Toxicology and Applied Pharmacology 349 (2018) 55–61

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

Glyphosate affects the secretion of regulators of uterine contractions in cows while it does not directly impair the motoric function of myometrium *in vitro*

Michael Hubert Wrobel

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tuwima Street 10, 10-748 Olsztyn, Poland

ARTICLE INFO

Keywords:
Herbicide
Glyphosate
Roundup
Ovary
Uterus
Cow

ABSTRACT

The effects of pure glyphosate and its most popular product (brand name: Roundup) on the secretion of hormones involved in the regulation of myometrial contractions as well as their direct effects on myometrial contractions were examined. Myometrial strips as well as uterine and ovarian cells were taken from cows during the oestrous cycle and they were treated with both compounds at concentrations from their environmental range. Glyphosate stimulated the secretion of oestradiol from granulosa cells while both herbicides increased and decreased oxytocin (OT) and progesterone secretion from luteal cells respectively. However only Roundup stimulated mRNA expression of the precursor of OT. Both compounds decreased the secretion of prostaglandins from endometrial cells while they exerted no effect on the basal and OT-stimulated force of myometrial contractions. The studied herbicides did not directly impair the motoric function of the myometrium. However our data indicate the potential of these compounds to disturb the secretory functions of the ovaries and uterus which can lead to the deregulation of uterine contractions and to the impairment of fertilisation or to difficulties in the maintenance of gestation.

List of abbreviations

AA	arachidonic acid
Act D	actinomycin D
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DMSO	dimethyl sulfoxide
E2	oestradiol
KRS	Krebs-Ringer's solution
MTT	tetrazolium salt
NP-I/OT	neurophysin-I/OT
OT	oxytocin
P4	progesterone
PGA	peptidyl-glycine-α-amidating monooxygenase
PG(s)	prostaglandin(s)
PGFM	13,14-dihydro-15-keto-PGF2α
TBP	TATA box binding protein

1. Introduction

Despite the protective effect of pesticides on crops animal fodder and water can be contaminated with their residues. Admittedly modern pesticides reach their target organisms effectively but they also inadvertently infiltrate non-target organisms. The bioaccumulation of pesticides in living bodies can alter the function of the endocrine system by binding with oestrogen (ER)androgen, (AR)arylhydrocarbon, (AhR) orphan, Steroidogenic Factor (SF-1), active/androstane receptor (CAR), pregnane X receptor (PXR) or other receptors (Andersen et al., 2003; Lemaire et al., 2006; Mlynarczuk et al., 2013; Xia et al., 2017). Hence it can lead to various chronic illnesses including reproductive disorders (Stefansdottir et al., 2014). Glyphosate [*N*-(phosphonomethyl) glycine] is one of the most broad-spectrum pesticides and one of the most used herbicides in global agriculture (Cai et al., 2017). Glyphosate which is never used alone in agriculture (Defarge et al., 2018) is the active substance in > 750 commercial formulations (Benbrook, 2016). Among them it is the primary active ingredient in Roundup-branded herbicides produced by Monsanto while the final composition is often confidential business information (Cai et al., 2017; Defarge et al., 2018). The uses of Roundup includes weed control in agriculture vegetation control in non-agricultural areas and harvesting aids as crop desiccants (Tarazona et al., 2017). Therefore it is no wonder that there are reports that confirm the existence of glyphosate or products of its degradation in plants (Barroso et al., 2018) water (Desmet et al., 2016) and soil (Napoli et al., 2016) in agricultural areas. The highest glyphosate contents were found in the smallest soil fractions which are easily inhaled. Hence the risk of glyphosate transport in dry sediment to off-target areas by wind can also be very high (Bento et al., 2017).

