Contents lists available at ScienceDirect

Toxicology Reports

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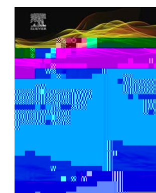

Effects of endosulfan isomers on cytokine and nitric oxide production by differentially activated RAW 264.7 cells

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A R T I C L E I N F O

Keywords:
Endosulfan
Macrophage
Inflammation
TNF
Nitric oxide

A B S T R A C T

Endosulfan is an organochlorine insecticide comprised of two isomers: endosulfan-α and endosulfan-β. Endosulfan exposure has been shown to elevate some inammatory factors, such as nitric oxide (NO) and tumor necrosis factor (TNF), in animals or cultures of animal cells. Because the two endosulfan isomers can vary in their biological activities, the goal of this study was to determine if individual endosulfan isomers differentially impact production of NO or TNF by the mouse macrophage cell RAW 264.7 at non-cytotoxic levels. We found elevated TNF with exposure to endosulfan- (not endosulfan-), but only at concentrations that were cytotoxic (100 μM), whereas neither endosulfan isomer altered baseline levels of NO at any concentration up to 300 μM. In interferon (IFN)-γ-activated cultures, NO levels were significantly suppressed by either endosulfan isomer at 10 μM (the lowest concentration examined), whereas only endosulfan- significantly lowered TNF levels at non-cytotoxic concentrations. In lipopolysaccharide (LPS)-activated cultures, both endosulfan isomers significantly reduced NO, but not TNF, at non-cytotoxic concentrations. These results suggest that the endosulfan isomers have some capacity to alter inflammatory responses differentially, particularly with IFN-γ stimulation.

1. Introduction

Endosulfan is an organochlorine insecticide of the cyclodiene group that typically contains two isomers: endosulfan-α (endosulfan I) and endosulfan-β (endosulfan II). Adverse effects have been reported in non-target species including neurological toxicity, endocrine disrup-

2. Material and methods

2.1. Cell culture and chemical exposures

Mouse RAW 264.7 cells (ATCC) were cultured at 37 °C in humidi ed air plus 5% CO_2 in medium comprised of DMEM (Life Technologies, cat #11965-092) plus 10% heat-inactivated fetal bovine serum (Hyclone), penicillin/streptomycin (100 U/100 g per mL), and sodium pyruvate (1 mM). Endosulfan- and endosulfan- (ChemService) were dissolved separately in dimethylsulfoxide (DMSO) for stock solutions at 100 mM which were stored at Š 20 °C until needed. Dilutions of endosulfan in DMSO were prepared fresh for each experiment such that addition of endosulfan to culture medium resulted in a uniform nal DMSO concentration of 0.1% (v/v) across exposure levels. Within each experiment, cultures contained medium, DMSO, or endosulfan at concentrations of 10, 33, 100 or 300 M. Lipopolysaccharide (LPS, E. coli 055:B5) and/or mouse interferon gamma (IFN-) were added to some cultures at 100 ng/mL or 6 ng/mL, respectively. Exposure to endosulfan, LPS and/or IFN- occurred simultaneously and for a duration of 24 h with a minimum of two replicate cultures for each condition.

2.2. Cytotoxicity assays

The reductive metabolism of cells, an indicator of cytotoxicity, was measured using a WST-1 cell cytotoxicity kit using the manufacturer's protocol (G-Biosciences). Brie y, 4.2×10^4 RAW 264.7 cells were added to wells of 96-well tissue culture plates and allowed to adhere overnight. The medium in each well was then replaced with 100 L of medium containing medium only, DMSO, or endosulfan. One L of LPS and/or 1.0 L of IFN-

LLP6352.7 (on7-4re)-389.6 (LPntaining)-288.9 96O,on8tainingovernulfan. si8n 67nntasubtna36containixicity si6nor so2nTh

concentration group (for each isomer) were pooled prior to statistically testing for differences across endosulfan concentrations. When compared to DMSO controls, endosulfan- was found to cause significant and concentration-dependent cytotoxicity at concentrations of 100 μM and higher (Fig. 1B). In contrast, endosulfan- exposure significantly enhanced formazan production (negative cytotoxicity) at low concentrations (10–33 μM). Relative to those low concentrations, 300 μM endosulfan- caused significantly increased cytotoxicity that was not different from controls.

3.2. Nitric oxide production

In the absence of activation with IFN-γ or LPS, NO production in control cultures (DMSO) was very low with nitrite levels reaching approximately 0.4 μM after 24 h (Fig. 2A and B). In the presence of either IFN-γ or LPS, the nitrite levels of control cultures were significantly increased by approximately 23-fold to an average of 8.8 μM. In the presence of both IFN-γ and LPS, nitrite levels were further significantly increased over 100-fold to approximately 35 μM. In the absence of stimulation, NO production was unchanged by endosulfan exposure. In contrast, both endosulfan isomers significantly reduced IFN-γ-stimulated NO production beginning at the non-cytotoxic 10 μM concentration and reaching a maximal effect at 100 μM (93% and 70% reduction, respectively). A similar but less potent impact of the different endosulfan isomers on NO production was observed in LPS-treated cultures and in cultures containing both IFN-γ and LPS.

3.3. TNF production

In the absence of activation with IFN-γ or LPS, low levels of TNF were detected (200 pg/mL) in control cultures (DMSO) (Fig. 2C and D). IFN-γ alone significantly increased TNF levels approximately 13-fold on average, and LPS alone significantly increased TNF levels more than 200-fold. When combined with LPS, IFN-γ stimulation did not significantly elevate TNF levels beyond that seen with LPS alone.

Endosulfan- exposure significantly altered TNF levels in both stimulated and unstimulated cultures, but only at concentrations that were significantly cytotoxic (100 μM and higher). It is unclear if these changes in TNF production were a cause or a consequence of that cy-

that 24 h exposures to non-cytotoxic concentrations of endosulfan (up to 10 M) caused significantly elevated levels of activated nuclear factor kappa-light-chain-enhancer of activated B cells (NF- B) and higher levels of NO, TNF and IL-6 [7]. A follow-up study by Kim et al. [8] showed that non-cytotoxic concentrations of endosulfan caused significantly elevated levels of reactive oxygen species which they suggested as a driving mechanism for the coincidently increased levels of activated NF- B, activator protein 1 (AP-1) and other inflammation-associated transcription factors [8]. Using RAW 264.7 cells, we found that individual or isomers of endosulfan caused no significant changes in NO levels after 24 h of exposure, even at cytotoxic con-

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