

Nanoparticles to identify Alzheimer disease by magnetic resonance imaging: abridged secondary publication

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KEY MESSAGES

1. We designed and tested an imaging material that can be used to detect Alzheimer disease (AD) by magnetic resonance imaging (MRI).
2. The material consists of magnetic iron nanoparticles coated with curcumin that binds the amyloid plaques in AD brain.
3. After injecting the nanoparticles, MRI distinguished AD from control model mice.
4. In examining slices of brains, the nanoparticles were highly concentrated at the plaques and significantly coincided with the dark spots seen on MRI.

5. The nanoparticles were non-toxic to cells grown in dishes.

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Introduction

Alzheimer disease (AD) is an incurable and progressive neurodegenerative disorder.^{1,2} Current AD drugs only provide partial symptomatic relief and do not slow degeneration. Some drug candidates may prove effective in delaying disease progression.^{1,2} However, large clinical trials have failed to show efficacy of the drug candidates in AD; there were only trends toward effectiveness among patients with mild AD. The current consensus is that the failure was not an inability of the drugs to accomplish their molecular action, but was a matter of timing: treatment beginning too late, after loss of brain function.^{1,2} Therefore, it is important to identify patients as early as possible to initiate treatment before irreversible brain injury takes place.

Positron emission tomography enables early diagnosis of AD by detecting amyloid plaques with a radioactive compound that binds plaques. However, this method is very expensive and is only available near a cyclotron that can generate the radioactively labelled reagent. Only one site in Hong Kong offers this service. Therefore, only limited patients may benefit from early diagnosis and potential treatment.

Magnetic resonance imaging (MRI) is more affordable and widely available. We aim to develop a method of labelling amyloid plaques for MRI. We developed magnetic particles that can enter the brain and specifically bind to amyloid plaques for detection by MRI. The particles consist of a microscopic iron core coated with curcumin (a natural compound) and polymers (long molecules

used in some drugs and foods). The curcumin binds both iron and amyloid plaques, thus temporarily anchoring the particles to plaques in the brain. The polymers protect the particles circulating in the blood. Curcumin can reach the brain and bind plaques in mice,³ and iron particles have been used for several MRI applications.⁴

Early diagnosis of AD enables early treatments; if effective, patient ability to function independently may be preserved, thus extending the duration of healthy life and reducing the burden on caregivers and costs to healthcare system. Preventive treatments, to be effective, must couple with cost-effective early diagnosis.

Methods

AD model mice and their normal siblings were used. Iron solutions were mixed under certain conditions to make a black powder consisting of nanoparticles. Curcumin and polymers were added to coat the nanoparticles. Their size, electric charge, and structure were analysed using several methods.

To determine whether the nanoparticles were safe to cells, cells were grown in dishes and nanoparticles were added. We then measured a chemical the cells produce when they are healthy; the amount of the chemical indicates the health of the cells. To determine whether the nanoparticles can enter the brain, we added them to liquid on one side of a layer of cells and measured the nanoparticles that crossed the layer to the liquid on the other side.

