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ORIGINAL ARTICLE

Static magnetic field increases the sensitivity of *Salmonella* to gentamicin

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Abstract The present study was carried out to evaluate the effects of static magnetic field (SMF) on antibiotic sensitivity of *Salmonella enterica* subsp. *enterica* serovar Hadar. We have evaluated antibiotic susceptibility using the disc diffusion method following exposure to SMF. Our results showed that exposure to a 200-mT SMF static magnetic field increased the efficiency ($p<0.01$) of gentamicin against *Salmonella* Hadar but did not affect the diameter of the inhibition zone of some other antibiotics actives on Enterobacteria: penicillin, oxacillin, cephalotin, neomycin, amikacin, tetracyclin, erythromycin, spiramycin, chloramphenicol, nalidixic acid and vancomycin.

Keywords Static magnetic field · *Salmonella* Hadar · Antibiotic susceptibility · Diameter of the inhibition zone

Introduction

Many investigations have shown that extremely-low-frequency electromagnetic fields (ELM-EMFs) generated by environmental sources, such as 50–60 Hz high voltage transmission lines and other electronic appliances, affected biological systems (Blank 1995). Many biological effects related to the ELF-EMF have been reported in the literature. Among these have been the effects on cell growth, proliferation and cell viability (Raylman et al. 1996; Potenza et al. 2004b; Ji et al. 2009; Obermeier et al. 2009), cell morphotype (Cellini et al. 2008), increased calcium influx (Galvanoskis et al. 1999; Amara et al. 2004), altered DNA in terms of point mutations (Potenza et al. 2004a), inhibition of nocturnal levels of melatonin (Brendel et al. 2000), protein synthesis (Goodman et al. 1993), transport of ions by cell membranes (D'Inzeo et al. 1993) and gene transcription (Phillips et al. 1992). In terms of electromagnetic field action in bacteria, several papers have reported alterations on growth, DNA molecules and gene expression (Galvanoskis et al. 1999; Potenza et al. 2004b; El May et al. 2009). *Escherichia coli* cells exposed to an extremely low frequency magnetic field (0.1 T) for 6.5 h exhibited changes in viability compared to unexposed cells, which had a viability 100 times higher than the control (Justo et al. 2006). El May et al. (2009) reported that a static magnetic field (200 mT) induced a decrease of colony-forming units (CFU) of *Salmonella enterica* subsp. *enterica* serovar Hadar (*S.* Hadar) and an overexpression of *rpoA*, *katN*, and *dnaK* mRNAs following 10 h of SMF exposure indicating a stress status. In eukaryotes and prokaryotes, the effect of SMF exposure could be mediated by a modulation of ion transport into the cells and a possible formation of free radicals (Strasak et al. 2002; Amara et al. 2004). *Staphylococcus epidermidis* biofilms had greatly increased susceptibility to gentamicin. There was a reduction of at least 50% in the minimum biofilm inhibitory concentration (CMI) of gentamicin after exposure to a pulsed electromagnetic field (PEMF) (Pickering et al. 2003).

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The application of static magnetic fields (5 Gauss) may enhance the activity of gentamicin against biofilm-forming *Pseudomonas aeruginosa* adherent to a polymer substrate (Benson et al. 1994).

Salmonella spp. is a leading cause of bacterial foodborne disease all over the world, causing a diversity of illnesses including typhoid fever, gastroenteritis and septicemia (D'Aoust 2000). In this study, we report an investigation on antibiotic sensitivity of *S.* Hadar, with the aim of detecting possible magnetic field-induced changes. In order to reach this aim, the disc diffusion method was used.

Materials and methods

Magnetic field application

The static magnetic field (SMF) was produced as described in El May et al. (2009) by a pair of cylindrical coils (each coil: diameter 20 cm, and length, 13 cm) (Beaudouin, Paris, France), powered by a transformer. The coils were water-cooled and the temperature inside the coils was regulated at the laboratory temperature of about 25°C. An Erlenmeyer-shaped glass double phial (external diameter, 6 cm; external height, 8 cm; internal diameter, 4 cm; internal height, 7 cm) was used as sample holder. Cultures for magnetic field exposure were fixed in the centre of the coil radius. The induction of SMF was measured and standardized using a Tesla meter. For this type of exposure, no shielding against the natural variation of terrestrial MFs was required, their intensity (0.075 mT) being insignificant compared with the applied SMF. The temperature was maintained at 37°C inside the glass double phial by water circulation, using an incubator system composed of pump and resistance. Control cultures were kept in the same conditions as the exposed ones except that the magnetic field was turned off.

