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Research Article

Manganese-enhanced MRI: Comparison of agents in the rat pancreas

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ABSTRACT

Mangafodipir was approved for use as an MRI contrast agent in the late 1990s for liver and pancreas imaging but it was removed from the European market for commercial reasons in 2012. Previously, preliminary work in mice and in diabetic patients showed that Mn^{2+} ions could be used as a contrast agent to monitor the function of insulin-producing β-cells by acting as a calcium analogue. Clinical translation of this work was hampered by a lack of available Mn contrast agents, but both mangafodipir and Mn gluconate are currently being used in clinical trials.

As a first step towards using Mn in diabetic patients to monitor treatment or disease progression, we imaged the pancreas of healthy rats using mangafodipir, Mn gluconate and Mn chloride (as a control). The hypothesis was that Mn gluconate produces pancreatic enhancement similar to that seen previously with mangafodipir and Mn chloride, with greater enhancement following glucose challenge vs saline challenge. 18 Wistar rats were imaged at 7 T and normalised plateau pancreatic enhancement over baseline was compared for saline vs glucose challenge, calculated from a sigmoid fit to the enhancement curve. For saline vs glucose challenge, mean increases in plateau height ± sd were: 22 ± 18% for Mn chloride, 31 ± 29% for mangafodipir and 41 ± 17% for Mn gluconate. A paired t-test indicated that enhancement was greater for glucose vs saline ($p=0.01$) and that there was no significant difference in the percentage enhancement between any of the compounds ($p>0.2$). In conclusion, all three contrast agents produced similar enhancement, with greater plateau height under glucose challenge vs saline challenge. Mangafodipir and Mn gluconate show potential for translation into a clinical study investigating beta cell imaging of the pancreas in type 1 diabetes mellitus and type 2 diabetes.

Introduction

Manganese ions (Mn^{2+}) are strongly paramagnetic, and have been used in magnetic resonance (MR) contrast agents since the 1980s [1]. In the pancreas, Mn^{2+} ions can act as a calcium analogue and enter insulin-producing β-cells through voltage-gated calcium channels [2]. Type 1 diabetes mellitus is an autoimmune disease that results in the gradual destruction [3–5] of these insulin-producing β-cells, therefore T_1 enhancement with Mn chloride has been proposed as a method for evaluating β-cell mass and function in mice [6–9]. In humans, one study

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has investigated Mn-enhanced MRI in patients with type 2 diabetes [10], a disease characterised by relative insulin deficiency and diminished β-cells [5]. This study showed that Mn-enhanced imaging could be used to distinguish normoglycaemic from type 2 diabetic patients by comparing T_1w signal enhancement in the pancreas.

Further work in this area has been limited by a lack of clinically available Mn-based contrast agents, but a Mn agent previously marketed for use in liver and pancreas imaging in humans (mangafodipir) as well as a new agent being evaluated in humans for cardiac applications [11] (Mn gluconate) are both licensed for human usage. In both cases, Mn^{2+} ions are the source of signal enhancement, but the delivery of unchelated Mn^{2+} (as in the case of manganese chloride) depresses myocardial function, reduces heart rate and causes vasodilation [12], making it unsuitable for use as a routine clinical agent. Mn gluconate was developed to mitigate this by simultaneously providing cardioprotective calcium gluconate in the Mn^{2+} solution [13]. In the case of mangafodipir, the Mn^{2+} ions are chelated with dipyridoxyl diphosphate (DPDP), but approximately 80% of Mn^{2+} ions dissociate from the DPDP molecule allowing entry into cells [14]. It is unclear how these different formulations might impact on enhancement of the pancreas. Whilst not currently being marketed at the time of this study, the agents are available to be manufactured and used in humans. Before using one of these agents in a study of diabetic patients, we wanted to confirm the equivalence of Mn gluconate and mangafodipir (the agent used in the previous human study) [10] as a pancreatic contrast agent in healthy rats subject to a glucose challenge, using Mn chloride as a control. This work was previously presented at the ISMRM 2018 Annual Meeting [15].

