn^{2+} entry may take place even when ec concentrations of Mn^{2+}, due to extensive plasma protein binding [13] are in the low μM range.[3-5] As shown in rat hearts,[5,14] about 30 μM seems to be a treshold for "free" Mn^{2+} above which transient Ca^{2+} channel occupancy by Mn^{2+} becomes so frequent and extensive that influx of trigger Ca^{2+} is gradually reduced. After cell Mn^{2+} uptake, an early washout phase involves residual ec Mn^{2+} plus an early cell Mn^{2+} efflux [3,5,7,9] most probably via NCX.[4] Cell retention properties reside first of all in an extensive Mn^{2+} binding to ic proteins, membrane phospholipid sites and small molecules keeping cytosolic Mn^{2+} at or below 1 μM.[27] Also mitochondrial storage [3] contributes to the apparently effective myocardial Mn^{2+} sink.

The most compelling in vivo evidence for validity of Mn^{2+}-Ca^{2+} competition has been delivered by Hu et al.[8] In anesthetized mice they showed a close concordance between positive (dobutamine) and negative (diltiazem) inotropic interventions during $MnCl_2$ infusion and recorded changes in MR signal intensity in the cardiac left ventricular wall. Furthermore, there were no apparent signs of heart failure (maintained left ventricular dimensions) during the 20 min period of Mn^{2+} washin.

Mn^{2+}- Ca^{2+} relationships with a new contrast medium (Figure 4).

The results from the present study can be interpreted in terms of the above Mn^{2+}- Ca^{2+} antagonism. As seen in Figure 4a we observed a mismatch between ec Mn^{2+} and ec Ca^{2+} during the washin perfusions. As expected with *Manganese* [5,7,9,10] contractile failure occurred with high perfusate [Mn^{2+}] (significant above 50 μM). Also as partly hypothesized [4,9] for *Manganese-Calcium,* contractile overshoot due to elevation of ec Ca^{2+} occurred with all

concentrations tested. Estimates reveal that during washin with *Manganese-Calcium* the perfusate Ca^{2+} concentrations rose from the control level of 1.25 mM to: 1.35 mM, 1.75 mM, 2.25 mM and 6.25 mM. These elevations of perfusate Ca^{2+} induced a transient cellular Ca^{2+} loading of an increasing intensity. Whereas arrhythmias or changes in HR were not observed and the recovery to normal contractility was rapid, it is fair to state that the high Ca^{2+} present did not confer any added benefit.

The potential safety issue with Ca^{2+} supplements may become more evident when known kinetics of Mn^{2+} containing agents in humans are considered. Thus Toft et al. showed [28] that iv injection (< 1 min) of 5 and 10 μmol/kg of MnDPDP in human volunteers raised plasma Mn metal concentrations to about 30 and 90 μM, respectively. Parallel elevations in plasma Ca^{2+} can be estimated to be 300 and 900 μM if the same MnDPDP had been supplemented with Ca^{2+} like in the present experiments. If one also considers even higher doses for rapid injection of *Manganese-Calcium* type media and the clinical use in patients with ischemic heart disease, it is likely that instead of improving safety the present high Ca^{2+} additive may promote cardiac side-effects. As also seen in Figure 4 a further implication of a Mn^{2+}-Ca^{2+} mismatch with *Manganese-Calcium* concerns the considerably lower cellular Mn^{2+} uptake and retention than seen with *Manganese.* A likely mechanism is that elevation of ec Ca^{2+}, at least with the highest (100-500 μM) concentrations tested have raised too far the gradient of Ca^{2+} to Mn^{2+} at critical slow channel binding sites and thus reduced the effective cell Mn^{2+} influx. Such an interpretation is supported by recent findings [9] in ex vivo rat hearts in which elevation or reduction of perfusate Ca^{2+} during infusion with $MnCl_2$ respectively lowered and raised the tissue Mn^{2+} uptake.

