Sub-acute Effect of Glyphosate on Antioxidant Status and Lipids of Rat Brain

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Abstract

Roundup, a glyphosate-based herbicide is one of the most commonly used herbicides. Indiscriminate use of this herbicide might have impact on non-target species including humans. The objective of this study was to determine the sub-acute effect of Roundup on malondialdehyde (MDA) and lipids of rat brain. Thirty two adult male rats (Wistar strain) divided into 4 groups of 8 rats each, were used for this study. Group one served as the control and was given distilled water. The test groups T3, T4 and T6 were given graded doses: 300, 400 and 600 mg Roundup Kg^{-1}body wt respectively, orally for 8 days. At the end of the treatment period the brain was excised. Alterations in the integrity of brain cell membrane was determined by the estimation of the MDA level, total cholesterol (CHOL), total phospholipid (TPL), phosphatidylcholine (PC) phosphtidylethanolamine (PE), PC/PE and TPL/CHOL molar ratios. Results showed that there were significant ($p<0.05$) reductions in MDA and cholesterol levels of all the Roundup-exposed rats relative to the control. However the MDA levels were elevated in the liver and kidney. The TPL/CHOL molar ratios were normal in the T4 and T6-treatment groups while the PC/PE molar ratios were significantly ($p<0.05$) increased relative to the control. The pattern observed for the T3-treatment group was opposite that observed for the T4 and T6-treatment groups. This study suggests that the rearrangement in the lipid composition of the test groups might be an adaptive mechanism for the maintenance of constant bilayer fluidity and consequently brain cell homeostasis.

Key Words: Roundup, membrane fluidity; lipids, brain, rat

Introduction

Pesticides are chemicals of economic importance used in preventing, eliminating or controlling the damaging effect of pests. Herbicides as a class of pesticides are chemicals used in preventing the growth of unwanted vegetation. The use of pesticides in agriculture has increased the yield of plants for food resulting in reduced food costs. However, they generally persist in agricultural products and in the environment, posing health hazards to humans and animals (Dallegrave *et al*., 2007). Glyphosate (n-phosphonomethylglycine) is the active ingredient and polyoxyethyleneamine (POEA) is the surfactant agent of the Roundup formulation, which is an organophosphate herbicide (Releya, 2005). Glyphosate is marketed as a non-selective, broad spectrum, post-emergence herbicide due to its high water solubility and extensive usage. It is used to control weeds in emerged grasses, broad-leaf weeds, pastures and cultures such as rice, corn and soy (Williams *et al*., 2000). Its residues may thus enter the food chain, and glyphosate and its metabolite such as aminomethylphosphonic acid (AMPA) and formaldehyde are found as environmental contaminants in soil and rivers (Temple and Smith, 1992; Schuette, 1998;WHO, 2003). Due to the high water solubility and extensive usage (especially in shallow water systems), glyphosate exposure of non-target aquatic organisms is a concern (Tsui and Chu, 2003).

It has been postulated that organophosphate pesticides cause oxidative stress in different tissues through the formation of reactive oxygen species (ROS) (Akhgari *et al*.,2003; Abdollahi *et al*.,2004; Mehta *et al*., 2009). Because of their high reactivity, these species can damage lipids, proteins, carbohydrates, and nucleic acids (Avellar *et al*., 2004), leading to serious health problems. Among these macromolecules, lipids are probably most susceptible to this attack. Also, the brain tissue is particularly susceptible to oxidative damage, as it is rich in polyunsaturated fatty acids which easily undergo peroxidation (Akhgari *et al*.,2003; Dringer, 2000). With the increase in the use of glyphosate-Roundup®, along with the ignorance on its toxicity in mammals, the objective of this study was to determine the sub-acute effect of the glyphosate-based herbicide, Roundup on malondialdehyde (MDA) levels and lipids of rat brain.

Materials and Methods

Animals and Treatment: Thirty two male albino rats (Wistar strain), weighing 180-250 g were obtained from The Animal House of the Anatomy Department of the University of Benin. They were allowed an acclimatization period of 2 weeks before the commencement of the study. The rats were allowed free access to food and water throughout the period of the study. The animals were randomly placed, into 4 groups of 8 rats each. The first group (T1) served as the control and were treated with distilled water while the remaining three groups (T3, T4 and T6) were treated with graded doses 300, 400 and 600 mg Roundup kg^{-1} body wt respectively for a period of 8 days. Roundup was administered orally by gavage. At the end of the treatment period, the animals were necropsized under chloroform anaesthesia. The brain was excised by cracking the skull of the animal, weighed and stored at -4^0C until required for biochemical analysis.

