Nanoparticle-enhanced Magnetic Field Induces Apoptosis in Nematode

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ABSTRACT

To investigate molecular consequence of magnetic field to C. elegans, Fe3O4 nanoparticles were incorporated into the culture media of C. elegans and the nematodes were grown under magnetic field for 4 days. For the expermental group, we observed a 30 to 50 percents reduction in the moving speed. However, only nematode at L4 stage exhibited this difference. To explore molecular mechanisms affected by this treatment, real-time RT-PCR was performed using primers specific to amplify 120 genes. Differential expression for genes belong to apoptosis was observed. Biochemical assay for Caspase 3 activity was performed. The magnetic field-treated nematode exhibited high levels of Caspase 3 activity consistent with real-time PRC. Fluorescence microscopy showed that apoptotic activity was associated with the Fe3O4 treatment. For further validation, a ced-3 mutant nematode under enhanced magnetic field showed less effect for the movement than wild-type. The current study indicated that nanoparticle-enhanced magnetic field induced apoptosis in nematode.

Keywords: apoptosis, nanoparticles, magnetic field, C. elegans

1 INTRODUCTION

It has been hypothesized that long term exposure to extremely low frequency (ELF) magnetic fields in the power frequency range of 50–60 Hz could increase the risk of breast cancer. The evidence of a suppressive effect of ELF magnetic field exposure on melatonin production is contradictory. While some epidemiological studies have related exposure to environmental exposure to extremely low-frequency electromagnetic field to an increased risk for certain types of adult and childhood cancer including leukemia, cancer of central nervous system and lymphoma [1–4], others [5–7] have failed to find such an association. If the relationship between EMF exposure and cancer is causal, the risk of developing cancer must follow an exposure-response pattern and should be evident in occupations with high exposure levels. However, studies on cancer incidences among occupationally exposed subjects like welders have also revealed inconsistent results [8–10].

The development of nanotechnology advances the synthesis of various nanoparticles including magnetic nanoparticles (MNP). Application of MNP to study biological effect of EMF might have the advantage of intensifying local magnetic field, thus enhances the biological response. In the current study, we took the advantage of the well-developed genetics of C. elegans. Combination of nematode, MNP, and magnetic field brought some surprising but interesting results.

2 RESULTS

Static magnetic field (SMF) reduces the mobility of nematode The static magnetic field (SMF) was established by assembling a sandwich-like device composed of 2 magnets and one culture plate at the center (Fig. 1). The intensity of the static magnetic field is approximately 0.5 T. The moving and crawling action of nematodes was monitored and recorded by video camera and analyzed by personal computer. Wild-type nematodes (N2) move approximately 7.5 mm/min at room temperature. In the presence of SMF during the measurement, the moving speed was reduced but insignificantly (Fig. 2.). Extended treatment of SMF for 2 hrs made negligible reduction in mobility. To enhance the effect of SMF, nematodes were fed with magnetic nanoparticles (MNP) (Fig. 3). The MNP-treated nematodes moved with similar speed as N2. To further enhance the magnetic field, MNP-treated nematodes were grown in the presence of SMF for 4 days. When examined under stereoscope, mobility of this group of nematode reduced to 3.3 mm/min, which is significantly different from the wild-type worms (Fig. 3.).

Genes differentially expressed in the presence of SMF

The combination of MNP and SMF significantly reduced the mobility of nematodes. It will be interesting to know genes affected by this treatment. We applied real time RT-PCR to screen for genes differentially expressed under the SMF. For the initial screening 120 genes were randomly selected for their association with cancer, apoptosis, development, stress response, and metabolism. Repeated assay indicated that at least expression of 26 genes was significantly affected (Table 1). These genes consistently exhibited differential expression in the presence of SMF. It is interesting to know that some of them are associated with apoptosis (ced-2, ced-3, ced 6), cancers (abl-1, cbp-1), stress response (hsp 16, hsp70, hsp90), and aging (age-1). At least 2-fold difference in expression was observed.