The experimental findings indicate that the 10:1 ratio for Ca^{2+} to Mn^{2+} used here was rather extreme. However, the ratio was chosen with due reference to the recent patent by Harnish et al.[18] which describes new contrast media for MRI of heart and other organs. In this patent the following molar ratios were claimed for Ca^{2+} to Mn^{2+}: from 2:1 to 40:1; preferably from 4:1 to 20:1; and, most preferably from 8:1 to 10:1. These ratios are far above what has been referred to (Ca^{2+} to Mn^{2+} 1:1) in an earlier paper by Schaeffer et al.[29] The patent excludes the use of chelates and is based on easily dissociating Mn^{2+} salts and various anions including gluconate. In a recent paper Storey et al.[12] presented preliminary results with EVP 1001-1 (Eagle Vision Pharmaceutical Corporation, Exton, PA) a contrast medium developed with background in the above cited patent. Basic principles behind EVP 1001-1 were presented, but its formulation was not disclosed and no recordings of physiology were presented. This lack of essential information makes it difficult to apply our findings on safety and efficacy to this particular paper which dealt with single pig heart experiments and use of EVP-1001-1 for perfusion MRI and MRI of ischemic myocardium.

Our findings of reduced efficacy with a *Manganese-Calcium* type medium confirm recent findings in in vivo rats [30] subjected to coronary artery ligation and after one week to late-enhancement MRI of myocardial infarction with use of contrast media containing gadolinium or Mn^{2+}. Two groups of rats received 1 min rapid infusion of 15 μmol/kg of either $MnCl_2$ (*"Manganese"*) or $MnCl_2$ with Ca-gluconate (*"Manganese-Calcium"*) in a Ca^{2+} to Mn^{2+} ratio of 8:1. In normal myocardium peak elevations of the longitudinal relaxation rate constant (ΔR_1) were 1.33 s^{-1} with *"Manganese"* but only 0.46 s^{-1} with *"Mangenese-Calcium"*. The ΔR_1 reduction with the 8:1 Ca^{2+} to Mn^{2+} ratio was inferred as the result of Ca^{2+}- Mn^{2+} competition for cell uptake, and reflects in magnitude closely the reduction in tissue Mn content with the 10:1 ratio employed in our experiments. In the same study the

potential superiority of Mn^{2+} releasing contrast media in predicting infarct size was well documented.

MR relaxography.

Relaxography and 2SX analysis of myocardial tissue specimens showed much similar results in the present guinea pig hearts as in previously studied rat hearts.[9] Accordingly, two-component T_1 behaviour was present with short ic $T_{1\text{-}1}$ and long ec $T_{1\text{-}2}$ and population fractions compatible with ic and ec water.[31] A main finding was that ic Mn^{2+} ions most probably due to ic protein binding [32] presented a high ic r_{1ic} of 31 $(s\ mM)^{-1}$, that is 4-5 times higher than shown for Mn^{2+} ions in vitro. However, r_{1ic} was considerably lower than found (60 $(s\ mM)^{-1}$) in rat hearts.[9] Likely explanations are that with the much higher tissue Mn contents (1620-3720 vs 500-700 nmol/g dry wt.) in the present than in the previous study, longitudinal relaxation was almost fully saturated ($T_{1\text{-}1}$ reductions by 85-92 %) and that T_1 relaxation data with lower and intermediate Mn contents are lacking in the present study. Another main finding was that of a slow water exchange regime as recently observed in rat cardiac tissue.[9] Thus long lifetimes for ic (~ 10 s) and ec (~ 6 s) water were found in the excised ventricular specimens. These values are much higher than reported or assumed in key references on myocardial perfusion.[26, 33] Whether these marked discrepancies relate to differences in experimental-analytical conditions, that is to results obtained with direct MR relaxography of excised cardiac tissue vs results obtained from secondary image analysis of perfused hearts, remains to be seen.

Methodological considerations.

The study was performed in a crystalloid perfused guinea pig heart and qualifications have to be taken before transferring obtained results to a clinical situation in humans. The strength of

the model are the possibilities to highlight in detail basic physiology, biochemistry and MR relaxography. This was demonstrated by the parallel recording of safety and efficacy aspects. Since species differences in cardiac handling of Ca^{2+} are small between guinea pig and man and Mn^{2+}-Ca^{2+} competition seems to be a general biological phenomenon [4], the basic observations and interpretations have therefore to be regarded as valid.

At least two important differences from in vivo conditions may have influenced our results. Firstly, in vivo plasma protein binding [13] would have limited ec Mn^{2+} activity to a low μM level and almost halved the Ca^{2+} present. This factor would have slowed contractile responses of both Mn^{2+} containing test substances in vivo. In the experiments we used a model with 4 repeated washin-washout perfusions with ascending concentrations of Mn^{2+} containing media. Whereas only the lowest concentrations are likely in plasma under in vivo conditions, the higher concentrations underlined the basic Mn^{2+}-Ca^{2+} mismatch with *Manganese-Calcium*. Also with *Manganese* the ascending concentration model showed that a cardiodepressive effect which might possibly justify a well balanced Ca^{2+} additive to $MnCl_2$, was first observed at or above 50 μM.