Some mice were injected with nanoparticles

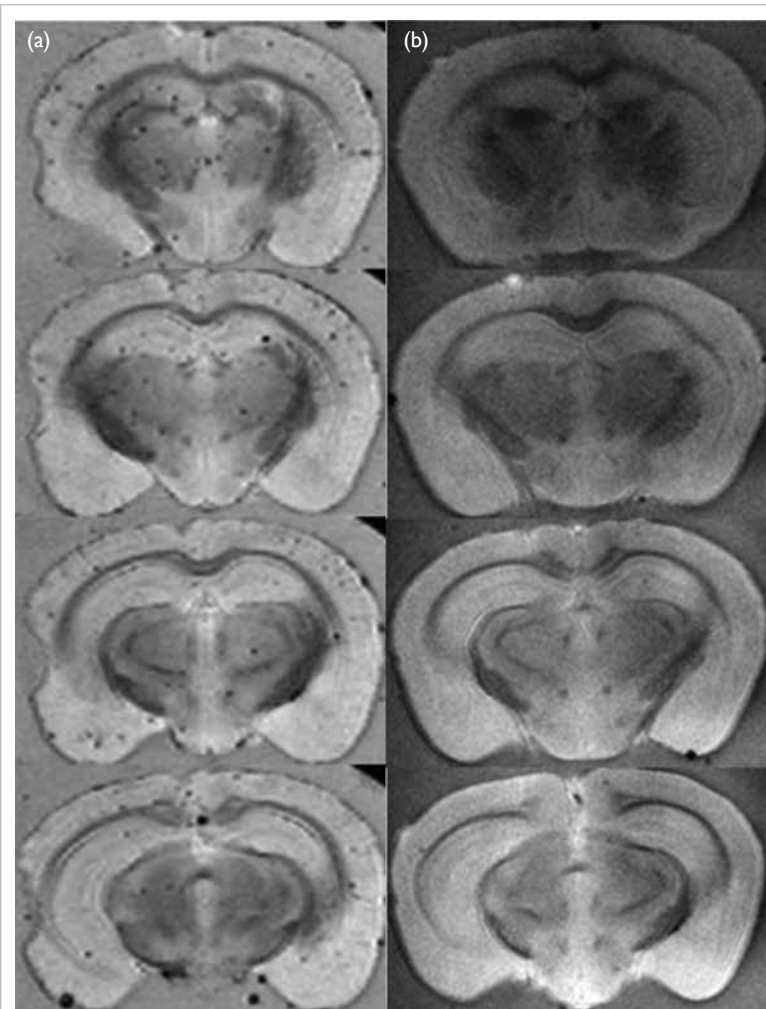


FIG 1. Magnetic resonance images of brains after nanoparticle injection and brain removal in (a) mice with Alzheimer disease and (b) normal mice

and then killed after 1, 3, 5, or 24 hours to assess distribution of iron in different organs.

The nanoparticles were injected into AD mice and their normal siblings. After 4 hours, mice were scanned by MRI and then killed, and their brains were removed and scanned again by MRI. The area covered by dark spots in MRI was measured.

Brains were thinly sliced and stained to show plaques (red) and iron (blue), and fluorescence showed curcumin (yellow).

To determine safety, memory of mice was tested 2 days after injecting nanoparticles.

Results

The mean diameter of nanoparticles was <100 nm. Their electric charge was almost neutral. X-ray analysis confirmed that the nanoparticles contained iron. Infrared analysis suggested that curcumin bound iron, and that polymers coated the nanoparticles. Baking off the coating of the nanoparticles left 14% of their weight, representing the iron. Another X-ray method supported the known structure of the nanoparticles: the middle containing iron and the outer layers containing carbon, a component of curcumin and polymers.

The nanoparticles were safe to cells. They crossed a layer of cells at a steady rate, suggesting that they may also be able to enter the brain.

After injecting nanoparticles into AD and normal mice, iron concentration rose in blood and brain but not in other organs tested (except for liver at 1 hour). We chose to perform MRI 4 hours after injection because 4 hours seemed likely to show the minimum non-specific binding in the brain.

After injecting nanoparticles, MRI showed significantly more dark spots in AD mice than in normal mice (Fig. 1), both before and after extracting the brains. Dark spots were concentrations of nanoparticles at amyloid plaques (Fig. 2). Without curcumin, iron particles do not show the increased plaques expected in AD mice. Young mice, which have few plaques, also showed more dark spots in AD mice than in normal mice.

In brain slices from mice injected with nanoparticles, amyloid plaques often appeared in the same places as the nanoparticles and dark spots in matching MRI images (Fig. 3).

The memory of mice given the nanoparticles 2 days before did not significantly differ from that of mice not given the nanoparticles.

