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Effects of electromagnetic pulse on polydactyly of mouse fetuses

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ABSTRACT

There is an increasing public concern regarding potential health impacts from electromagnetic radiation exposure. Embryonic development is sensitive to the external environment, and limb development is vital for life quality. To determine the effects of electromagnetic pulse (EMP) on polydactyly of mouse fetuses, pregnant mice were sham-exposed or exposed to EMP (400 kV/m with 400 pulses) from Days 7 to 10 of pregnancy (Day 0 = day of detection of vaginal plug). As a positive control, mice were treated with 5-bromodeoxyuridine on Days 9 and 10. On Days 11 or 18, the fetuses were isolated. Compared with the sham-exposed group, the group exposed to EMP had increased rates of polydactyly fetuses (5.1% vs. 0.6%, $P < 0.05$) and abnormal gene expression (22.2% vs. 2.8%, $P < 0.05$). Ectopic expression of *Fgf4* was detected in the apical ectodermal ridge, whereas overexpression and ectopic expression of *Shh* were detected in the zone of polarizing activity of limbs in the EMP-exposed group and in the positive control group. However, expression of *Gli3* decreased in mesenchyme cells in those two groups. The percentages of programmed cell death of limbs in EMP-exposed and positive control group were decreased (3.57% and 2.94%, respectively, $P < 0.05$, compared with 7.76% in sham-exposed group). In conclusion, polydactyly induced by EMP was accompanied by abnormal expression of the above-mentioned genes and decreased percentage of programmed cell death during limb development.

1. Introduction

Modern technology has caused both the types and intensities of electromagnetic radiation (EMR) to increase rapidly. Every day, humans are exposed to various artificial sources of EMR. An electromagnetic pulse (EMP) is a high-energy electromagnetic wave with an extremely fast rising time and a broad bandwidth [1]. There are many sources of EMP signals, including strong electrical field apparatuses, such as high-pressure gas switches and Tesla transformer generators under certain occupational conditions. In addition, EMPs are used extensively in medical, security screening, and military applications [2,3]. The distinguishing properties of EMP have raised concerns regarding their biological effects and possible health hazards to humans, especially to some workers and researchers who are regularly exposed. Different countries and associations have issued different recommendations regarding reasonable limits to exposure. For example, in occupational cases, the Institute of Electrical and Electronics Engineers limits whole-body exposure (including exposure of the head) to 100 kV/m [4]. However, the International Commission on Non-Ionizing Radiation Protection reference level for occupational exposure to electric fields that vary over time is 137 kV/m [5].

There is increasing interest in the link between EMR and human health [6,7]. Many researchers have suggested a link between EMR and various adverse effects on the

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nervous, endocrine, cardiovascular, and reproductive systems [8–11]. Some reports have also described teratogenicity, including polydactyly, when pregnant animals were exposed to EMR [12]. However, the specific mechanism underlying EMR-induced polydactyly has not been investigated. In the present study, pregnant mice were used as a model to explore mechanisms of EMP-induced polydactyly. In this study, pregnant mice were exposed to EMP during the period of organogenesis, which is highly sensitive to teratogens [13].

Developmental systems of vertebrate limbs are accurately controlled by complex cellular and molecular mechanisms, depending on reciprocal interactions between the two signaling centers, namely the zone of polarizing activity (ZPA) and the apical ectodermal ridge (AER). In these two centers, sonic hedgehog (SHH) and fibroblast growth factors (FGFs), respectively, are the key signaling molecules [14].

It is well established that Shh is associated with patterning of the anterior-posterior (A-P) axis and that it mediates the activity of the ZPA. It is also necessary for continuous limb growth. Interactions of Shh with AER help to establish all three of the limb axes (proximal-distal axis, anterior-posterior axis, and dorsal-ventral axis). The sonic hedgehog–Patched–Gli (Shh-Ptch-Gli) pathway begins with the secreted protein Shh, which initiates a chain of events in target cells that leads to the activation and repression of target genes by transcription factors in the Gli family [15]. Although Gli1 and Gli2 are apparently indispensable during limb development, Kruppel family member 3 (Gli3) is especially crucial because all Gli3-associated human congenital diseases comprise limb malformations. *Shh* downregulates *Gli3* expression in mesenchymal cells of the developing limb bud [16].