Materials and methods

Ethics statement

All animal studies were ethically reviewed by the University of Edinburgh Animal Welfare and Ethical Review Board and carried out in accordance with the Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.

Animals and diet

Eighteen male adult Wistar rats 12-14 weeks old with a mean ± SD weight of 280 ± 22 g were housed four per cage under a standard 12 hr light/dark cycle at a constant temperature (24 ± 2 ˚C) with *ad libitum* access to standard chow diet and water. After two weeks of acclimation, rats were fasted overnight, and tail venous blood glucose levels were measured by OneTouch glucometer before each scan. At the end of the first imaging session the rats were placed in an incubator under close observation until they regained consciousness (within 30 min). After the second imaging session they were euthanised.

Image acquisition

Each rat (18 Wistar rats in total) underwent two imaging sessions 1-2 weeks apart at 7 T (Agilent Technologies, Santa Clara, USA). In each imaging session, the animals were weighed and anaesthetised using Isofluorane (4% in O_2 for induction and 1.5-2% in air:O_2 (50:50) for maintenance). The tail vein was cannulated and the animal was placed in the magnet using a volume coil for signal reception. Respiration, heart rate and temperature were continuously monitored. The imaging protocol included anatomical T_1w and T_2w multislice TSE (1.5 mm slice thickness). These images were used to plan a coronal slice through the pancreas for the dynamic series, which was a respiratory-gated 2D T_1w spoiled gradient echo acquisition (TR=100 ms, TE=1.35 ms, α=60˚, slice thickness=2 mm, matrix=128x128, FOV=60 mm). After approximately 10 dynamic images, either saline (first imaging session, 2 ml/kg) or glucose (second imaging session, 50% glucose, 2 ml/kg, i.e. 1g/kg body weight glucose) was injected using a syringe driver over 1 minute, followed after 2 minutes by the contrast agent injection. Each subject received either Mn chloride, (100 μmol/kg, 6 rats), Mn gluconate (100 μmol/kg, 7 rats) or mangafodipir (125 μmol/kg, 5 rats). A higher dose of mangafodipir was used to ensure an equal amount of Mn ions available for uptake, because 80% dechelation occurs after injection [14] . In each case, the contrast agent was injected over 20 minutes using an infusion pump, and dynamic imaging continued for 40 minutes after the Mn injection was complete, making a total of approximately 60 minutes (140-180 frames).

Image analysis

For each visit, the anatomical images were used as a guide to outline the pancreas and the liver on the dynamic image stack, avoiding large vessels in the liver. The mean signal in the pancreas and liver regions was calculated and plotted vs time, then the mean signal in the baseline was subtracted from the whole curve. A sigmoid function (Eq. (1)) was fitted to each curve using python (ver 3.6.0) and the scipy module (1.1.0):

$$y = \frac{A}{\left(1 + e^{-b(t-t_0)}\right)} \tag{1}$$

Where y is the baseline-subtracted signal intensity, A is the plateau value, b is the slope, t is the timepoint number and t_0 is a time offset parameter. The free parameters in the fit were A, b and t_0, and uncertainties in the fitted parameters were calculated from the fit covariance matrix.

The fitted plateau height for the pancreas was normalised by dividing by the plateau height for the liver [6], and this normalised plateau height was compared for glucose vs saline challenge over all agents using a paired t-test. The percentage difference in normalised plateau height for saline and glucose challenges was compared between agents using an unpaired t-test. A p value of <0.05 was considered significant.

Results

One rat died in an early experiment where Mn chloride was infused over 10 minutes, leading to the 20 minute infusion used for all subsequent experiments. All other rats appeared fit and healthy after the first imaging session. Five rats were removed from analysis due to technical issues with the injections or imaging in either the saline or the glucose examinations, leaving 12 complete datasets (3 Mn chloride, 5 mangafodipir, 4 Mn gluconate). The mean time between the two imaging sessions was 8.8 days, and mean blood glucose was 6.6 ± 0.7 mmol/l before scan 1 (saline infusion) and 6.4 ± 0.8 mmol/l before scan 2 (glucose infusion), within normal limits.