Discussion

An ideal nanoparticle should be stable and small (<100 nm) to get through tiny blood vessels and remain in the blood long enough to enter the brain. It should reach the brain and bind amyloid plaques. It should be safe to cells and animals. Our

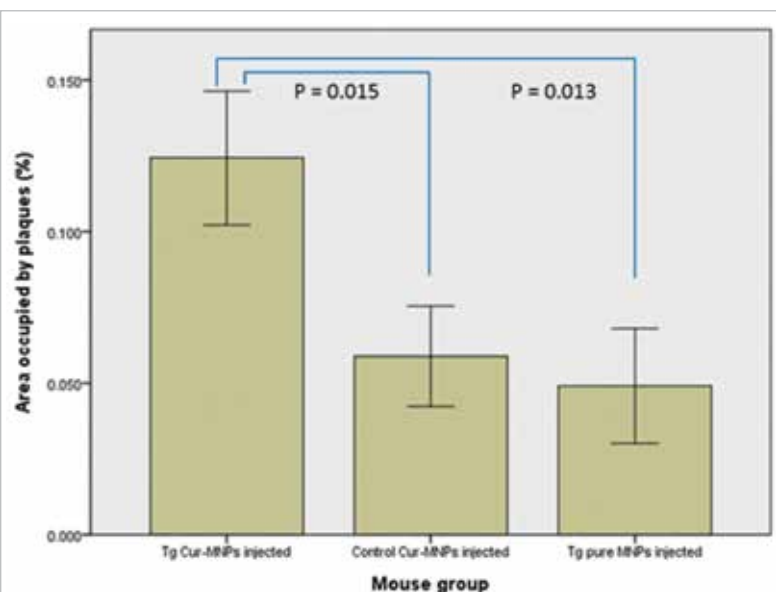


FIG 2. Area occupied by plaques in magnetic resonance images of live mice after injecting nanoparticles in Alzheimer disease mice (left), normal mice (middle), and Alzheimer disease mice after injecting iron particles without curcumin (right)

nanoparticles have these desired attributes.

Multiple methods confirmed that the nanoparticles have the predicted structure: a core of iron, a layer of curcumin, and an outer layer of polymers. The polymers lent particles longevity in the blood and ability to penetrate the blood-brain barrier. The nanoparticles were safe to cells and could penetrate a cell monolayer. Thus, the nanoparticles may be able to enter the brain. We chose 4 hours as the time for MRI imaging because non-specific binding appears to be minimised at around that time.

MRI and brain slices proved that the nanoparticles entered the brain and bound to plaques. MRI of AD mice displayed significantly more black spots, representing nanoparticles. This occurred even in young mice, with few plaques. Thus, nanoparticles may help detect AD early. They can be further tested in human clinical trials in the future.

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Disclosure

The results of this research have been previously

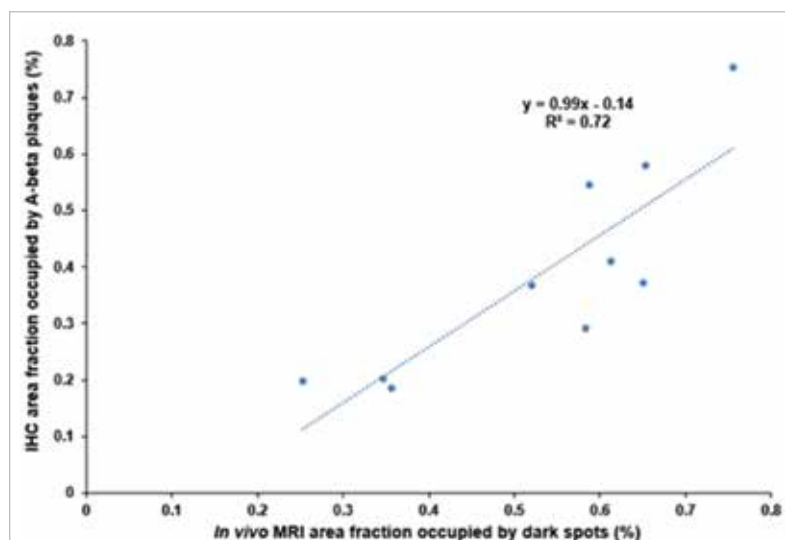


FIG 3. Areas of plaques in brain slices of Alzheimer disease mice correlate with area of nanoparticles in corresponding magnetic resonance images

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1. Cheng KK, Chan PS, Fan S, et al. Curcumin-conjugated magnetic nanoparticles for detecting amyloid plaques in Alzheimer's disease mice using magnetic resonance imaging (MRI). *Biomaterials* 2015;44:155-72.

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