Reproductive Biology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Reproductive Biology

journal homepage: www.elsevier.com/locate/repbio

The pesticide Lindane induces dose-dependent damage to granulosa cells in an *in vitro* culture

Maria Grazia Palmerini[a,*], Gulmira Zhurabekova[b], Aru Balmagambetova[b], Stefania Annarita Nottola[c], Selenia Miglietta[c], Manuel Belli[a], Serena Bianchi[a], Sandra Cecconi[a], Valentina Di Nisio[a], Giuseppe Familiari[c], Guido Macchiarelli[a]

[a] *Dept of Life, Health and Environmental Sciences, University of L'Aquila, L'Aquila, Italy*
[b] *Dept of Normal and Topographical Anatomy, West Kazakhstan Marat Ospanov State Medical University, Aktobe, Kazakhstan*
[c] *Dept of Anatomy, Histology, Forensic Medicine and Orthopaedics, La Sapienza University of Rome, Rome, Italy*

ARTICLE INFO

Keywords:
Lindane
Reproductive toxicity
Granulosa cells
In vitro culture
Ultrastructure

ABSTRACT

Lindane, which is one of the most persistent organochlorine pesticide contaminating the Aral Sea region, is associated with numerous pathologies of the female reproductive system, including infertility, due to its gap junction blocker activity. By using an *in vitro* model of reproductive toxicity consisting of mouse parietal granulosa cells (GCs) exposed to increasing concentrations of Lindane ranging from 1 to 100 μM (L1; L10; L100), we aimed to ascertain the Lindane toxicity by evaluating the ultrastructure and expression of the cell death protein p53. GCs exposed to L1 showed an early sign of degeneration as chromatin marginalization and initial reduction of cell-to-cell contacts. Such effects increased at L10 with nuclear membrane invagination, cytoplasmic blebbing, reduction of microvilli and intercellular connections. L100 induced evident cellular damages with an extensive presence of vacuoles, cytoplasmic fragments, nuclear membrane vesiculation and abundant cellular debris. A dose-dependent increase of p53 expression was evident in the L1 and L10 groups but not in L100. These data provide evidence for a dose-dependent reproductive toxicity of the gap junction blocker Lindane, as seen in mouse GCs cultured *in vitro* by ultrastructural damage compatible with apoptosis. Since gap junctions may play a critical role in FSH-stimulated progesterone production, the ultrastructural damage here evidenced could explain the increase in the prevalence of reproductive pathologies and infertility in exposed women. Finally, this study provided a useful and repeatable model of reproductive toxicity *in vitro*, which is applicable to evaluate the detrimental effects of toxicants or the reversing effect of protective substances.

1. Introduction

The disastrous retraction of the Aral Sea began in the 1960s because of excessive water consumption, which was mainly for cotton field irrigation. Thousands of tons of pesticides, including organochlorines (OCPs), were extensively used in Karakalpakstan, which is now the most polluted near the Aral Sea [1]. OCPs, as persistent lipophilic chemicals, accumulate in the adipose tissue and jeopardize reproductive and developmental systems [2]. Ovarian follicle GCs are a highly susceptible target of pesticides with a consequent alteration of steroidogenesis [3,4]. Major pollutants found in the maternal/cord blood and milk from Karakalpakstan women are the OCP residues of Lindane (α-HCH and β-HCH), DDTs and dioxins [5]. Lindane is classified as an endocrine disruptor [6] and an intra-uterine growth retardant, and preterm births and unfavorable outcomes of pregnancy and birth have been correlated to its chronic bioaccumulation [7,8].

