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Absence of relevant effects of 5 mT static magnetic field on morphology, orientation and growth of a rat Schwann cell line in culture

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Summary. The aim of this study is to observe possible changes in the morphology, orientation or cell growth of an in vitro cultured Schwann cell line by 24 h exposure to 5 mT static magnetic fields. The magnetic field generator basically consists of a pair of circular coils in a Helmholtz arrangement and enables temperature to be controlled (37±0.1°C). We did not find any statistically significant differences in the cell growth rate between control and exposed cells, nor did we observe any differences in cell morphology or orientation.

Key words: Static magnetic field, Growth rate, Morphology, Schwann cell

Introduction

The rapid development of electrical and electromagnetic devices in industry, medical practice as well as in domestic life has meant that human beings are more exposed than ever to a wide range of electromagnetic fields (EMF) of different intensities. This has led to a growing interest in the study of the biological effects of EMF and possible impact on human health (Aldrich et al., 2001).

Cell cultures might represent the most simple and reliable systems for obtaining trustworthy data on the biological effects of static magnetic fields (SMF). However, the results obtained from the studies on the effects of SMFs on cell cultures are so variable that they range from no effects to important morphological and functional consequences.

Two variables are particularly relevant in the study of these possible effects: cell type and SMF intensity. As regards cell type, there are reported effects of SMF as well as a lack of any effects in both non-tumour and tumour cells. Reports of absence of effects of SMF on non-tumour cells, include human lung fibroblasts (Sato et al., 1992; Wiskirchen et al., 2000) or lymphocytes (Fanelli et al., 1999; Aldinucci et al., 2003). Lack of effects on tumour cells such as HeLa cells (Hiraoka et al., 1992), for leukemia cells (Sakurai et al., 1999; Teodori et al., 2002b) or human glioblastoma cells (Teodori et al., 2002a) have been found. In contrast, other studies detected SMF effects on non-tumour cells such as astrocytes (Hisamitsu et al., 1997), neurons (Calvo and Azanza, 1999; Pacini et al., 1999), fibroblasts (Pacini et al., 2003), lymphocytes (Onodera et al., 2003), monocytes (Salerno et al., 1999) and smooth muscle cells (Iwasaka et al., 2003). Further, different kinds of alterations on tumour cells resulting from SMF exposure have been described, including: leukemia cells (Sabo et al., 2002), melanoma cells (Short et al., 1992) and lymphoid tumour cells (Aldinucci et al., 2003).

Moreover, effects and absence of effects have been found after exposure of cell cultures to a wide range of SMF intensities. Absence of any effect has been described with exposure to SMF intensities of 6 mT (Teodori et al., 2002a,b), 0.2 to 1.5 T (Pacini et al., 1999; Wiskirchen et al., 2000), 4.75 to 7 T, (Aldinucci et al., 2003; Schiffer et al., 2003), and 10 T (Nakahara et al., 2002; Hirose et al., 2003). In contrast, other studies have shown an extreme range of effects caused by SMF exposure with intensities of 0.5-6 mT (Buemi et al., 2001; Dini and Abbro, 2005) 0.1-0.5 T (Rosen, 2003; Salerno et al., 1999), 1-4.7 T (Aldinucci et al., 2003; Sabo et al., 2002) and 7-14 T (Raylman et al., 1996; Iwasaka et al., 2003).

The aim of this study is to determine whether a 5 mT SMF affects cell growth, morphology or orientation of

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an *in vitro* cultured Schwann cell line. We selected Schwann cells mainly due to their morphological and functional relationship with nerve fibres and their physiological ability, subsequent to nerve lesion, to dedifferentiate, proliferate, migrate and myelinate regenerated neurites.

Materials and methods

Magnetic system

For SMF exposure we constructed a magnetic field generator with a pair of identical circular coils in a Helmholtz arrangement. This arrangement was used to generate a fairly uniform magnetic field in the middle of the coils where a platform was placed to fit onto the cell culture flask. The coils were made with 1 mm diameter copper wire and 400 turns. Coil radius was 10 cm. The Helmholtz coils were connected to a 2A power supply (PROMAX). With this arrangement we obtained a maximum magnetic field of 6 mT. A magnetic field sensor (LOHET II, Honeywell Control Systems) was located on the platform close to the cell culture flask and connected to an amplifier and digital multimeter (PROMAX).

For temperature control, we used a DS1620 Digital Thermometer and Thermostat (Maxim/Dallas Semiconductor), also placed on the platform close to the culture flask. This device can control temperature to within 0.1°C and is programmed to store the temperature each minute.