To further verify the pathways that are affected by SMF, ced-3, ced-6, and cbp-1 were selected for further investigation. Among these genes we selected ced-3, ced-6, and cbp-1 for further investigation. The ced-3 gene encodes a caspase required for apoptosis, and ced-3 controls the

apoptosis of C. elehgans. Ced-6 does not participate in apoptosis directly; however Ced-6 is involved in the pathways to regulate cell corpse engulfment. Cbp-1 encodes a homolog of the mammalian transcriptional cofactors CBP and p300 that have been shown to possess histone acetyltransferase activity, and which, when mutated, lead to Rubinstein-Taybi syndrome and colorectal cancer.

Apoptosis pathway mediates SMF-induced mobility reduction To investigate the correlation between ced-3, ced-6, and cbp-1 to SMF we obtained mutant nematodes from worm base. Mobility of these mutant nematodes was measured in the presence and in the absence of SMF. The mutants were also treated by nanoparticles including TiO_2, Fe_2O_3, and Fe_3O_4 (Fig. 4). For wild-type nematode (N2, Fig. 4. upper left), SMF-treatment significantly reduced the mobility about 40%. Treatment with Fe_2O_3 and Fe_3O_4 further slowed down the movement. Effect of TiO_2 is insignificant. Ced-3 mutant worms, on the other hand, were insensitive to magnetic field. Ced-6 and cbp-1 mutants, on the other hand, are affected by SMF treatment. Because ced-3 is directly involved in apoptosis, ced-6 is indirectly involved, and cbp-1 in involved in cancer, thus apoptosis pathway is very likely mediated the effect of SMF on the mobility reduction of nematode.

Biochemical evidence showing direct involvement of apoptosis in SMF-induced mobility reduction To explore biochemical evidence of apoptosis, we characterized caspase 3 activity of SMF-treated nematodes. Caspase 3 activity is the hallmark for apoptosis. Significant increase in Caspase 3 activity was observed consistent with the genetic evidence (Fig. 5).

Apoptosis occurred majorly at the intestinal region of nematode To identify tissue that is affected by SMF, fluorescence microscopy was performed to SMF-treated worms. The staining is specific to caspase 3 activity. Fluorescent image indicated that apoptosis was localized at the intestinal adduct where magnetic NPs were distributed (Fig. 6).

3 CONCLUSION

Application of static magnetic field to nematode at the strength of 0.5 T for 4-days significantly reduced the mobility of worms. Feeding the worms with super magnetic nanoparticles further enhanced the damaging effect of magnetic field. By applying real time RT-PCR we were able to spot genes whose expression was affected by SMF-treatment. These genes are associated with apoptosis, cancer, stress response, and aging. Further genetic analysis indicated that apoptosis pathway mediates the SMF-induced mobility reduction. Biochemical evidence also supported the direct involvement of apoptosis. Fluorescence microscopy localized the site of action is close to intestinal adducts.

The current study provided the first evidence that magnetic field is associated with apoptosis. It is also worthy to know that application of nanoparticles can accelerated novel studies, and in the current example, biological response to magnetic field.

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Fig. 1. The experimental setup to observe the effect of SEF on the mobility of C. elegans. The sandwich id composed of two magnets and a culture plate (upper left). The action of nematode was recorded by video camera directly on the stereoscope (lower left). Structure and dimension of nematode was also drawn (lower right).