Fig. 1 shows example images, normalised pancreatic enhancement curves and fits from one subject. Fig. 2 shows sigmoid fit plateau height within the pancreas normalised to that from the liver for saline and glucose challenges for each rat. A paired t-test for saline vs glucose normalised plateau height showed a significantly greater enhancement during glucose challenge ($p = 0.0004$), with only one rat showing smaller plateau enhancement with mangafodipir.

For glucose vs saline challenge, the mean percentage increases in plateau height ± sd were: 22 ± 18% for Mn chloride, 31 ± 29% for mangafodipir and 41 ± 17% for Mn gluconate, with no difference between these values (t-test, $p > 0.2$).

Discussion

Manganese-based contrast agents were proposed in the 1980s, and have been used in humans in a limited number of applications (Liver [16], cardiovascular [17,18], Pancreatic masses [19]). Mangafodipir was used in a study of diabetes [10] where T_1w signal enhancement

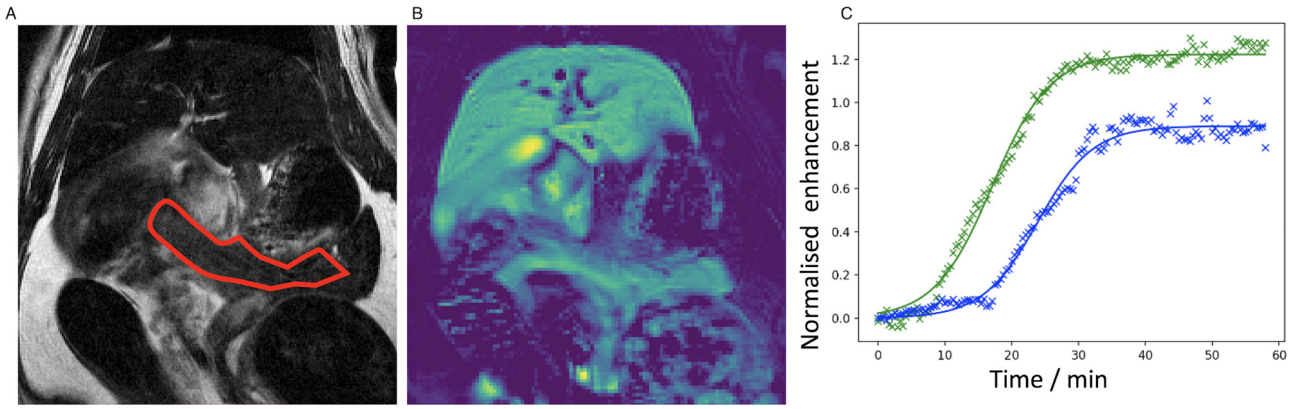

Fig. 1. Example images and curves. (A) example anatomical T2w image with pancreas outlined in red, (B) subtraction image for pre vs post contrast dynamic image, (C) enhancement curves (normalised to liver plateau value) from one subject. Blue curve – saline challenge, green curve - glucose challenge.