The other main difference from in vivo conditions was the absence of autonomic control. The negative inotropic response here observed with *Manganese* may have been masked in vivo by sympathetic stimulation as previously shown in conscious dogs.[14] Furthermore, it is more likely that potential activation of sympathetic reflexes by $MnCl_2$ is induced by nitric oxide mediated vasodilation [34] and not by a more distant tendency to cardiodepression.

Conclusions.

The present study indicates that two of the three strategies to improve the safety during intravenous injection or infusion of Mn^{2+} containing contrast media, seem to be inadequate. Thus with pure $MnCl_2$ and rapid release of Mn^{2+} vasodilation and associated phenomena like flushing and, in accidental situations with a nonintended rapid injection of a too high dose, the plasma capacity for Mn^{2+} binding and the autonomic defense may be overwhelmed with ensuing hypotension and heart failure. However, with Ca^{2+} supplemented Mn^{2+} salts the issues are twofold and conflicting: firstly to raise ec Ca^{2+} with the intention to preserve a hardly threatened contractile function; and secondly to avoid that high ec Ca^{2+} reduces cell Mn^{2+} uptake and impairs ic relaxation properties. It is therefore the authors' view that the use of slow-to-intermediate Mn^{2+} releasing chelates is a more attractive principle and should be advanced towards clinical use. In this context, recent experience in human volunteers with a low dose of MnDPDP for myocardial T_1 relaxation enhancement [35] bears promise for future MnMRI in patients with ischemic heart disease.

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Table 1. Tissue content of Mn, PCr and ATP

Test substance	n	Mn (nmol/g dry wt.)	PCr (µmol/g dry wt.)	ATP (µmol/g dry wt.)
Control	5	<40	34.12 (7.08)	17.09 (2.82)
Manganese	5	3720 (500)[*]	36.69 (2.54)	19.76 (1.94)
Manganese-Calcium	5	1620 (160)[*†]	31.36 (8.10)	20.47 (1.60)

PCr indicates phosphocreatine; ATP indicates adenosine triphosphate. Mean (SD) values are presented.
[*]Significantly different from *Control*, [†]Significantly different from *Manganese*.

Table 2. Tissue T_1 relaxation parameters: apparent values

Test substance	n	T_1 (ms)	$T_{1\text{-}1}$ (ms)	$T_{1\text{-}2}$ (ms)	p_{01} / p_{02} (%)
Control	4	1031 (45)	596 (61)	2017 (348)	46 / 54 (6)
Manganese	5	159 (95)*	47 (8)*	683 (339)*	59 / 41 (7)
Manganese-Calcium	4	162 (34)*	83 (10)*	1025 (298)*	61 / 39 (9)*

T_1 indicates the average longitudinal relaxation time; $T_{1\text{-}1}$ is the short component of T_1; $T_{1\text{-}2}$ is the long component of T_1; p_{01} is the ic population fraction; p_{02} is the ec population fraction. Mean (SD) values are presented. *Significantly different from *Control*, †Significantly different from *Manganese*.

Table 3. Intrinsic values from 2SX analysis of pooled T_1 relaxation data

R_{1ec} (s^{-1})	r_{1ic} (s nmol/g dry wt.)$^{-1}$	R_{1ic0} (s^{-1})	τ_{ic} (s)	τ_{ec} (s)	p_{ic} / p_{ec} (%)
0.44	0.00627	1.15	9.6	5.9	62/38

R_{1ec} is the ec relaxation rate constant; r_{1ic} is the ic relaxivity; R_{1ic0} is the ic relaxation rate constant at $[Mn^{2+}]$ = 0 nmol/g dry wt.; τ_{ic} is the ic water lifetime; p_{ic}/p_{ec} is the ic/ec population fraction; τ_{ec} is the ec water lifetime. τ_{ec} was calculated from the following formula: $\tau_{ec} = \tau_{ic} * (1 - p_{ic})/ p_{ic}$.

