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Development of non-pyrogenic magnetosome minerals coated with poly-l-lysine leading to full disappearance of intracranial U87-Luc glioblastoma in 100% of treated mice using magnetic hyperthermia

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ABSTRACT

Magnetic hyperthermia was reported to increase the survival of patients with recurrent glioblastoma by 7 months. This promising result may potentially be further improved by using iron oxide nanoparticles, called magnetosomes, which are synthesized by magnetotactic bacteria, extracted from these bacteria, purified to remove most endotoxins and organic material, and then coated with poly-L-lysine to yield a stable and non-pyrogenic nanoparticle suspension. Due to their ferrimagnetic behavior, high crystallinity and chain arrangement, these magnetosomes coated with poly-L-lysine (M-PLL) are characterized by a higher heating power than their chemically synthesized counterparts currently used in clinical trials. M-PLL-enhanced antitumor efficacy was demonstrated by administering 500–700 μg in iron of M-PLL to intracranial U87-Luc tumors of 1.5 mm^3 and by exposing mice to 27 magnetic sessions each lasting 30 min, during which an alternating magnetic field of 202 kHz and 27 mT was applied. Treatment conditions were adjusted to reach a typical hyperthermia temperature of 42 °C during the first magnetic session. In 100% of treated mice, bioluminescence due to living glioblastoma cells fully disappeared 68 days following tumor cell implantation (D68). These mice were all still alive at D350. Histological analysis of their brain tissues revealed an absence of tumor cells, suggesting that they were fully cured. In comparison, antitumor efficacy was less pronounced in mice treated by the administration of IONP followed by 23 magnetic sessions, leading to full tumor bioluminescence disappearance in only 20% of the treated mice.

1. Introduction

Every year, 25,000 patients in the United States and in Europe are diagnosed with glioblastoma (GBM) [1,2], a dreadful disease with a low 5-year survival rate of 10% with standard treatments [3,4]. New treatments are under development to improve this poor prognosis [5–13]. Among them, different types of nanotherapies [14] or thermotherapies such as whole-body hyperthermia, ultrasound waves, radiofrequency microwaves, phototherapy and magnetic hyperthermia have been or could be tested [15]. Compared with other thermotherapies, magnetic hyperthermia treatment [5–21], in which iron oxide nanoparticles are administered to tumors and heated under alternating magnetic field (AMF) application, requires lower heating temperatures of 43–50 °C to be efficient. This is due to a more localized heat that improves efficacy and safety. Using chemical superparamagnetic iron oxide nanoparticles (SPION), the magnetic hyperthermia treatment of patients

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with GBM was associated with an increase in patient survival following the diagnosis of first tumor recurrence of 7 months compared with conventional therapies [4,22,23]. To improve further the efficacy of magnetic hyperthermia, magnetic nanoparticles with better heating properties than those of SPION could be used. Such properties may be achieved by stable magnetic single domain iron oxide nanoparticles, which are either doped with cobalt to increase magnetocrystalline anisotropy [24], or that possess a large size, typically between 40 and 100 nm, leading to ferrimagnetic properties.

In this article, instead of introducing a toxic compound such as cobalt in nanoparticles, we use magnetotactic bacteria to synthesize large nanominerals called magnetosomes. The latter are cubo-octahedric iron oxide minerals composed of magnetite or maghemite depending on their level of oxidation, which are surrounded by biological material and usually organized in chains. Compared with SPION, magnetosomes are larger and better crystallized, yielding improved magnetic properties useful in a series of different applications, including magnetic hyperthermia [25]. In addition, due to their chain arrangement, magnetosomes are not prone to aggregation and lead to homogenous tumor temperature distribution [26–31]. In previous studies, suspensions of chains of magnetosomes isolated from magnetotactic bacteria were administered to MDA-MB-231 breast tumors xeno-grafted subcutaneously under the skin of mice and were exposed to several AMF applications, yielding more efficient antitumor efficacy than SPION [26,27]. Despite these appealing features, magnetosomes suffer from two drawbacks that have hindered their industrial development. On the one hand, the biological material surrounding their mineral core is difficult to fully characterize and obtain reproducibly with the same composition. In addition, in the absence of a specific treatment, they contain lipopolysaccharide since magnetosomes originate from gram-negative magnetotactic bacteria. On the other hand, with most current methods of bacterial growth, magnetosome production yield is relatively low, typically below 10 mg/L/day [32].

