Iron-induced myocardial injury: an alarming side effect of superparamagnetic iron oxide nanoparticles

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Introduction and background

Superparamagnetic iron oxide nanoparticles (SPION), as magnetic resonance (MR) imaging contrast agents or magnetic targeting carriers, have potential applications in diagnostics, imaging, cell and drug/gene delivery for cardiovascular diseases. SPION are highly magnetic particles that cause magnetic field perturbations, which can be identified on T2* weighted images [1]. Clinically, SPION allows non-invasive detection of the region of myocardial infarction and the peri-infarct area based on a multiparametric cardiovascular MR approach [2, 3], characterization of acute MI pathology by detecting infiltrating macrophages and altered perfusion kinetics [4] and non-invasive visualization of the aorta and aortic diseases [5]. Preclinically, a large number of animal studies have been performed with SPION and cardiac magnetic resonance imaging to deliver, track or determine the efficacy of stem cell therapy in the heart in the past 10 years [1]. More recently, magnetic targeting has emerged as a promising and novel strategy for ischaemic heart disease [6–10], in which SPION can direct drugs, genes or cells to the target site under a magnetic field gradient.

Superparamagnetic iron oxide nanoparticles’ biocompatibility with the target organ is the first prerequisite for clinical translation, and iron oxide nanoparticles have long been believed to have low toxicity and are well-tolerated in the human body. However, with the expanding application of SPION, toxic effects, such as oxidative stress and inflammatory reaction, have increasingly attracted attention. Iron oxide nanoparticles accumulate in lysosomes (following cellular internalization), in which the low pH breaks the iron oxide core down into iron ions. It has been reported that iron oxide nanoparticle inhalation exposure may induce lung cytotoxicity via oxidative stress and biphasic inflammatory responses in Wistar rats [11]. In vitro studies have also suggested that iron oxide nanoparticles mediate activation of microglia in the brain [12] and differentiation of blood mononuclear cells into pro-inflammatory macrophages to secrete higher levels of pro-inflammatory cytokines [13]. In addition, iron oxide particles stabilized with coatings, such as dextran or citric acid, induced toxic effects on the behaviour and function of endothelial cells [14–16] and activated the expression of genes related to oxidative stress [17]. Moreover, the oxidative injury caused by SPION can be suppressed via antioxidant poly (trollox) nanoparticles binding to and internalizing in endothelial cells [16]. Thus, could the invasion of SPION produce similar side effects in the myocardium?

Iron oxide nanoparticles with systemic administration were mainly cleared by the reticuloendothelial system and renal excretion, resulting in cytopathological effects on the lungs, liver and kidneys, while the heart and brain remain free from adverse effects because of limited iron deposits [18]. A recent clinical study also showed that a single dose of intravenous iron oxide administration has a beneficial effect on LV remodelling in patients with acute ST-elevation myocardial infarction [19], in which the underlying iron deficiency with a decline in iron circulating levels was reported [20]. However, this situation is quite different from local delivery of SPION-mediated therapeutic agents (stem cells, gene or drug) in the treatment of ischaemic heart disease. First, intramyocardial injection of SPION-mediated agents contains large amounts of iron oxide nanoparticles, and the local quantity of SPION deposition in the myocardium is higher than that reported in previous intravenous studies [21–23], in which SPION was administered systemically and proved to be a relatively safe and efficient MR contrast agent. Second, the heart is not a monocyte-macrophage organ, and iron clearance occurred more slowly in the heart than in the liver [24]. Thus, it is difficult for macrophages to migrate away from the massive SPION introduced by SPION-mediated agents. Moreover, SPION-mediated therapeutic agents target the ischaemic or injured lesion rather than the normal myocardium. Thus, the injected SPION easily accumulates in situ for a prolonged period of time due to the lack of blood flow and mechanical...
contraction in the ischaemic or necrotic region [24, 25]. Magnetic
resonance monitoring of SPION-containing stem cells in an animal
model of myocardial infarction demonstrated that the iron particles
could persist in the infarct lesion for several months [25, 26]. Third,
this situation is even worse in the context of SPION-based magnetic
targeting therapy introduced in cardiovascular diseases [6, 7, 27].
Magnetic attraction could attenuate the loss of SPION-containing
therapeutic drugs/cells via venous drainage, and subsequently
increase the heart stay by approximately 3–10-fold [7]. SPION may
accumulate in the ischaemic myocardium in a highly clustered fash-
ion when employed as magnetic carriers in targeting therapy. Thus,
local delivery of SPION-mediated therapeutic agents might induce
myocardial iron overload, particularly in the setting of myocardial
infarction or magnetic targeting.