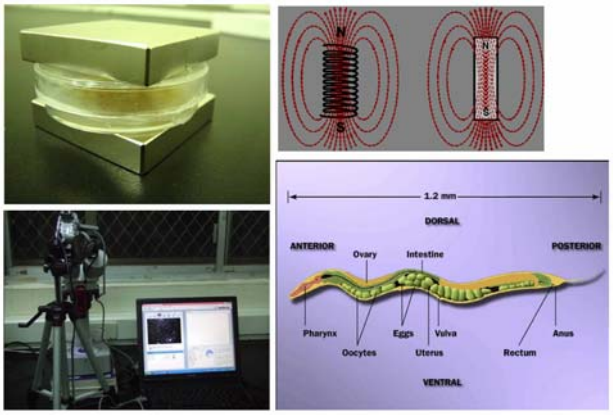

Fig. 2. Mobility assay for nematodes treated with static magnetic field. Temporary treatment of SMF does not affect the mobility of nematode. 2-hr treatment of SMF (N2+MF) or simply feeding worms with Fe_3O_4 (N2+ Fe_3O_4) does not significantly change the mobility of worms. When the Fe_3O_4-fed worms were treated with SMF for 4 days, significant difference in mobility was observed (N2+ Fe_3O_4+MF).

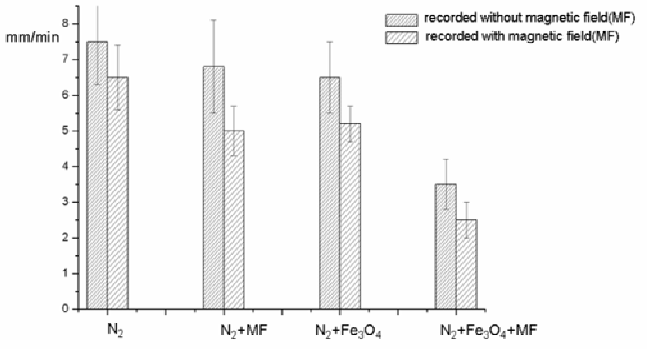

Fig. 3. Images of nematodes fed with Fe_3O_4 nanoparticles. The brown area indicates NPs and localized at intestinal adduct.

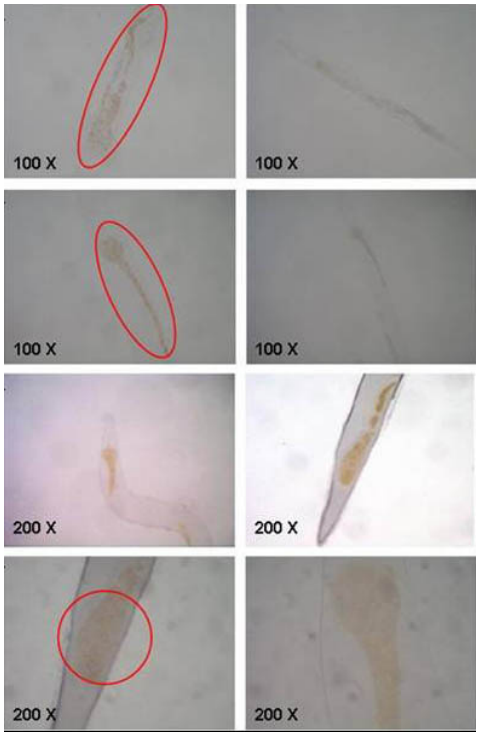

Fig. 4. Effect of SMF on the mobility of wild-type nematode (N2) and mutants (Ced-3, Ced-6, and Cbp-1). Several kinds of nanoparticles were incorporated into the growth media of nematode. These NPs include TiO_2, Fe_2O_3, and Fe_3O_4. The nematodes were assayed for mobility, with or without 4-day treatment of SMF (MF).