Extraction of Lipids from the Brain: Total lipids in the brain were extracted by the method of Atsushi and Radin (1978). One gram of brain was homogenized in 18ml of hexane: isopropanol (3:2) for 30s and the suspension was filtered through a sintered glass Buchner funnel (medium porosity). The homogenizer, funnel and residue were washed 3 times with 2ml portions of hexane:isopropanol solution, by re-suspending the residue each time and letting the solvent soak for 2 minutes before applying air pressure. The non lipids in the extract were removed by mixing the pooled filtrates for at least 1 minute with 12ml of aqueous sodium sulphate. The contents were poured into a separating funnel and two layers were formed. The lipids were in the upper hexane rich layer. The lower layer was run into a container while the upper layer containing the lipids was collected. The lipid extract was evaporated to dryness and the residue re-dissolved in 2ml of hexane.

Biochemical Estimations: Malondialdehyde (MDA) level was estimated or determined by the method of Buege and Aust (1978). Aliquots of brain homogenate (1ml) were added to 2ml of (1:1:1 v/v/v) TCA-TBA-HCl reagent (TBA 0.375% w/v, 15% TCA w/v and 0.25N HCl) and mixed thoroughly by whirling. The solution was heated for 15minutes in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifugation at 2500 rpm for 10minutes. The absorbance of the clear supernatant was measured at 535nm against a reference blank.

Total cholesterol level was determined by the method of Richmond (1973) using already prepared kit from Randox laboratories (UK). The Total lipid content was estimated by the method of Zollner and Kirsch (1962). In this method, lipid reacts with sulphuric acid and phosphovanilin reagent to form a pink coloured complex which is measured spectrophotometrically at 530 nm. The estimation of total phospholipid was based on the method of Bartlett (1959). The Phospholipid in the lipid extract was estimated by phosphorus determination after acid digestion. The released inorganic phosphate was reacted with ammonium molybdate to form a coloured complex which is determined spectrophotometrically at 800nm. The lipid extract was first resolved into its individual phospholipid constituents by thin layer chromatography (Curzner and Davidson, (1967). Each resolved constituent was then quantified by the method of Bartlett (1959). All scrapings were digested and analyzed according to Bartlett's modification of the Fiske-Subbarow reaction (Fiske and Subbarow, 1925) as described for the estimation of total phospholipid, the only difference being that the mixture was centrifuged before colour development was achieved, so as to remove the silica gel.

Statistical Analysis

Values were expressed as mean ± SD. Statistical analysis of data was performed by one-way analysis of variance (ANOVA) using SPSS package version 16.0. Differences between the means were compared by Duncan's test. The level of significance was set at $p < 0.05$.

Results and Discussion

The effect of the glyphosate-based herbicide, Roundup on malondialdehyde (MDA) levels in the brain, liver and kidney of rats is shown in Table 1. Relative to the control, results show a significant ($p<0.05$) increase in the MDA levels in the kidney and liver of the rats that were treated with 600 mg Roundup Kg^{-1} body wt. Contrary to this pattern, brain MDA levels of all the Roundup treated rats were significantly ($p<0.05$) reduced relative to the control. Results also show significantly ($p<0.05$) reduced total cholesterol levels in the brain of all the Roundup-treated rats relative to the control. When compared to controls (Group T1) that received only normal saline, the animals in the test group treated with graded doses of the insecticide were found to have significantly ($p < 0.05$) higher amounts of total phospholipids (Table 2).

Table 1: Effect of the glyphosate-based herbicide, Roundup (Rdp) on Malondialdehyde (MDA) levels in the Brain, Liver and Kidney of Rats.

Group	Treatment (mg Rdp/Kg bwt)	MDA Brain (mg/g tissue)	MDA Liver (mg/g tissue)	MDA Kidney (mg/g tissue)
Control	Distilled Water	14.44±0.69	0.98 ± 0.07	7.90 ± 0.65
T3	300	9.09 ±0.31[a]	1.08 ± 0.14	8.63 ±0.87
T4	400	8.72 ± 0.60[a]	1.20 ± 0.16	8.46 ±0.98
T6	600	8.15 ± 0.45[a]	2.95 ± 0.02 [a]	10.50 ±0.51[a]

Data are reported as mean ± SD.
[a] Values are Significantly($p<0.05$) different from control.

Table 2: Brain Total Lipid (TL), Total phospholipid (TPL) Total cholesterol (CHOL) and Total phospholipid/Total cholesterol Molar Ratio (TPL/CHOL) of Rats Treated with Roundup

Group	Treatment (mg Rdp/Kg bwt)	TL (mg/g tissue)	TPL (mg/g tissue)	TCHOL (mg/g tissue)	TPL/CHOL ratio
Control	Distilled water	4.52±0.54	1.09 ± 0.05	16.24 ±1.55	1.07 ±0.12
T3	300	4.53±0.41	1.24 ±0.14	9.69 ± 0.55[a]	0.60 ±0.08[a]
T4	400	4.81±0.70	0.79 ±0.14	13.23 ± 1.17[a]	1.16 ±0.20
T6	600	4.67±0.88	1.63 ±0.24[a]	12.07 ± 0.27[a]	1. 19 ±0.19

Data are reported as mean ± SD.
[a]Values are Significantly($p<0.05$) different from control.