Proximal-distal limb outgrowth is controlled by the AER that expresses members of the FGF family. Experiments on chicks suggested that *Shh* expression in the ZPA is maintained by *fibroblast growth factor 4* (*Fgf4*) expression in the AER, and vice versa, providing a molecular mechanism for coordinating the activities of these two signaling centers. The AER factor *Fgf4* can be induced by ectopic expression of *Shh* [17]. This SHH/FGF4 feedback loop model is supported by genetic evidence showing that *Fgf4* is not expressed in $Shh^{-/-}$ mouse limbs [18].

Embryonic development is dynamic, encompassing a continuous progression of cell division, movement, differentiation, and death. Abnormal regulation of this process is associated with a wide variety of human diseases, including immunological and developmental disorders, neurodegeneration, and cancer [19]. Many researchers have reported that programmed cell death (PCD) plays a crucial role in organogenesis and tissue remodeling. During digit formation in higher vertebrates, PCD eliminates interdigital webs, primarily via apoptotic machinery [20]. However, exaggerated or defective PCD during embryogenesis may cause developmental abnormalities. Nakamura et al. [21] reported that 5-bromodeoxyuridine (BrdU) changed PCD and gene expression during limb development and induced time-specific limb malformations during fetal development. EMR has been reported to induce cell death in several *in vitro* studies [22,23].

The main goal of the study was to explore the effects of EMP exposure on the development of mouse limbs and to evaluate whether polydactyly was associated with PCD or abnormal gene expression. In this study, BrdU was used as a positive control to observe the proportion of PCD and the gene expression of *Fgf4*, *Shh*, and *Gli3*, which are related closely to vertebrate limb development.

2. Materials and methods

2.1. Animals

Kunming mice, 120 females and 60 males, weighing 25 to 30 g, were purchased from the Experimental Animal Center, Fourth Military Medical University, China. All studies were performed with the approval of the experimental animal care committee of the Fourth Military Medical University. The temperature and relative humidity of the animal room were 21 ± 1 °C and 60 ± 7%, respectively. The room was illuminated with artificial light for 12 hours daily and was dark for 12 hours at night. The animals were allowed free access to standard food pellets and tap water.

Female and male mice were caged together at a ratio of 2:1. Every morning, the female mice were examined for vaginal plugs. When a vaginal plug was detected, the day was designated as Day 0 of pregnancy. Mice with vaginal plugs were randomly allocated into three groups: sham-exposed group, positive control group, and EMP-exposed group. In the positive control group, mice were treated with 300 mg/kg of BrDU intraperitoneally on Days 9 and 10 of pregnancy.

2.2. EMP exposure

The EMP simulator comprised four parts: generator, gigahertz transverse electromagnetic mode cell, operation box, and field intensity monitor. During exposure, the free-moving mice were kept in a pure plastic cage. The device was as described by Lihua Zeng [24]. Mice in the EMP-exposed group were exposed to 400 pulses of 400 kV/m daily from Days 7 to 10. Sham-exposed mice were placed under identical conditions for the same interval, but they were not exposed to EMP.

2.3. Experimental protocols

Two sets of experiments were conducted to study the effects of EMP exposure on limb development of mouse fetuses and to identify probable underlying mechanisms. In the first part of our study, 60 female mice with vaginal plugs were randomly allocated into three groups. Morphological analysis of polydactyly was performed using the Alcian blue–Alizarin red staining method on Day 18. During the second part of the study, the levels of *Fgf4*, *Shh*, and *Gli3* expression were detected using whole-mount *in situ* hybridization (WHISH), and the difference in the proportion of PCD cells between the three groups was investigated using flow cytometry. In each of the experiments of this part, six pregnant mice from each group and six fetuses from each pregnant mouse were evaluated.