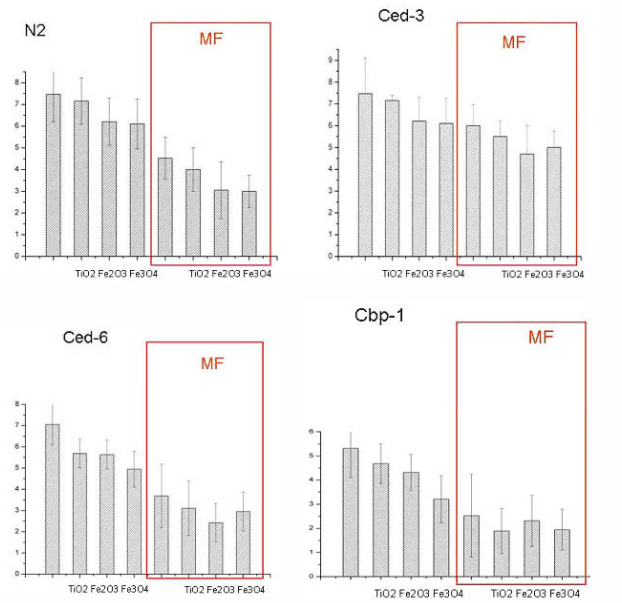

Fig. 5. Caspase 3 activity assay on the SMF-treated nematodes. N2 was also treated with cytochalasin D to induce apoptosis, and served as positive control (100%) in this experiment.

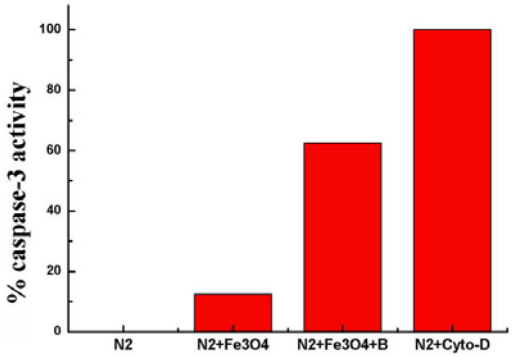

Fig.6. Fluorescent image of nematode stained with caspase 3 activity. N2 was also treated with cytochalasin D to induce apoptosis, and served as positive control in this experiment (Cyto D). Treatment of SMF induced apoptosis-like activity in the intestinal region of nematode (B).

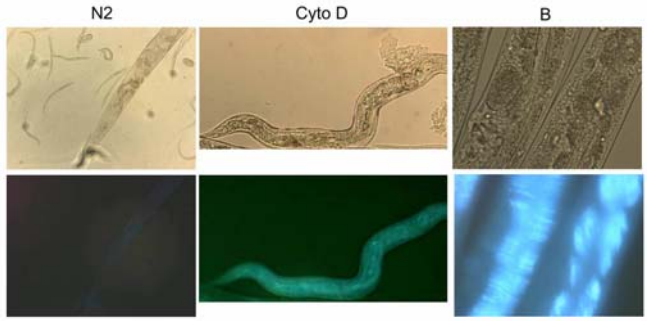

Table 1 List of differentially expressed genes derived from Real time RT-PCR

Name*	△△Ct**	SD***	Fold****
abl-1	5.56	0.17	47.18
alx-1	-1.32	0.26	0.40
bir-1	2.43	0.51	5.39
ced-3	4.16	1.00	17.88
ced-2	2.71	0.36	6.54
ced-6	-0.71	0.22	0.61
ced-8	4.27	1.64	19.29
che-13	-0.641	0.09	0.64
mel-26	3.43	0.88	10.78
T27F7.2	2.05	0.68	4.14
tir-1	4.83	0.50	28.44
nft-1	1.06	0.56	2.08
par-4	4.63	0.42	24.76
bub-1	5.98	0.34	63.12
daf-18	2.35	0.46	5.10
cbp-1	3.23	0.88	9.38
dic-1	5.5	0.43	45.25
hoe-1	-1.35	0.29	0.39
cyp-44A1	-1.82	0.53	0.28
sod-2	2.14	1.22	4.41
hsp16	2.67	0.78	6.36
hsp70	2.94	0.22	7.67
hsp90	3.68	0.57	12.82
act-1	4.85	0.65	28.84
age-1	1.86	0.98	3.63
dif-2	5.62	1.24	49.18

*This list contains 26 genes which are selected from result of 120 genes. The selected genes are consistently differentially expressed with p-value less than 0.05 in the Student t-test. The values are averaged from at least 6 sets of experiments.

**△△Ct, is defined as the threshold cycle number relative to the control genes. In the current study we used ribosomal genes L18 and L21 as control for expression.

***SD: standard deviation.

****Fold: is fold increase or decrease comparing to the gene expression of N2 nematode n the absence of magnetic field.

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