Another important question is whether SPION accumulation
has toxicity effects on ischaemic myocardium. Although there is
little information concerning the biological effects of SPION on
myocardial tissues, the myocardium toxicity of excess non-SPION
iron have been extensively explored. First, both primary haemo-
chromatosis (a genetically determined condition resulting in iron
overload) and secondary hemochromatosis (such as repeated
transfusion, thalassaemia or sickle cell anaemia) can result in iron
overload cardiomyopathy, with the pathogenic mechanism of that
myocardial iron overload induces the formation of reactive oxygen
species (ROS) via the Fenton reaction [28, 29]. The myocardium
is one of the most sensitive tissues to iron, as demonstrated by
the fact that myocardial injury and heart failure are a common
presentation of hemochromatosis [24]. In chronic iron overload,
iron toxicity is dose-dependent [30]. Second, recent studies have
demonstrated that haemorrhagic myocardial infarction can result in
local iron depositions within the infarct zones, which can be a
source of prolonged inflammatory burden in the chronic phase of
myocardial infarction, most likely resulting in LV negative remodelling
[31] and ventricular arrhythmias [32]. Third, acute myocardial
ischaemia (specifically after reperfusion) can generate ROS via
activation of the oxidative stress system [33] and then directly
injuring the cell membrane of cardiomyocytes and induce cell
death [34]. SPION deposition might further enhance oxidative
stress levels in ischaemic myocardium, thereby promoting more
cardiomyocyte death.

The free radical-mediated pathway is the principal mechanism of
iron toxicity in cardiomyocytes [35]. Iron can be taken up by ventricu-
lar myocytes via the sarclemmal L-type Ca2+ channel [36] in a dose-
and time-dependent manner [37]. Iron excess produces highly toxic
hydroxyl radicals via the Fenton-catalysed Haber-Weiss reaction,
which damages the lipid-rich cell membrane, and is known as lipid
peroxidation. Cellular lipid peroxidation produces polyunsaturated
fatty acids and increases toxic aldehydes. The aldehyde products can
form a covalent link to proteins (aldehyde-protein adducts), rendering
the loss of cell membrane integrity. Structures located on the cell
membrane, such as Na⁺-K⁺ ATPase and 5'-nucleotidase, were
affected thereafter. Oxidative stress-mediated iron toxicity also affects
other cellular organelles and their functions. Consequently, iron-
induced myocardial injury occurred.

Hypothesis

Based on the available studies, it is logical to assume that local
myocardial delivery of SPION-mediated therapeutic agents might
produce myocardial iron overload, resulting in deterioration of myo-
cardial injury and exacerbating cardiac function via oxidative stress-
mediated iron toxicity, and undermining therapeutic effects. This
hypothesis could be confirmed in an animal study. First, SPION-
mediated therapeutic agents (such as SPION-labelelled stem cells,
etc.) are intramyocardially injected into peri-infarcted zones in an
acute myocardial infarction rat model. Second, it should be investi-
gated whether SPION deposition in the heart causes myocardioocyte
loss and deteriorates the structure and function of the ventricle.
For example, T2-star magnetic resonance (MR-T2*) was used to
accurately evaluate cardiac iron status and detect early global vent-
ricular dysfunction; lipid peroxidation products (8-iso-PGF2α and
malondialdehyde, etc.) in the myocardium reflect the oxidative
stress mechanism; and histology was performed to examine myo-
cyte apoptosis, inflammation and fibrosis. Third, the efficacy of
novel SPION coated with antioxidants (such as N-Acetylcysteine or
Trolox) was investigated in attenuating oxidative stress-mediated
cardioc injury, further validating the SPION’s adverse effects and its
mechanism.

Implication

The evaluation of SPION compatibility with myocardium, particularly
with the ischaemic myocardium, is an urgent problem that needs to
be resolved before the clinical translation of SPION in the cardiovas-
cular field. If our hypothesis is true, then protective measures
should be taken into consideration before developing clinical applica-
tions. Given that SPION toxicity mainly stems from oxidative stress,
surface modification with an antioxidant (such as N-Acetylcysteine
or Trolox) may be a new method used to suppress oxidative damage
and injury.

