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Effects of co-exposure to extremely low frequency (ELF) magnetic fields and benzene or benzene metabolites determined in vitro by the alkaline comet assay

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Abstract

In the present study, we investigated in vitro the possible genotoxic and/or co-genotoxic activity of 50 Hz (power frequency) magnetic fields (MF) by using the alkaline single-cell microgel-electrophoresis (comet) assay. Sets of experiments were performed to evaluate the possible interaction between 50 Hz MF and the known leukemogen benzene. Three benzene hydroxylated metabolites were also evaluated: 1,2-benzenediol (1,2-BD, catechol), 1,4-benzenediol (1,4-BD, hydroquinone), and 1,2,4-benzenetriol (1,2,4-BT). MF (1 mT) were generated by a system consisting of a pair of parallel coils in a Helmholtz configuration. To evaluate the genotoxic potential of 50 Hz MF, Jürkat cell cultures were exposed to 1 mT MF or sham-exposed for 1 h. To evaluate the co-genotoxic activity of MF, the xenobiotics (benzene, catechol, hydroquinone, and 1,2,4-benzenetriol) were added to Jürkat cells subcultures at the beginning of the exposure time. In cell cultures co-exposed to 1 mT (50 Hz) MF, benzene and catechol did not show any genotoxic activity. However, co-exposure of cell cultures to 1 mT MF and hydroquinone led to the appearance of a clear genotoxic effect. Moreover, co-exposure of cell cultures to 1 mT MF and 1,2,4-benzenetriol led to a marked increase in the genotoxicity of the ultimate metabolite of benzene. The possibility that 50 Hz (power frequency) MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Keywords: Extremely low frequency magnetic fields; ELF-MF; Benzene; Benzene metabolites; Primary DNA damage; Alkaline comet assay; Genotoxicity; Co-genotoxicity

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1. Introduction

During the last two decades, concerns have arisen regarding a possible association between exposure to extremely low frequency (ELF) electric and magnetic fields (EMF) and cancer incidence (e.g. childhood acute leukaemia, cancer of the nervous system, and lymphomas). Exposure to ELF-MF is mainly related to the generation, transmission, distribution, and use of electric energy. The frequency of electric power current is 50 Hz (in European and Asian countries) or 60 Hz (in North America). This range falls within the extremely low frequency (ELF) region of the electromagnetic spectrum (frequencies from 3 to 3000 Hz) (Poole and Ozonoff, 1996). As a consequence of the very large wavelengths (approximately 6000/5000 km at 50/60 Hz, respectively) in the ELF range, electric and magnetic fields (EF and MF, respectively) propagate effectively uncoupled (Polk and Postow, 1995). Moreover, EF is easily shielded by trees, walls, and other materials, whereas MF usually penetrates nonferrous material and readily enters the body. Thus, it is most likely that any biological effect is due to secondary currents induced in the body by the MF and recent researches have focused on potential adverse biological effects of exposure to MF.

Wertheimer and Leeper (1979) firstly reported an excess of cancer mortality among children living in homes located near power lines and presumably exposed to elevated MF. Subsequently, a large number of epidemiological studies investigated the possible association between residential or occupational exposure to ELF-MF and cancer (e.g. leukaemia, cancer of the central nervous system, and lymphoma). Some of these epidemiological studies did support the hypothesis of Werthemier and Leeper (Savitz et al., 1988; Feychting and Ahlbom, 1993), but others did not (Verkasalo et al., 1993; Coghill et al., 1996; Linet et al., 1997; Tynes and Haldorsen, 1997). Reviews and meta-analyses of epidemiological studies focused on the potential role of 50/60 Hz MF in the aetiology of cancer suggested that the epidemiological evidence to support the association between exposure to ELF-MF and the risk of childhood leukaemia is less consistent than what was observed in the mid 1980s, with an OR of about 1.5 (Portier and Wolfe, 1998; Repacholi and Greenebaum, 1999; Ahlbom et al., 2000). However, higher OR values were recently reported (Greenland et al., 2000).

In parallel with epidemiological studies, direct mechanistic studies using in vitro and in vivo model systems have been conducted with the effort to determine a link, if any, between MF and mutagenesis and to determine the possible mechanism of cancer risk. However, despite the large number of studies performed (comprehensive reviews in Juutilainen and Lang, 1997; Löscher and Liburdy, 1998; McCann et al., 1998; Moulder, 1998), the effect of ELF-MF on genotoxicity is still not clear. The IARC has recently conducted a comprehensive review of the results retrieved in the literature considering epidemiological reports, animal carcinogenicity data, as well as the outcomes of in vitro studies and proposed to apply the 2B category (possible human carcinogen) to ELF-MF (IARC, 2002).

The association between occupational exposure to benzene and an increased risk of leukaemia is well documented (IARC, 1987). However, the mechanism by which benzene exerts its long-term toxic effects, such as causing acute myeloid leukaemia and myelodysplastic syndromes in exposed workers, remains largely unclear (Smith, 1996; Yin et al., 1996). Exposure to benzene is strictly related to some industrial activities (e.g. coating applications, rubber, chemical, and shoe production). However, the presence of this xenobiotic in cigarette smoke and gasoline (i.e., especially after the reduction of lead content and the consequent decrease in octane number) renders benzene exposure an environmental as well as an occupational problem.

Benzene, which is not a direct mutagen, is metabolized in the liver by the activity of cytochrome P450 (CYP). However, none of the prominent metabolites of benzene are hard electrophiles. Benzene is first hydroxylated in the liver by the activity of CYP2E1 to yield phenol (Gut et al., 1996). When the activity of CYP2E1 results in the formation of benzene oxide, it can rearrange non-enzymatically to form phenol, or can lead to the formation of 1,2-benzenediol (1,2-BD, catechol). Phenol can be further hydroxylated to form catechol or 1,4-benzenediol (1,4-BD, hydroquinone), both of which can be further hydroxylated to 1,2,4-benzenetriol (1,2,4-BT). Moreover, 1,4-benzenediol may be oxidized to *p*-benzoquinone which can be reduced to 1,2,4-benzenetriol following a two-electron reduction catalyzed by DT-diaphorase (Snyder and Hedli, 1996). The ultimate metabolite, 1,2,4-benzenetriol, although formed in small quantities, is capable of potent toxic effects. This toxic metabolite