Bacterial strain and growth conditions

The bacterial strain used in this study was *Salmonella enterica* subsp. *enterica* serovar Hadar (*S.* Hadar, isolate 287) (antigenic formula: 6.8; Z10/e,n,X) isolated from chicken at the Institut Pasteur de Tunis (Tunisia) and stored at −80°C. Cells were grown on Nutrient broth (Pronadisa; Hispanleb, Madrid, Spain) (5 g polypepton and 3 g meat extract per liter of distilled water, pH 7) and Nutrient agar (15 g per liter).

Fresh bacterial cultures were used through the experiments. *Salmonella* Hadar cells were cultivated overnight at 37°C in 5 ml of culture medium in an 18-mm diameter tube. The bacterial culture was then diluted with culture medium to give the same initial concentrations (0.1 OD_{600}). Control cultures were kept in the same conditions as the exposed ones. Then, 1 ml aliquot was withdrawn under sterile conditions from both the control and exposed cultures for examination by light spectrophotometry at 600 nm.

Antibiotic susceptibility

Antimicrobial susceptibility tests were performed using the agar diffusion method on nutrient agar.

An amount of 300 µl of each bacterial suspension (an even cell suspension) were spread on agar plates. Antibiotic disks were disposed manually. Antibiograms were performed using the following antibiotics (BioMérieux, France): penicillin 10 U (P), oxacillin 1 U (Ox), cephalotin 30 µg (CF), neomycin 30 µg (N30), amikacin 30 µg (AN), tetracycline 30 µg (TE), erythromycin 15 µg (E), spiramycin 100 µg (SP), chloramphenicol 30 µg (C30), nalidixic acid 30 µg (NA), vancomycin 30 µg (VA) and gentamicin 10 µg (GM). Following incubation for 18 h at 37°C, zone diameters were measured by the standard methods.

Antibiograms were performed with increasing exposure time measured against a no treatment control. For the control experiments, the bacterial cultures were similarly positioned; except that the magnetic field was turned off. The diameter of inhibition zones of SMF (200 mT)-exposed bacteria and untreated control during 12 and 24 h incubation were determined. Antibiograms were done for three exposure experiments.

Statistical analysis

The differences between control and exposed cells for each time point were determined using Student's t test. Means are given with ±SD of three different measurements of inhibition zone diameters and the level of significance was set at $p<0.05$.

Results

In order to determine the biological effects of static magnetic field (200 mT) during 12 and 24 h on antibiotic susceptibility to *S.* Hadar isolate 287, a standardized bioassay using the disc diffusion method was applied. The diameter of inhibition zones of exposed bacteria and untreated control for each time point (12 and 24 h incubation) were determined.

Effects of static magnetic field (200 mT, 12 h) on antibiotics

Bacterial cells in liquid culture broth were exposed to a 200-mT static magnetic field for up to 12 h of growth (log phase); antibiograms were then performed for the exposed

Fig. 1 Inhibition zone diameters of gentamicin by *Salmonella* Hadar. **a** Control (after 12 h incubation), **b** static magnetic field (SMF)-exposed bacteria (200 mT, after 12 h), **c** SMF-exposed bacteria (200 mT, 24 h)

bacteria and controls. As shown in Fig. 1, there was a 2 mm difference between the gentamicin inhibition zone diameter of the exposed cells (23 mm) and the controls (21 mm) after 12 h incubation. The compared analysis of the antibiograms showed a significant increase ($p<0.01$) of the gentamicin inhibition zone diameter for the exposed bacteria (Fig. 2).

There was no significant effect of SMF (200 mT, 12 h) exposure on the susceptibility to the other tested antibiotics: penicillin, oxacillin, cephalotin, neomycin, amikacin, tetracyclin, erythromycin, spiramycin, chloramphenicol, nalidixic acid and vancomycin (Fig. 2).

Effects of static magnetic field (200 mT, 24 h) on antibiotics

Bacterial cells in liquid culture broth were exposed to a 200-mT static magnetic field during 24 h (stationary phase); antibiograms were then performed for the exposed bacteria and controls. As shown in Fig. 1, there was a 2.2 mm difference between the inhibition zone diameter of the exposed cells (26.2 mm) and the controls after 24 h incubation (24 mm). The compared analysis of the antibiograms showed that a longer exposure time resulted in a significant improvement ($p<0.01$) of effectiveness of gentamicin after 24 h (Fig. 3).

There was no significant effect of SMF (200 mT, 24 h) exposure on the susceptibility to the other tested antibiotics (data not shown).