In conclusion, local delivery of SPION-mediated therapeutic
agents might produce massive and persistent iron overload in ischae-
mic myocardium, consequently deteriorating myocardial injury. Thus,
antioxidant coating may be a new strategy used to suppress the
harmful properties of SPION.

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Conflicts of interest

The authors indicate no potential conflicts of interest.
References

1. Vandsburger M. Cardiac cell tracking with MRI reporter genes: welcoming a new field. J Cell Mol Med. 2012; 16: 1203–5.
2. Vandergriff AC, Hensley TM, Henry ET, et al. Magnetic targeting of cardiosphere-derived stem cells with superparamagnetic nanoparticles for treating rats with myocardial infarction. Biomaterials 2014; 35: 8528–39.
3. Srinivas A, Rao PJ, Selvam G, et al. Oxidative stress and inflammatory responses of rat following acute inhalation exposure to iron oxide nanoparticles. Hum Exp Toxicol. 2012; 31: 1113–31.
4. Yilmaz A, Rosch S, Yildiz H, et al. First multiparametric cardiovascular magnetic resonance study using ultrasmall superparamagnetic iron oxide nanoparticles in a patient with acute myocardial infarction: new vistas for the clinical application of ultrasmall superparamagnetic iron oxide. Circulation. 2012; 126: 1932–4.
5. Cheng K, Li TS, Malliaras K, et al. Magnetic targeting enhances engraftment and functional benefit of iron-labeled cardiosphere-derived cells in myocardial infarction. Circ Res. 2010; 106: 1570–81.
6. Zang Y, Li W, Ou L, et al. Targeted delivery of human VEGF gene via complexes of magnetic nanonanoparticle-adenoviral vectors enhanced cardiac regeneration. PLoS ONE. 2012; 7: e39490.
7. Huang Z, Pei N, Shen Y, et al. A novel method to deliver stem cells to the injured heart: spatially focused magnetic targeting strategy. J Cell Mol Med. 2012; 16: 1203–5.
8. Hamm B, Staks T, Taupitz M, et al. Contrast-enhanced MR imaging of liver and spleen: first experience in humans with a new superparamagnetic iron oxide. J Magn Reson Imaging. 1994; 4: 659–68.
9. Onishi H, Murakami T, Kim T, et al. Safety of ferucarbotran in MR imaging of the liver: a pre- and postexamination questionnaire-based multicenter investigation. J Magn Reson Imaging. 2009; 29: 106–11.
10. Richards JM, Shaw CA, Lang NN, et al. In vivo mononuclear cell tracking using superparamagnetic particles of iron: feasibility and safety in humans. Circ Cardiovasc Imaging. 2012; 5: 509–17.
11. Anderson LJ, Westwood MA, Holden S, et al. Myocardial iron clearance during reversal of siderotic cardiomyopathy with intravenous desferrioxamine: a prospective study using T2* cardiovascular magnetic resonance. Br J Haematol. 2004; 127: 348–55.
12. Terrovitis J, Stuber M, Youssf A, et al. Magnetic resonance imaging overestimates ferumoxide-labeled stem cell survival after transplantation in the heart. Circulation. 2008; 117: 1555–62.
13. Kawamura M, Miyagawa S, Fukushima S, et al. Enhanced survival of transplanted human induced pluripotent stem cell-derived cardiomyocytes by the combination of cell sheets with the pedicled omental flap technique in a porcine heart. Circulation. 2013; 128: 587–94.
35. Lekawanvijit S, Chattipakorn N. Iron overload thalassemic cardiomyopathy: iron status assessment and mechanisms of mechanical and electrical disturbance due to iron toxicity. *Can J Cardiol*. 2009; 25: 213–8.

36. Oudit GY, Sun H, Trivieri MG, et al. L-type Ca^{2+} channels provide a major pathway for iron entry into cardiomyocytes in iron-overload cardiomyopathy. *Nat Med*. 2003; 9: 1187–94.

37. Parkes JG, Hussain RA, Olivieri NF, et al. Effects of iron loading on uptake, speciation, and chelation of iron in cultured myocardial cells. *J Lab Clin Med*. 1993; 122: 36–47.