In this study, we have developed a magnetosome synthesis method that uses MSR-1 magnetotactic bacteria and leads to a large amount of magnetosomes (~100 mg per liter of growth medium). In this method, magnetosomes were first isolated from magnetotactic bacteria, most biological material was removed, and the magnetosome mineral core was then stabilized with a poly-L-lysine coating, leading to nanoparticles called M-PLL. M-PLL properties such as composition, surface charge, magnetic parameters, stability, cytotoxicity, pyrogenicity, and systemic and brain toxicity, were determined and compared with those of chemically synthesized iron oxide nanoparticles (IONP) currently used for magnetic hyperthermia [33,34]. Then, we studied *in vitro* whether M-PLL and IONP induce U87-Luc cell death in the presence (or not) of an AMF and whether cell death occurs through an apoptotic or necrotic mechanism.

The anti-tumor efficacy of M-PLL and IONP was also examined *in vivo* by first growing U87-Luc human GBM tumor cells inside the brain of nude mice. When tumors reached a size of ~1.5 mm^3, 2 μl of a suspension containing 500 μg in iron of M-PLL or IONP was administered at the site of tumor cell implantation and mice were then exposed 23 or 27 times for 30 min to an AMF of strength 27 mT and frequency 202 kHz. When the tumors re-grew despite the magnetic treatments, we re-administered 200 μg in iron of M-PLL or IONP at 47 days following tumor cell implantation (D47). To compare the efficacy of both treatments, the maximum mouse survival day reached with M-PLL and IONP was estimated. Possible mechanisms responsible for antitumor activity were also examined and we distinguished between those taking place in heated and unheated regions.

2. Materials and methods

2.1. Classification of the different suspensions of nanoparticles as a medical device of class III

Suspensions containing IONP and M-PLL were classified as medical devices of class III, since medical products containing nanomaterials, which were also activated by an external source of energy and used to treat cancer, were categorized as such [35,36].

2.2. Preparation of the M-PLL suspension

2.2.1. Growth of MSR-1 magnetotactic bacteria

MSR-1 magnetotactic bacteria were purchased from DSMZ (DSM-6361, Braunschweig, Germany). After cultivation in an agar gel, several colonies of these bacteria were collected and amplified in a pre-culture growth medium without iron up to an optical density measured at 565 nm (OD_{565}) of ~0.5–2. Cells were then grown in a fermenter under batch fed conditions using an acid solution containing an iron source that maintained the pH of the growth medium at 6.9. During fermentation, oxygen was bubbled in the growth medium with an air compressor to promote bacterial growth while maintaining oxygen concentration below 0.1% to enable magnetosome synthesis. After 75 h, we obtained a bacterial suspension with OD_{565} ~ 10–12 containing ~100 mg of magnetosomes in iron per liter of growth medium, as deduced by the iron assay. The growth protocol of magnetotactic bacteria is detailed in the supplementary information (SI).

2.2.2. Preparation of uncoated magnetosome minerals, M-Uncoated

Following fermentation, suspensions of bacteria were concentrated and re-suspended successively in several solvents (1 M NaOH, 1X PBS, Triton X-100 and 1% SDS, phenol, chloroform) under different sonication and temperature conditions and for various times to remove most organic material originating from the magnetotactic bacteria. The resulting suspension contained mainly the mineral cores of the magnetosomes. The suspension was autoclaved for sterilization. The method used to prepare suspensions of M-Uncoated is detailed in SI.

2.2.3. Preparation of suspensions of magnetosome minerals coated with poly-L-lysine, M-PLL

Suspensions of M-Uncoated at 20 mg/mL in iron were mixed under sterile conditions with a poly-L-lysine solution at 40 mg/mL at pH 9.5 under sonication. The supernatant containing free poly-L-lysine in excess was removed. Nanoparticle suspensions were washed with sterile water. Water was removed and replaced with 5% glucose. A sterile and injectable suspension of M-PLL mixed in 5% of glucose was thus obtained. The method used to prepare suspensions of M-PLL is detailed in SI.

2.3. Preparation of the IONP suspension

Ferrimagnetic chemically synthesized iron oxide nanoparticles (IONP) were purchased from Micromod, reference: 10-00-102 [34]. Prior to their administration in mice and to *in vitro* studies, IONP suspensions were centrifuged at 14,000 rpm (12 × 4 g) for 30 min and were then washed 3 times with a sterile injectable solution of 5% glucose.

2.4. Measurements on the different suspensions

To measure iron concentrations, nanoparticle sizes, morphology, surface charge, stability, surface and core composition, and magnetic properties, we used a series of different