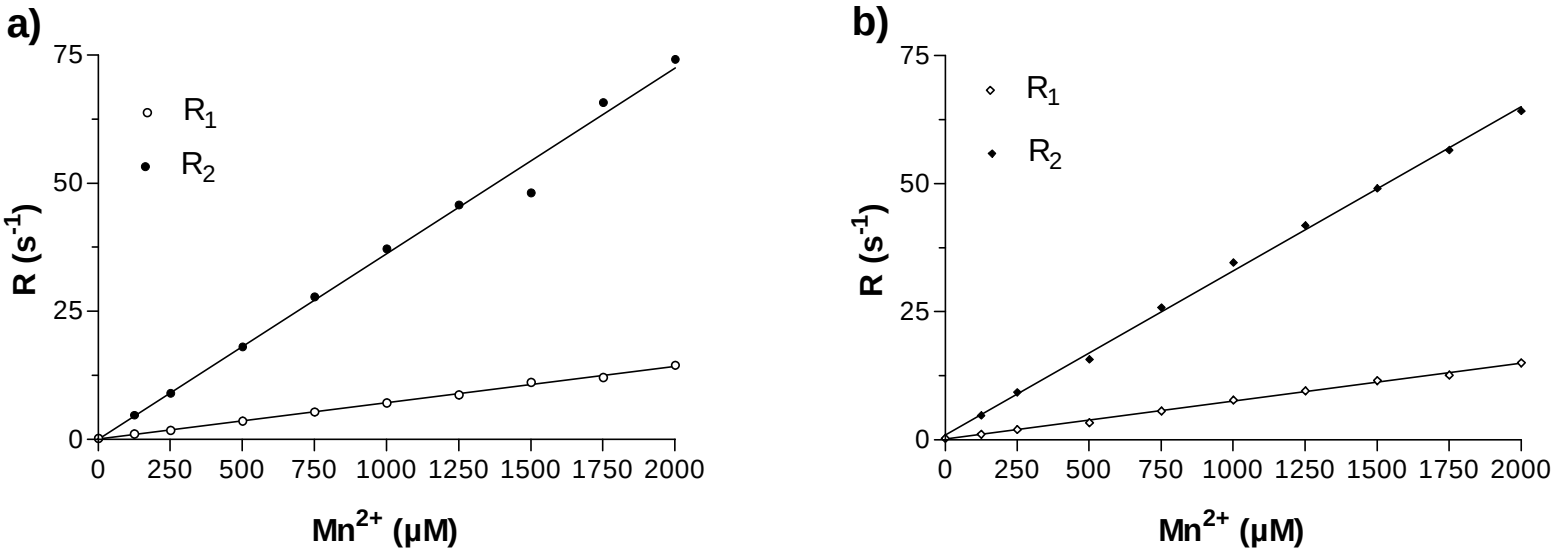

Figure 1. *In vitro* experiments: Relationship between $MnCl_2$ concentrations with (a) *Manganese* and (b) *Manganese-Calcium* in homogenous solution (water) and proton relaxation rate constants R_1 and R_2. Relaxivity values with *Manganese*: $r_1 = 7.1$ (s mM)$^{-1}$ and 36.2 (smM)$^{-1}$. Relaxivity values with *Manganese-Calcium*: $r_1 = 7.4$ (s mM)$^{-1}$ and 32.1 (s mM)$^{-1}$.