Lindane is a Cx43 gap-junction blocker in GCs [9], which can alter folliculogenesis by abolishing the oocyte-directed follicle-organizing activity [10]. In bovines, it was shown to affect the oocyte maturation rate *in vitro* in a dose-dependent manner and even if effects were at a low concentration (7.25 μg/ml) they were revealed only in late embryonic development [11]. *In vitro*, concentrations ranging from 10 to 100 μM altered first meiotic spindle formation and polar body extrusion in mice [12]. Exposure of developing oocytes *in vivo* led to an increase of irreversible damages in two-cell embryos [13]. A marked reduction of female and male germ cell formation was found *in vivo* and *in vitro*, which was perhaps *via* apoptotic cell death [14]. Reproductive toxicity is probably a consequence of the gap-junction blocker activity on GCs or the ability to alter intracellular calcium homeostasis, maturation promoting factor (MPF) activity and formation of the bipolar mitotic

* Corresponding author.
E-mail address: mariagrazia.palmerini@univaq.it (M.G. Palmerini).

http://dx.doi.org/10.1016/j.repbio.2017.09.008
Received 18 June 2017; Received in revised form 28 September 2017; Accepted 30 September 2017
1642-431X/ © 2017 Society for Biology of Reproduction & the Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn. Published by Elsevier Sp. z o.o. All rights reserved.

