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The effect of glyphosate-based herbicide Roundup and its co-formulant, POEA, on the motoric activity of rat intestine – *In vitro* study

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ABSTRACT

The study was aimed at evaluating the effect of Roundup, polyoxyethylene tallow amine (POEA) and mixture of glyphosate and POEA in different levels on the motoric activity of jejunum strips. The incubation in the Roundup solutions caused a significant, mostly miorelaxant, reversible reaction of smooth muscle; only in the highest tested dose which is equivalent to the agricultural concentration (1% corresponding to 1.7 g glyphosate/L) there was an irreversible disturbance of the spontaneous contractility and reactivity. The incubation in POEA solutions in the range of low doses (0.256; 1.28; 6.4 mg/L) resulted in a biphasic muscle reaction (relaxation and contraction); whereas in the range of high doses, *i.e.* 32; 160 and 800 mg/L (agricultural spray concentrations) induced only a miorelaxant, irreversible response. The results indicate very high toxicity of POEA which exceeds the toxicity of the commercial formulations. Besides, it is postulated that glyphosate and POEA may display antagonistic interaction towards the motoric activity of gastrointestinal tract.

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1. Introduction

Pesticides containing glyphosate (*N*-phosphonomethylglycine) are nowadays the most used herbicides worldwide. They gained tremendous popularity thanks to their high effectiveness in crop plants protection against weeds and low toxicity of the glyphosate towards mammals, including humans (Dill et al., 2010; Williams et al., 2000). However, during the last decades there has been a rising number of data revealing the possible toxicity of glyphosate and its commercial products on animals. Interestingly, the results of various experiments indicate biological activities of glyphosate containing pesticides and glyphosate itself that are not always possible to observe while conducting toxicological studies required for the registration of plant protection products (PPP) (Mesnage et al., 2015).

It is generally accepted that the toxicity of commercial glyphosate herbicides exceeds significantly the toxicity of glyphosate. This was confirmed in numerous *in vivo* and *in vitro* studies (Contardo-Jara et al., 2009; El-Shenawy, 2009; Howe et al., 2004; Mesnage et al., 2015; Richard et al., 2005). The exposure to high doses of Roundup-type products causes serious poisonings in human (Chang et al., 1999; Roberts et al., 2010; Stella and Ryan, 2004) although the toxicity of glyphosate alone towards mammals is very low. It is worth emphasizing that though glyphosate is approved as an active substance for the use in plant protection products (Commission Directive 2001/99/EC) and as such is widely considered, its activity towards the target organisms (weeds) is also questionable. This is due to the fact that the recommended agricultural dilutions of the herbicide formulations contain the active ingredient in a concentration exhibiting probably no herbicidal activity (Séralini, 2015) what suggests that glyphosate should be considered as a declared active ingredient. It is speculated that the higher toxicity of commercial products might result from the presence of so called inert ingredients (Brausch et al., 2007; Mann et al., 2009; Mesnage et al., 2015, 2014, 2013; Moore et al., 2012). Nowadays, one of the most commonly used herbicides is Roundup. It is an aquatic solution of glyphosate, used in the form of isopropylamine salt and other co-formulants which are confidential for regulatory purposes but certainly include polyethyloxylated tallow amine

Abbreviations: ACh, acetylcholine; POEA, polyethyloxylated tallow amine; Isop, isoproterenol; MK-HS, modifies Krebs-Henseleit solution; PPP, plant protection product.

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(POEA). The data obtained in numerous studies point out very high toxicity of POEA towards animals which exceeds clearly the toxicity of glyphosate and its commercial products (Folmar et al., 1979; Howe et al., 2004; Mesnage et al., 2015, 2013; Moore et al., 2012; Seok et al., 2011; Servizi et al., 1987; Tsui and Chu, 2003). The results of experiments on cell models and animals as well as the clinical signs observed in case of intoxication might indicate that POEA is the ingredient that is responsible for most of the toxic effects of glyphosate containing herbicides. Besides, this data points out the possible interactions between the declared ingredient and the co-formulant (Benachour and Seralini, 2009; Frontera et al., 2011; Guilherme et al., 2012; Kim et al., 2013; Mesnage et al., 2015; Song et al., 2012a).

The results of our previous studies revealed a significant effect of glyphosate on the motoric activity of the gastrointestinal tract (Chłopecka et al., 2014). The alterations of jejunal smooth muscle activity were observed, if glyphosate was used in doses reflecting its concentrations measured in the blood of human with only slight or no clinical symptoms of glyphosate poisoning (Aris and Leblanc, 2011; Roberts et al., 2010). Our interest in understanding the effect of glyphosate on gastrointestinal motoric activity results from a pharmacokinetic study which revealed that shortly after oral exposure over 30% of administered dose of glyphosate was found in the wall of small intestine. Consequently, the amount of glyphosate found there clearly exceeds the amounts found in other tissues (Brewster et al., 1991). Therefore, the next object of our studies included the verification of the effect of a commercial herbicide containing glyphosate and POEA on the motoric activity of gastrointestinal preparations, as well as the evaluation of the interaction between the declared active ingredient and the POEA towards the motoric activity of intestine smooth muscle.