Discussion

In the present work, we studied the effects of SMF (200 mT) on *S.* Hadar sensitivity to antibiotics. As it is more difficult to evaluate the effect of low-intensity magnetic fields because it may interfere with the earth's magnetic field and because results are often conflicting, we selected a high induction (200 mT) field. The static magnetic field exposure (200 mT) induced a stress status in *S.* Hadar (El May et al. 2009). Furthermore, Pickering et al. (2003) showed that the *Staphylococcus epidermidis* biofilms had greatly increased susceptibility to gentamicin. There was reduction of at least 50% in the minimum biofilm inhibitory concentration of gentamicin after exposure to a pulsed electromagnetic field (PEMF). This PEMF

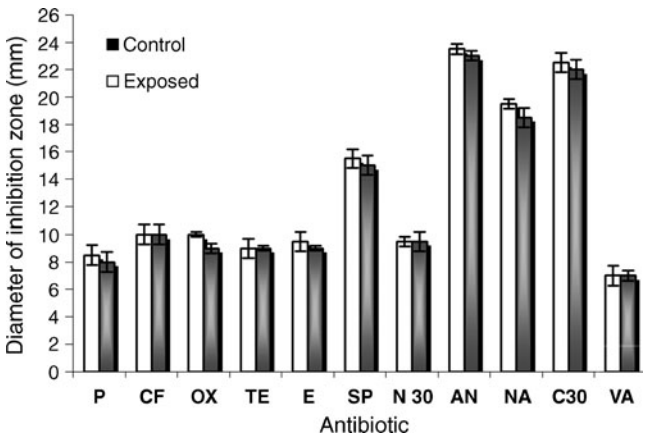

Fig. 2 Effect of static magnetic field (200 mT, 12 h) exposure on susceptibility of *Salmonella* Hadar to antibiotics. Comparison of the diameter of the inhibition zones of different antibiotics. penicillin (*P*), oxacillin (*OX*), cephalotin (*CF*), neomycin (*N30*), amikacin (*AN*), tetracyclin (*TE*), erythromycin (*E*), spiramycin (*SP*), chloramphenicol (*C30*), nalidixic acid (*NA*), vancomycin (*VA*), gentamicin (*GM*). The values are the means ± SD of three different measurements of inhibition zone diameters (Student's *t* test)

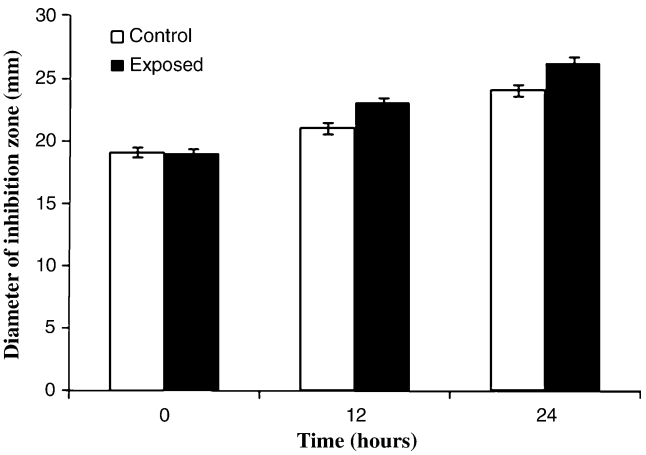

Fig. 3 Effect of static magnetic field (200 mT) exposure time (12 and 24 h) on susceptibility of *Salmonella* Hadar to gentamicin. Comparison of the diameter of the inhibition zones of gentamicin at different time. The values are the means ±SD of three different measurements of inhibition zone diameters (Student's *t* test)

has an effect on the efficacy of antibiotics in the treatment of infection of orthopaedic implants (Pickering et al. 2003).

Our results ("Statistical analysis" and "Results" sections) showed a significant increase in the effectiveness of gentamicin (GM) on *S.* Hadar when exposed to a SMF (200 mT, 12 and 24 h) (Figs. 1 and 3). However, the SMFs did not induce modification of the susceptibility to the other tested antibiotics (Fig. 2). The nature of antibiotics, their mode of penetration inside the cell, or their mechanism of action could be involved in this differential behavior. The main theories that try to explain the biological effects of electromagnetic fields are based on the possible effects on the permeability of ionic channels in the membrane (Galvanoskis and YfdSandblom 1999). To explain the increase of the *Salmonella* sensitivity to gentamicin, it could be hypothesized that penetration of antibiotic into bacteria was enhanced. Further research is needed to elucidate the related mechanism.

The presence of membrane potential sensitive ion channels in *E. coli* (Berrier et al. 1993). Gentamicin, as with other aminoglycoside antibiotic, is a cationic antibiotic which binds reversibly to anionic sites of the bacterial cell membrane dependent on concentration (Taber et al. 1987). In our experiments, the result of SMFs subtly altering the bacterial charge thereby was encouraging a relatively higher accumulation of gentamicin. It may also be possible that the static magnetic fields have an effect on the active transport mechanisms in the bacterial cell membrane.