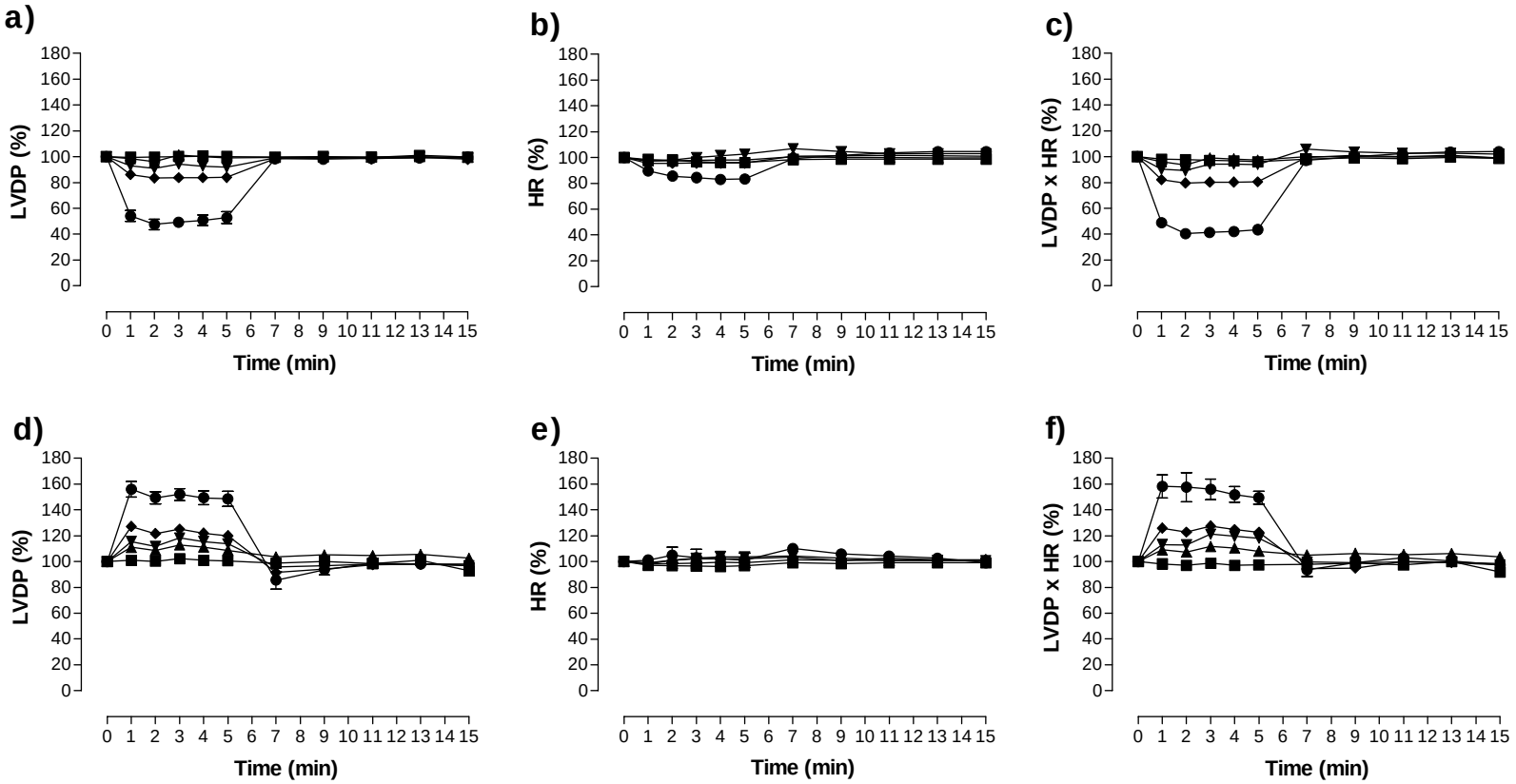

Figure 2. Changes in physiology during (5 min) and following (10 min) infusions of ascending concentrations of *Manganese* (a-c) and *Manganese-Calcium* (d-f).Values are expressed in % of those obtained at end of the initial control period. Symbols were: ■ 0 μM, ♦ 100 μM, ▼ 50 μM, ▲ 10 μM, ● 500 μM $MnCl_2$.