E-mail address: m.wrobel@pan.olsztyn.pl.

https://doi.org/10.1016/j.taap.2018.04.031
Received 11 December 2017; Received in revised form 19 April 2018; Accepted 24 April 2018
Available online 26 April 2018
0041-008X/

Moreover they both have been detected in samples of bovine and human milk (Jensen et al., 2016) as well as in the serum of women (Kongtip et al., 2017). It is not surprising that the effect of glyphosate has been the subject of regular assessments by national and international regulatory agencies (Tarazona et al., 2017). Admittedly glyphosate has a relatively low toxicity in mammals (Tarazona et al., 2017) but it was described as probably carcinogenic for humans (Guyton et al., 2015) and mice (George et al., 2010). While its potential risk for reproduction needs further evaluation (de Araujo et al., 2016).

Environmentally relevant and low concentrations of glyphosate possessed oestrogenic activity (Thongprakaisang et al., 2013; Mesnage et al., 2017). Oestradiol (E2)which secretion in bovine granulosa cells is stimulated by FSH (Zheng et al., 2008)affects smooth muscle contractions directly since it induces uterine motility *in vivo* in cows (Hawk, 1983) and increases the force of bovine myometrial strips contractions *in vitro* (Wrobel et al., 2005). MoreoverE2 also affects myometrial contractions indirectly since it increases the synthesis of receptors for prostaglandin (PG) F2α and oxytocin (OT) (Richter et al., 2004; Huang et al., 2015). BothPGF2 and OT are the most potent stimulators of uterine motility (Ruckebusch and Bayard, 1975; Fuchs et al., 1992; Olson, 2003). Prostaglandins originate from arachidonic acid (AA; Goff, 2004) while neurophysin-I/oxytocin (NP-I/OT) and peptidyl glycine-amidating monooxygenase (PGA) are involved in the first and final steps of OT synthesis respectively (Sheldrick and Flint, 1989). In contrast luteal secretion of progesterone (P4) which is enhanced by LH (Goravanahally et al., 2007)abolishes the effect of OT on the motor activity of the uterus. It is followed by uterine quiescence during gestation (Lye and Porter, 1978) while the stimulation of PGE2 receptors promotes uterine relaxation before parturition (Olson, 2003).

Previously the effect of chlorinated pesticides which were removed from US and European markets was examined in detail (Mlynarczuk et al., 2010; Wrobel et al., 2009, 2014, 2015; Wrobel and Mlynarczuk, 2017) and the model for the study of the effect of modern pesticides on the bovine reproductive system was constructed. The aim of this study was to investigate whether glyphosate and its most popular trade product (Roundup) affect myometrial contractions and what is their effect on the ovarian and uterine secretion of direct (OT and PGs) or indirect (E2 and P4) regulators of uterine motility.

2. Material and methods

2.1. Chemicals

Glyphosate and Roundup (No. 101667948A2 < 140720 > Monsanto Europe) were dissolved (0.11 or 10 ng/ml final concentrations at culture media) in dimethyl sulfoxide (DMSO; HPLC purity grade). The final concentration of DMSO in the culture media did not exceed 0.1%. Hence the control samples were supplemented with 0.1% DMSO. All materials used in these studies were purchased from Sigma-Aldrich (PL) unless otherwise stated. Each medium was supplemented with antioxidants: ascorbic acid (20 μg/ml; Merck USA) sodium selenite (5 ng/ml; ICNUSA) and transferrin (5 μg/ml)and antibiotic: amphotericin (2 μg/ml) and gentamycin (20 μg/ml). The media did not contain phenol red.