Gentamicin is an antibiotic present in some bone cements and also in antibiotic beads used to treat infection. This can affect ion transport into the cells and can result in biological changes. The other possible effect is the formation of free radicals caused by magnetic field exposure. It is essential to establish the scope of this technique and hence its potential value.

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References

Amara S, Abdelmelek H, Sakly M (2004) Effect of acute exposure to magnetic field on ionic composition of frog sciatic nerve. Pak J Med Sci 20:91–96

Berrier C, Coulombe A, Houssin C, Ghazi A (1993) Voltage-dependent cationic channel of Escherichia coli. J Membr Biol 133:119–127

Benson DE, Grissom CB, Burns GL, Mohammad SF (1994) Magnetic field enhancement of antibiotic activity in biofilm forming *Pseudomonas aeruginosa*. ASAIOJ 40(3):M371–M376

Blank M (1995) Electromagnetic fields: biological interactions and mechanisms. BioSystems 35:175–178

Brendel H, Niehaus M, Lerchl A (2000) Direct suppressive effects of weak magnetic fields (50 Hz and 16 2=3 Hz) on melatonin synthesis in the pineal gland of Djungarian hamsters (*Rhodopus sungorus*). J Pineal Res 29:228–233

Cellini L, Grande R, Di Campli E, Di Bartolomeo S, Di Giulio M, Robuffo I, Trubiani O, Mariggio MA (2008) Bacterial response to the exposure of 50 Hz electromagnetic fields. Bioelectromagnetics 29:302–311

D'Aoust JY (2000) Salmonella. In: Lund BM, Baird-Parker TC, Gould GW (eds) The microbiological safety and quality of foods. Aspen, Gaithersberg, Maryland, USA, pp 1233–1299

D'Inzeo G, Pisa S, Tarricone L (1993) Ionic channel gating under electromagnetic exposure: a stochastic model. Bioelectrochem Bioenerg 29(3):289–304

El May A, Snoussi S, Ben Miloud N, Maatouk I, Abdelmelek H, Aissa R, Landoulsi A (2009) Effects of static magnetic field on cell growth, viability, and differential gene expression in *Salmonella*. Foodborne Pathog Dis 6(5):547–552

Galvanoskis J, YfdSandblom J (1999) Periodic forcing of intracellular calcium oscillators. Theoretical studies of the effects of low-frequency fields on the magnitude of oscillations. Bioelectrochem Bioenerg 46:161–174

Galvanoskis J, Sandblom J, Bergqvist B, Galt S, Hammerius (1999) Cytoplasmic Ca^{++} oscillations in human leukemia T-cells are reduced by 50 Hz magnetic fields. Bioelectromagnetics 20 (5):269–276

Goodman EM, Greenebaum B, Marron MT (1993) Altered protein synthesis in a cell-free system exposed to a sinusoidal magnetic field. Biochim Biophys Acta 1202(1):107–112

Ji W, Huang H, Deng A, Pan C (2009) Effects of static magnetic fields on *Escherichia coli*. Micron 40(8):894–898

Justo OR, Perez VH, Alvarez DC (2006) Growth of E. coli under extremely low frequency electromagnetic field. Appl Biochem Biotechnol 134:155–163

Obermeier A, Matl FD, Friess W, Stemberger A (2009) Growth inhibition of Staphylococcus aureus induced by low-frequency electric and electromagnetic fields. Bioelectromagnetics 30 (4):270–279

Phillips JL, Haggren W, Tomas WJ, Ishida-Jones T, Adey WR (1992) Magnetic induced-changes in specific gene transcription. Biochim Biophys Acta 1132:140–144

Pickering SA, Bayston R, Scammell BE (2003) Electromagnetic augmentation of antibiotic efficacy in infection of orthopaedic implants. J Bone Joint Surg Br 85(4):588–593

Potenza L, Cucchiarini L, Piatti E, Angelini U, Dachà M (2004a) Effects of high static magnetic field exposure on different DNAs. Bioelectromagnetics 25:352–355

Potenza L, Ubaldi L, De Sanctis R, De Bellis R, Cucchiarini L, Dacha M (2004b) Effects of a static magnetic field on cell growth and gene expression in Escherichia coli. Mutat Res 561 (1–2):53–62

Raylman RR, Clavo AC, Wahl RL (1996) Exposure to strong static magnetic field slows the growth of human cancer cells in vitro. Bioelectromagnetics 17:358–363

Strasak L, Vetterl V, Smarda J (2002) Effects of low-frequency magnetic fields on bacteria Escherichia coli. Bioelectrochemistry 55(1–2):161–164

Taber HW, Mueller JP, Miller PF, Arrows AS (1987) Bacterial uptake of aminoglycoside antibiotics. Micromiol Rev 51:439–457