The coils with the flask and sensors were placed in an aluminium container with double closing and a small fan and lamp to lower or increase respectively the inner temperature controlled by the thermostat. The culture temperature was set at 37° C.

Cell culture

A rat Schwann cell line (RN 22) obtained from The European Collection of Cell Culture (ECACC) was used. The cells were grown in a 25 cm^2 culture flask (Falcon), in D-MEM low glucose with L-glutamine (Gibco) supplemented with 10% FBS (Gibco) and 1% penicillin/streptomycin (Gibco) in an incubator at 37°C, 100% humidity and 5% CO_2.

Magnetic field exposure

Twenty four hours before SMF exposure, $1.5x10^4$ cells were seeded in each culture flask. On exposure day, culture medium was replaced with Leibovitz L-15 medium CO_2 independent (Sigma), supplemented with 10% FBS (Gibco), 1% penicillin/streptomycin (Gibco) and 0.1% L-Glutamine (Sigma). Before seeding the cells, the flask bottom was marked using a rubber stamp with 24 circular zones, 2 mm in diameter. Three of these zones, numbers 2, 12 and 23, were photographed under phase-contrast before and after each experimental session with a NIKON Eclipse TE300 Inverted Microscope, equipped with a NIKON Dxm1200 digital Camera. These zones: anterior-left (num. 2), mid-centre (num. 12) and posterior-right (num. 23) were chosen considering the possible variability in cell beheaviour caused by the cell position inside the magnetic field.

We performed six experimental sessions. Each experimental session comprised two samples: control and exposed. The control sample was incubated for 24 h in a stove at 37°C in CO_2 free atmosphere without exposure to magnetic field. The exposed sample was subject, in the magnetic field generator, to 5 mT SMF at 37°C also for 24h. Once the three predetermined zones were photographed under phase-contrast microscopy, the samples were stained with Giemsa. The number of cells in the photographs was counted and the growth rate was obtained for each area dividing the number of cells after by the number before the experimental sessions. The results were contrasted by Student's T for paired samples considering a p-value <0.05 statistically significant.

Results

At the beginning of the experimental sessions, the RN 22 cells in both the control (Fig. 1A) and the experimental samples (Fig. 1C) were spindle-shaped with no preferential direction. The control samples were cultured for 24h without SMF exposure. After this period, the cell number increased with a mean growth rate of 1.96±0.33. These cells did not show any observable changes either in morphology or orientation (Fig.1B). After 24h exposure to 5 mT SMF, the experimental samples also increased in the number of cells with a mean growth rate of 1.84±0.45, differences with the control group not proving statistically significant. The morphology of exposed cells (Fig. 1D) was similar to that of the control group. We did not find any preferential orientation in the exposed cells (Fig. 1D). The differences in growth rate among the three studied zones were not statistically significant.

Discussion

These results agree with previous studies in which exposure to SMFs from 6 mT to 10 T does not alter cell cultures. Negative results were obtained from studies carried out on both tumor (Teodori et al., 2002a,b; Hirose et al., 2003) and non-tumor cell lines (Nakahara et al., 2002; Aldinucci et al., 2003; Schiffer et al., 2003). Based on these observations (Miyakoshi, 2005) in a recent review, considers that the evidence supports the idea that there are no lethal effects or alterations on cell growth or survival in culture under static magnetic fields up to 10 T.

However, changes in morphology, apoptosis, necrosis and cell proliferation have been described in cell cultures exposed to different SMF intensities. A decrease in apoptosis and cell proliferation with an increase in cell necrosis was observed in Vero cells exposed to 0.5 mT SMF, although, in the same conditions, rat astrocytes showed a significant increase