Phosphatidylcholine (PC), phosphatidylethanolamine (PE) and PC/PE ratio were significantly ($p<0.05$) increased in the group exposed to the highest dose of Roundup relative to the control (Table 3). However, only phosphatidylcholine and PC/PE ratios were significantly ($p<0.05$) increased in the group that was treated with 400 mg Roundup Kg^{-1} body wt. In both groups of Roundup-treated rats, the TPL/CHOL ratios were statistically ($p>0.05$) similar to the control. Relative to the control, the TPL/CHOL was significantly ($p<0.05$) reduced while PE was significantly ($p<0.05$) increased in the T3 treatment group.

Table 3: Effect of the Glyphosate-based herbicide, Roundup on Brain Phosphatidylcholine (PC), Phosphatidylethanolamine (PE) and Phosphatidylcholine/Phosphatidylethanolamine Molar Ratio (PC/PE).of Rats

Group	Treatment (mg Rdp/Kg bwt)	PC(mg/ml)	PE(mg/ml)	PC/PE ratio
Control	Distilled water	3.82±0.13	4.04 ± 0.13	0.90 ± 0.08
T3	300	3.90 ± 0.16	5.48 ± 0.34[a]	0.94±0.07
T4	400	8.65 ± 0.66[a]	4.13 ± 0.06	1.55±0.33[a]
T6	600	5.60 ± 0.78[a]	4.87±0.68[a]	1.28±0.05[a,]

Data are reported as mean ± SD.
[a]Values are Significantly($p<0.05$) different from control.

Many environmental pollutants have been reported to cause toxicity by the generation of reactive oxygen species (ROS) such as, hydrogen peroxide (H_2O_2), the superoxide anion (O_2^-), and the hydroxyl radical (·OH) (Ahmad et al., 2000; Harish and Murugan, 2011) which can damage the cell membrane and modify cell function. Hence they must be continuously inactivated to keep only a small amount necessary to maintain normal cell function. Oxidative stress arises when reactive oxygen species overwhelms the body's natural antioxidant defense system. The Blood Brain Barrier (BBB) is a highly selectively permeable barrier that protects the brain from variations in blood composition and toxins. In the absence of BBB, slight changes in the composition of the interstitial fluid in the CNS can lead to uncontrolled brain activity. Hence the endothelial cells forming the BBB are highly specialized to allow precise control over the substances that enter or leave the brain. However, molecules with high lipid solubility are reported (Laterra *et al.*, 1999) to move across the BBB by simple diffusion while the movement of polar compounds is mediated through specific transport proteins in the plasma membranes of endothelial cells. Since Glyphosate is highly soluble in water (Kollman and Segawa (1995), it is possible that the low solubility of this herbicide in lipids and / or absence of a possible Roundup/brain transport protein, could have blocked it from passage into the brain. Hence the significantly reduced malondialdehyde levels in the brain of all the Roundup-treated rats, observed in this study. It is also possible that the mechanism of Roundup toxicity of the brain does not involve the disruption of the antioxidant system. However, recent reports indicate elevated MDA levels in the liver of Roundup-exposed fish (Jin *et al.*, 2013). In the present study, a similar pattern was followed in the MDA levels of the liver and kidney of all the rats exposed to the highest dose of the herbicide.

The cholesterol levels in the brain of all the herbicide-treated rats in this study were significantly reduced relative to that of the control. Cholesterol is a major constituent of the brain and in humans, the brain contains 20% of the body's total cholesterol (Orth and Bellosta, 2012). This important macromolecule is required for synapse and dendrite formation as well as axonal guidance. Hence, its depletion leads to synaptic and dendritic spine degeneration, impaired neurotransmission and decreased synaptic plasticity (Koudinov and Koudinova, 2005). Orth and Bellosta (2012) reported that brain cholesterol, unlike those in other peripheral organs, is derived by *de novo* synthesis and that in the intact BBB of vertebrates, the uptake of lipoproteins from circulation does not occur.

Early reports by Cook *et al.*(1987) showed that alterations in protein cytoskeleton or lipid composition of erythrocyte membrane can affect membrane fluidity and ultimately the micro - viscosity or flow properties of the cell. In the present study, PC/PE and TPL/CHOL molar ratios were used as indices of membrane fluidity to determine whether or not Roundup altered this important membrane phenomenon in the brain of rats. We have used these molar ratios as indices of membrane fluidity in our earlier reports (Adaikpoh and Obi, 2009). These parameters have also been used by others (Hirata and Axelrod, 1980; Bangur *et al.,* 1995; Eriyamremu *et al.,* 2006). It is interesting to note that, of the two molar ratios used in the present study, only the PC/PE molar ratio indicated a reduction in membrane fluidity when rats were exposed to high doses of Roundup. An increase in the PC/PE molar ratio has been reported by Bangur, (1995) to suggest a transitory reduction in membrane fluidity. We therefore suggest that the changes in the lipid composition of the cell membrane, observed in this study, might be an adaptive mechanism by the cell to maintain a relatively constant bilayer fluidity and consequently internal homeostasis of the cell.

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