2. Materials and methods

2.1. Chemicals and media

Acetylcholine chloride (ACh), isoproterenol hemisulfate (Isop), glyphosate (*N*-phosphonomethyl-glycine) (Sigma Chemicals Co, St. Louis, USA, CAS: 1071-83-6), Roundup ULTRA 170 SL (170 g of glyphosate in the form of isopropylamine salt/L and 80 g/L of POEA corresponding to 17% and 8%, respectively) (Monsanto Europe S.A./N.V., Antwerpia, Belgium), polyethyloxylated tallow amine containing oxide/tallow amine ratio: 15:1 (POEA) (Dr. Ehrenstorfer GmbH, Augsburg, Germany, CAS: 61791-26-2), $CaCl_2$ (Merck, Darmstadt, Germany), NaH_2PO_4 (Fluka Chemie, AG, Buchs, Switzerland), NaCl, KCl, $MgSO_4$, $NaHCO_3$ and glucose (Avantor, Gliwice, Poland) were used for preparing and conducting the experiments. Modified Krebs-Henseleit solution (MK-HS) containing NaCl (123.76 mM), KCl (5 mM), $CaCl_2$ (2.5 mM), $MgSO_4$ (1.156 mM), $NaHCO_3$ (14.5 mM), KH_2PO_4 (2.75 mM) and glucose (12.5 mM) was used as the incubation medium. MK-HS maintained the pH value of 7.35 (7.30–7.40) throughout long-term experiments while heated up to 37 °C and bubbled continuously with carbogen (95% O_2 + 5% CO_2). All substance used to the experiments were dissolved in MK-HS.

2.2. Animals

The experiments were carried out on intestinal strips isolated from male Wistar rats (weighting approx. 250 g). The use of animals, all procedures involving animals and their tissues were approved by the Local Ethics Committee (approval number 8/2011). The rats had free access to feed and water. The animals were euthanized in chambers filled with carbon dioxide (CO_2) (Everitt and Gross, 2006).

Table 1
The concentrations of formulants (glyphosate and POEA) at decreasing dilutions of Roundup ULTRA 170 SL.

Formulation/Formulant/	Units	Concentrations					
R-p	%	**100**[a]	1[b]	0.2	0.04	0.008	0.0016
G	g/L	**170**	1.7	0.34	0.068	0.014	0.03
POEA	mg/L	**8x10**^{4}	800	160	32	6.4	1.28

Note that in experiments with POEA the concentration of 0.256 and 0.051 mg/L were additionally tested.
G – glyphosate.
POEA – polyoxyethylene tallow amine.
R-p – Roundup.
[a] Concentrated formulation.
[b] Agricultural spray (in-use dilution).

2.3. Preparation of intestine strips and registration of their motoric activity

Immediately after rat's euthanasia the abdominal cavity was opened and fragments of jejunum were incised. Intestine strips were placed in warm (37 °C) MK-HS and prepared as described in former experiments (Chłopecka et al., 2007). The preparations were incubated in MK-HS in the chambers of Schuler Organ Bath set (Hugo-Sachs Elektronik, Harvard Apparatus, USA). The experiments were carried out under isotonic conditions, under a load of 0.5 g. The registration of the data was performed through a bridge amplifier (DBA, type 660, Hugo-Sachs Elektronik, Harvard Apparatus, USA) and PowerLab (ADInstruments, Australia). Subsequently, the graphical records were analyzed by Chart v7.0 program and Excel (MS Office XP Professional).

2.4. Design of experiments

Each experiment started with 60-min preincubation supplemented with three exchanges of the incubation medium every 20 min. Subsequently, the strips were exposed to two reference substances, ACh and Isop, used in the reference concentrations of 1 μM and 0.1 μM, respectively (Chłopecka et al., 2007). The response of strips to the reference substances was registered and MK-HS was exchanged. Once the spontaneous motoric activity of jejunum strips stabilized after flushing with MK-HS, the incubation chambers were filled with tested solutions. The intestine strips were incubated for 3–15 min in selected solutions of tested compounds and then flushed with fresh MK-HS. At the end of each experiment all preparations were re-exposed to ACh in the reference concentration in order to verify their viability. If it was necessary, tested solutions were buffered with 0.1 M sodium hydroxide to obtain a pH value of 7.35. In experiments aimed at evaluating the effect of Roundup non-buffered solution were used as well.

2.4.1. Experiments with Roundup

The incubation chambers were filled with Roundup solution at concentrations amounting to 0.003, 0.014, 0.068, 0.34, and 1.7 g of glyphosate, *i.e.* declared active ingredient (d.a.i.)/L. The range of tested doses corresponds to the highest environmental levels, concentrations found in human blood and finally, concentrations used for agricultural dilution. The highest used concentration of Roundup referred to the amount of glyphosate in the field dilution of the herbicide used at the low application rate (*i.e.* 1% of Roundup containing 170 g of glyphosate/L and 800 mg/L of POEA). The intestine strips were incubated in the presence of Roundup and then flushed with fresh MK-HS (pH 7.35). The dilutions of Roundup and concentrations of main formulants (glyphosate and POEA) used in particular treatments are presented in Table 1.

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