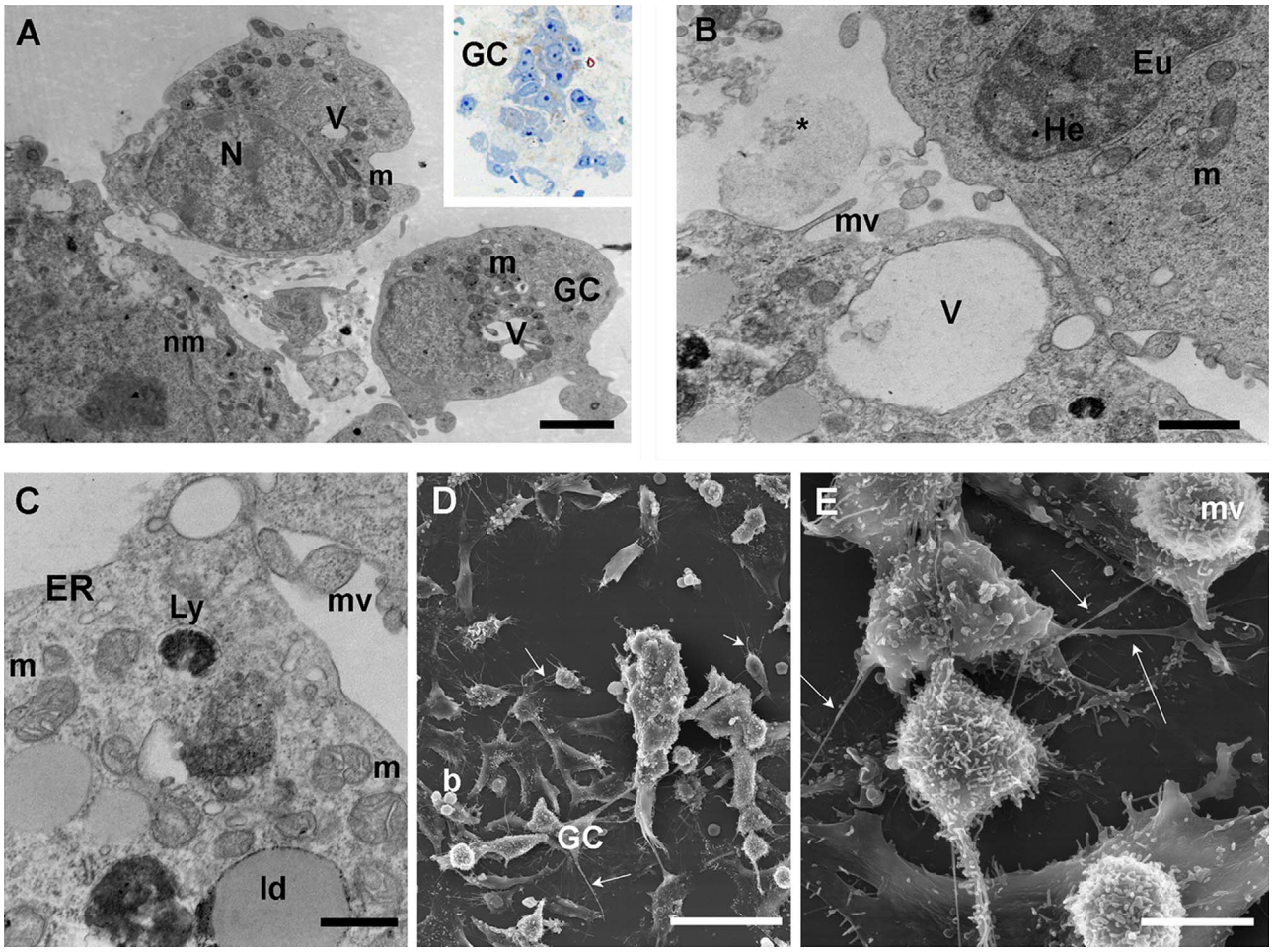

Fig. 1. ***Control.*** **A**. The ultrastructure of granulosa cells (GCs) with a large nucleus (N) delimited by a continuous nuclear membrane (nm), numerous round/ovoid mitochondria (m) and vacuoles (V) (TEM. Bar: 2 μm). ***Inset in A:*** A representative image of a semithin section of GCs (LM. Mag: 40×). **B**. Nuclear content of Heterochromatin (He) and Euchromatin (Eu). Long and thin microvilli (mv) and a large vacuole (V) are visible. M: mitochondria; *: atresic GCs showing signs of physiological turnover (TEM. Bar: 800 nm). **C**. lysosomes (Ly), lipid droplets (ld), and endoplasmic reticulum (ER); mv: microvilli (TEM. Bar: 400 nm). **D**. Adjacent GCs connect each other by means of pseudopodia (arrows); b: blebbing. The surface shows densely packed microvilli (mv) (SEM. Bar: 40 μm) magnified in **E** (SEM. Bar: 8 μm).

spindle [9,15]. Starting from this assumption and considering that the above cited studies referred to oocyte toxicity, we focused on the morphological effects on the somatic nourishing compartment of the mammalian follicle, whose action is functional in the FSH *plus* TGF beta1-stimulated progesterone production *via* the Connexin43 gap junction [9]. Therefore, by using one of the best models of reproductive toxicity *in vitro*, we aimed to investigate the ultrastructure of mouse GCs when cultured with an increasing concentration of Lindane (1–100 μM) though the use of light (LM), transmission (TEM) and scanning (SEM) electron microscopy and to correlate morphological data with the expression of the cell death protein p53.

2. Materials and methods

2.1. Chemicals

All of the materials were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless stated otherwise.

2.2. Animals and experimental design

Mice were housed in the animal facility under controlled temperature (21 ± 1 °C) and light (12 h light/day) conditions with free access to food and water. Prepubertal females (21 to 23 days old), were treated i.p. with 5IU of PMSG (pregnant mare serum gonadotropin). After 48 h, the animals were sacrificed by cervical dislocation [16]. For each experimental condition, at least 10 animals were used and each experiment was repeated at least three times. The local committee on "animal care and use" approved the experimental protocols, which were also compliant with the international standards of animal care and veterinary medical practice.

2.3. GC isolation and experimental protocol

The ovaries were collected, washed in PBS (phosphate buffered saline, pH = 7–7.4) at 37 °C and transferred into culture dishes (Becton Dickinson and Company, Franklin Lakes, NJ, USA) containing MEM Hepes (Life Technologies Italy, Monza MB, Italy). The ovaries were punctured with insulin syringe needles to release the mural GCs. GCs were then transferred into culture dishes for *in vitro* culture (IVC) [4]. We assessed Lindane toxicity from 1 to 100 mM [3] with the following experimental groups: control, control in vehicle, Lindane 1 μM (L1), Lindane 10 μM (L10), and Lindane 100 μM (L100).

2.4. In vitro culture (IVC) of mouse GCs

Aliquots of viable GCs (3 ml, ~5×10^3 cells) were cultured in DMEM (Dulbecco's modification of Eagle's medium, GE Healthcare, Little Chalfont, Buckinghamshire, UK) containing 5% FBS (Fetal Bovine Serum) supplemented with 2 mM L-glutamine and antibiotics (100 mM

Download English Version:

https://daneshyari.com/en/article/8393207

Download Persian Version:

https://daneshyari.com/article/8393207

Daneshyari.com