2.2. Collection and preparation of material

Ovaries and uteri from cows between the 8th and 12th days of the oestrous cycle were obtained in a commercial slaughterhouse. The stage of the oestrous cycle was identified as described by Fields and Fields (1996). Myometrial strips of longitudinal smooth muscle layer (6–7 mm long and 3–4 mm wide; four from each cow) were immersed in 2 ml of aerated (95% air and 5% $CO_2$4°C) physiologic salt solution (116 mM NaCl4.6 mM KCl 1.16 mM $NaH_2PO_4{\cdot}H_2O$ 1.16 mM $MgSO_4 \times 7H_2O$ 21.9 mM $NaHCO_3$ 1.8 mM $CaCl_2{\cdot}2H_2O$ 11.6 mM dextrose 0.03 mM CaNa EDTA; pH = 7.4)as described by Wrobel et al. (2005, 2015). Granulosa cells were obtained by vigorous aspiration of follicular fluid from 10 to 15 follicles (> 1 cm in diameter) for each experiment. The pull of cells from 2 to 4 corpa lutea for each experiment were obtained by perfusion with collagenase as described by Okuda et al. (1992). Myometrial and endometrial cells were obtained by enzymatic dispersion according to Wrobel et al. (2015). Cell viability (only above 80% was used for further studies) was estimated by exclusion of 0.04% trypan blue dye. The cell suspensions were transferred into plates (for the studies of the cytotoxic effect of the herbicides and hormone determinations: 2×10^5/ml of cells 48-wells plates; for measurement of mRNA expression: 5×10^5/ml of cells 6-wells plates; Nunclon Δ-Surface NUNCNL). Cells were pre-cultured (95% air and 5% $CO_2$100% humidity38°C; Memmert INCO 180D) for 24 h (granulosa and luteal cells) 72 h (endometrium) or 96 h (myometrium) to allow them to attach to the bottom of the wells. Next the cells were washed twice with M199 and the medium was replaced with DMEM/HAM-12 supplemented with 0.1% BSA.

2.3. Determination of the amount of glyphosate in the myometrium

The amount of glyphosate in the studied myometrial strips was commercially determined at the Plant Protection Institute National Research Institute Laboratory of Pesticide Residues(Bialystok Poland) by QuEChERS with modifications (Lozowicka et al., 2016).

2.4. The effect of herbicides on the viability of cultured cells

Cell viability was measured after incubation (72 h 38 °C) of granulosa (n = 5)luteal (n = 6) myometrial (n = 5)or endometrial (n = 4) cells with glyphosate or Roundup (both at the highest dose used here of 10 ng/ml). Each treatment was performed in quadruplicate. According to description of Actinomycin D (Act D; No. A1410 SIGMA) it inhibits RNA synthesis and in this way Act D reduces the synthesis of proteins including dehydrogenases and greatly reduces the survival of cells. Hence Act D (500 ng/ml) was used as a positive control similar to previous studies (Wrobel et al., 2009, 2015).

2.5. The effect of herbicides on hormone secretion from ovarian and endometrial cells

The granulosa luteal and endometrial cells (each n = 5) were treated (48 h) with glyphosate or Roundup (each at dose of 0.11 or 10 ng/ml). FSH (AFP-5679C 100 ng/ml) LH (AFP-11743B 100 ng/ml) and AA (20 μg/ml) were used as positive controls respectively. Each treatment was performed in duplicate. The media were stored (−20 °C10 μl of 0.3 M EDTA in 1% acetylsalicylic acid; Meyer et al., 1989) for determination of E2 in granulosa cell culturesP4 and OT in luteal cell cultures and PGFM (the main metabolite of PGF2α) and PGE2 concentrations in endometrial cell cultures.

2.6. The effect of herbicides on expression of mRNA for genes involved in OT synthesis in luteal cells

Luteal cells (n = 4) were incubated (24 h) with glyphosate and Roundup (10 ng/ml) in duplicate according to Wrobel et al. (2015). Next the cells were covered with Phenozol (300 μl for each well; A&A Biotechnology PL) and real-time PCR analysis of the mRNA expression of NP-I/OT and PGA was performed.

2.7. The effect of herbicides on myometrial contractions

Myometrial strips were incubated (48 h) with glyphosate (n = 4 cows) or Roundup (n = 4 cows) each at the dose of 0.11or 10 ng/ml. Next the force of spontaneous and OT-stimulated (10^{-7} M) myometrial contractions was recorded as described by Wrobel et al. (2005).