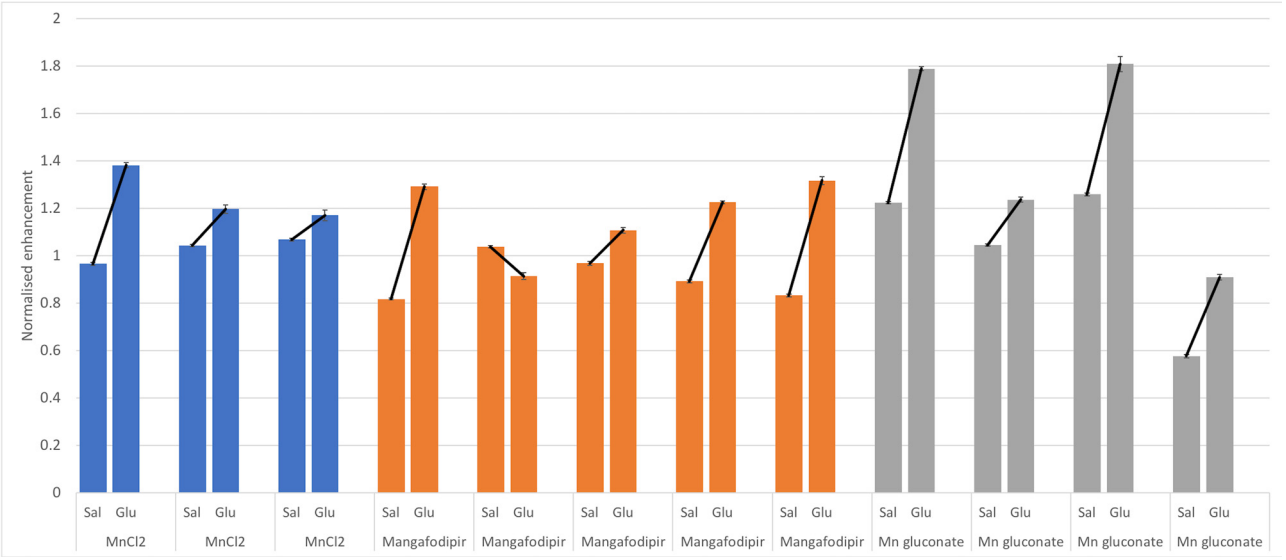

Fig. 2. Plateau height normalised to liver for saline and glucose challenges. For each subject, Sal indicates saline challenge, Glu indicates glucose challenge. Error bars show parameter uncertainty calculated from fit covariance matrix

could distinguish normoglycaemic patients from those with type II diabetes, but it was subsequently removed from the market for commercial reasons. Manganese gluconate is currently undergoing clinical trials (NCT01989195) and in our institution it is currently being clinically evaluated for use in the assessment of myocardial viability [11].

In a preclinical setting, Mn-based contrast agents have been used to study murine diabetes models, showing decreased Mn-induced signal enhancement in the pancreas of diabetic mice [6] that correlated with loss of beta-cell mass [9]. Further studies used change in relaxation rate [8] and modelling of intracellular relaxation rates [7] to quantify loss of beta cell mass and function.

As a first step towards continuing this work in humans with diabetes with either the previously-used mangafodipir or Mn gluconate, we aimed to examine whether these agents resulted in similar enhancement in the rat pancreas. Our results show that there is greater enhancement in the rat pancreas with Mn-based contrast agents after glucose challenge vs saline challenge, as predicted, and that Mn chloride, mangafodipir and Mn gluconate all result in similar increases in enhancement for glucose challenge vs saline challenge. It is unclear why one rat showed a smaller enhancement on glucose with mangafodipir. Our finding of a 20-40% change of normalised plateau signal enhancement between saline and glucose challenges is of similar range (50%) as found by Antkowiak et al [6], though this was in mice rather than rats and would depend heavily on injection (intraperitoneal in these mice) and imaging protocols.

The limitations of this study are that the number of subjects in each group was small, outlining the pancreas was challenging in some cases, and we used signal enhancement as a surrogate for change in T_1 rather than measuring it directly. In addition, the amount of pancreas included in the dynamic imaging slice (and hence the amount noise and partial volume effect) varied between subjects. Despite the small number of subjects and difficulty in outlining the pancreas, we still saw significantly larger enhancement in the pancreas for glucose vs saline challenge. In future work, a direct measurement of the change in T_1 could provide more robust quantification of enhancement [8].

An obvious next step would be to apply these contrast agents in a rat model of diabetes. However, our increasing experience with mangafodipir in human cardiovascular imaging has led us directly to human studies of diabetes and we feel that it would be unnecessary and unethical to continue preclinical work.

Conclusions

This study has shown that, in healthy rats, Mn gluconate produced pancreatic enhancement similar to the Mn contrast agent used previously in humans with type 2 diabetes (mangafodipir). This was a first

step in verifying its potential as a marker of beta cell function, which will be the focus of future clinical studies of type 1 and type 2 diabetes at our centre.

Sources of funding

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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