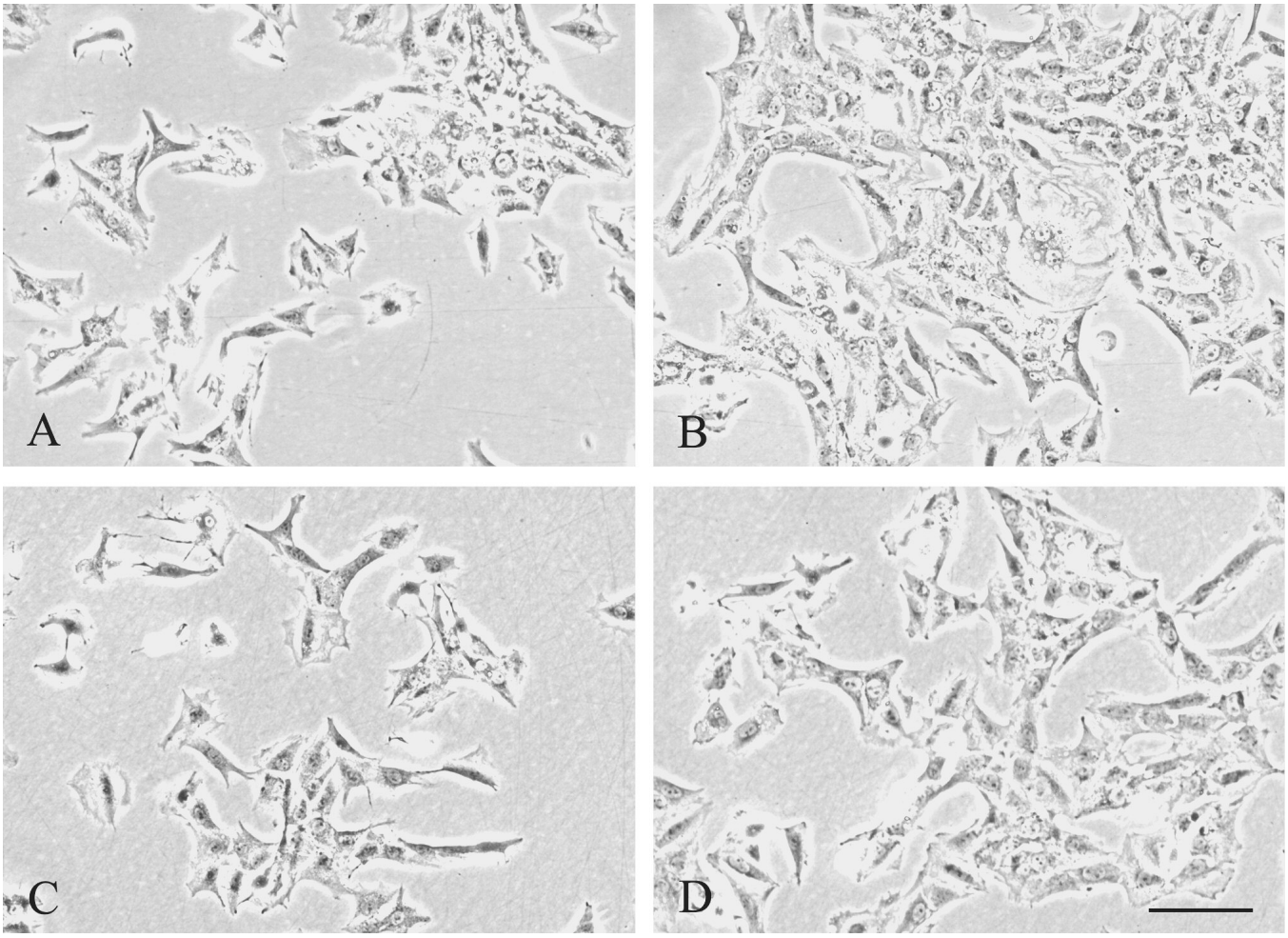

Fig. 1. Phase-contrast microscopy of cultured RN22 cells, non-exposed (**A and B**) and exposed to 5 mT for (**C and D**). The cells were photographed 24h after seeding (**A and C**) and 24h later (**B and D**). No significant differences in cell growth, morphology or orientation were observed between non-exposed and exposed cultures. Scale bar: 200 μm.

in these three parameters (Buemi et al., 2001). A reduction in viable cell numbers has been described in cancer cells in vitro exposed to 7 T SMF (Raylman et al., 1996). Moreover, Aldinucci et al. (2003) observed a statistically significant decrease in proliferation in Jurkat cells exposed to 4.75 T SMF, although normal human peripheral blood mononuclear cells similarly exposed did not display these effects. These results seem to indicate that tumour cells are sensitive but that normal cells are not. Accordingly, human peripheral blood mononuclear cells exposed to 10 T SMF did not show significant differences from non-exposed ones, although magnetic field exposure significantly increased cell death if cell division was previously stimulated with phytohemagglutinin (Onodera et al., 2003). However, in other experiments non-tumour cells such as human skin fibroblasts displayed modified cell morphology and a decrease in thymidine incorporation when cultured under 0.2 T SMF (Pacini et al., 2003). An increase in intracellular calcium has been proposed (Dini and Abbro, 2005) as a possible explanation for the disagreement on the bioeffects depending on cell type. An increase in intracellular calcium seems to produce different effects on different cellular systems, proving antiapoptotic on some cell types (Fanelli et al., 1999; Teodori et al., 2002a) and proapoptotic on others (Teodori et al., 2002b). Although our experiments did not reveal any observable changes in cell morphology, orientation or proliferation we do not rule out the occurrence of more subtle modifications or effects in this or other cellular systems.

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