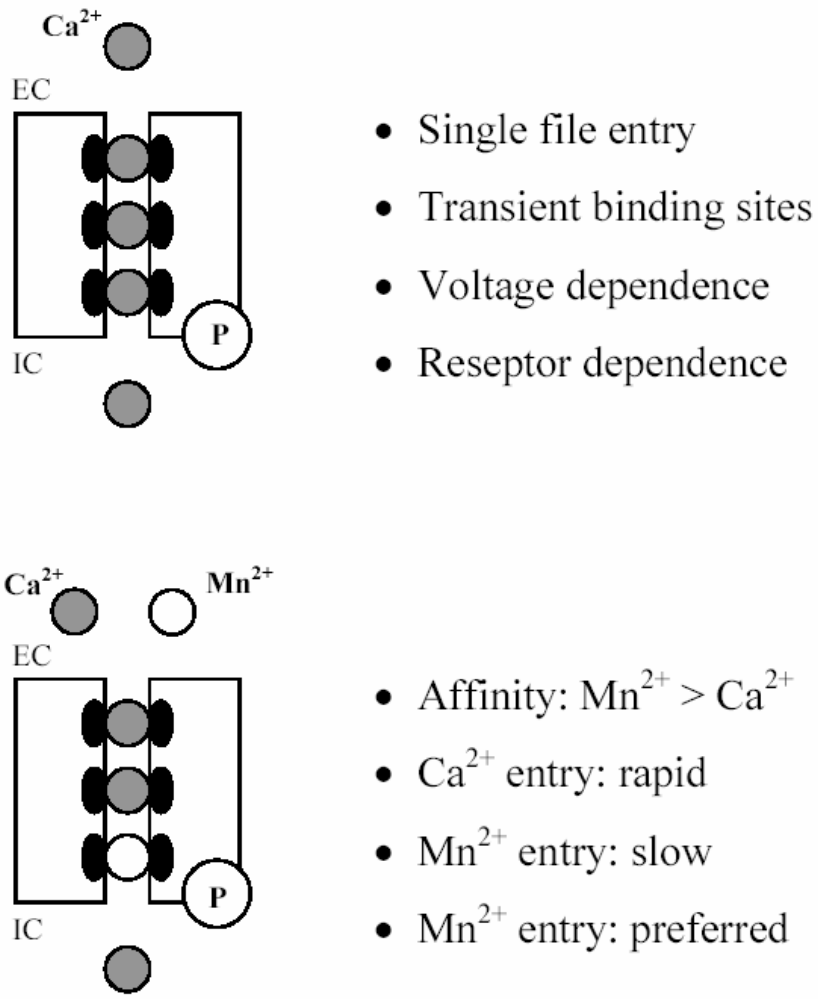

Figure 3. Slow Ca^{2+} channel function (upper panel) and Mn^{2+}- Ca^{2+} competition (lower panel). Depolarization of the sarcolemma and electrochemical gradients leads to entry of (trigger) Ca^{2+} through (voltage operated) slow channels[4]. Ca^{2+} ions move from the extracellular (ec) to the intracellular (ic) space in a single file from successive binding sites which also function as a selectivity filter. Trigger Ca^{2+} induces release of main contractile Ca^{2+} from ic storage sites (sarcoplasmic reticulum). Phosphorylation (P site) by adrenergic stimuli increases the number of active slow channels and enhances Ca^{2+} influx. Mn^{2+} has an intermediate affinity to channel binding sites: higher than for Ca^{2+}, but not too high inducing a permanent blockade. Accordingly, Mn^{2+} is a more preferred species for entry but the influx rate is slower than seen with Ca^{2+}. These overall properties explain why μM Mn^{2+} is able to compete with mM Ca^{2+} for cell influx. They also explain why inhibition of Ca^{2+} influx requires the transient Mn^{2+} occupancy of a considerable number of slow channels. Conversely, a marked rise in ec Ca^{2+} may reduce Mn^{2+} influx. Assumedly[4] a similar binding pattern and Mn^{2+}- Ca^{2+} competion exists for the sarcolemmal Na^{+}- Ca^{2+} exchanger (NCX).

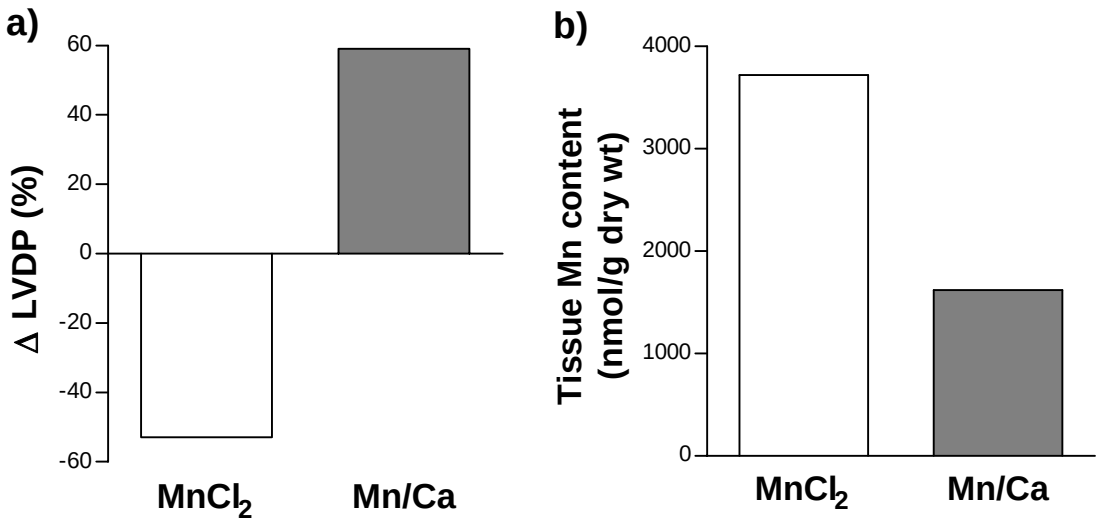

Figure 4. Physiology vs efficacy with *Manganese* and *Manganese-Calcium.* Changes in LVDP during Mn^{2+} washin (a) and the tissue Mn content (b) at end of experiments are compared.