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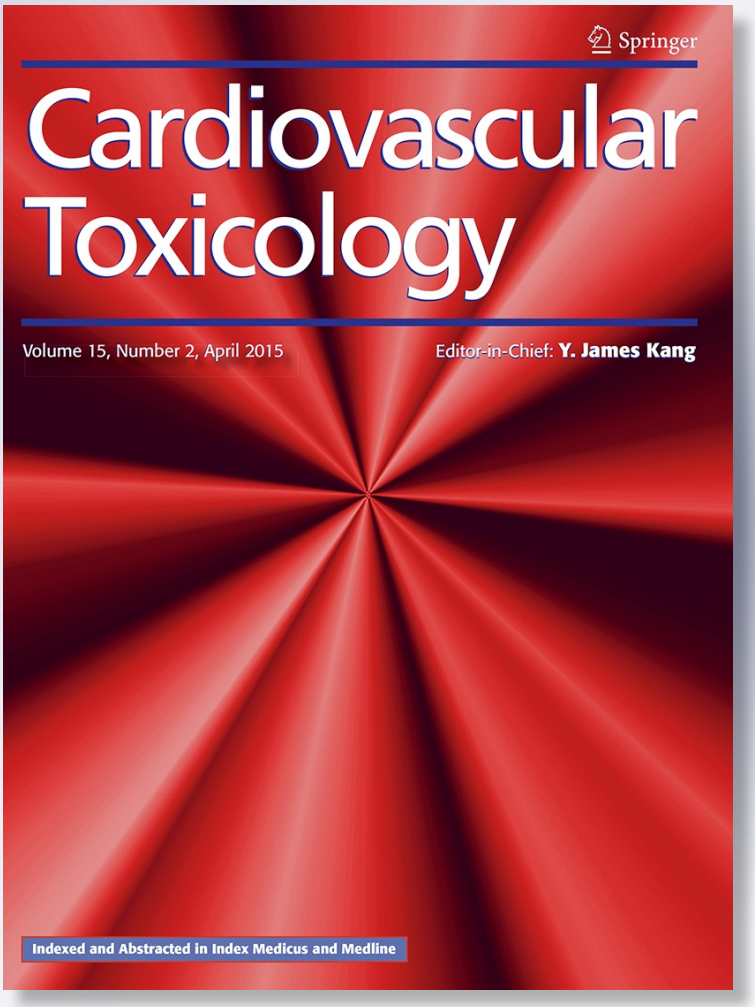

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Abstract In glyphosate (G)-based herbicides (GBHs), the declared active principle G is mixed with several adjuvants that help it to penetrate the plants' cell membranes and its stabilization and liposolubility. Its utilization is growing with genetically modified organisms engineered to tolerate GBH. Millions of farmers suffer poisoning and death in developing countries, and occupational exposures and suicide make GBH toxicity a worldwide concern. As GBH is found in human plasma, widespread hospital facilities for measuring it should be encouraged. Plasma determination is an essential prerequisite for risk assessment in GBH intoxication. Only when standard ECGs were performed, at least one abnormal ECG was detected in the large majority of cases after intoxication. QTc prolongation and arrhythmias along with first-degree atrioventricular block were observed after GBH intoxication. Thus, life-threatening arrhythmias might be the cause of death in GBH intoxication. Cardiac cellular effects of GBH were reviewed along with few case reports in men and scanty larger studies. We observed in two mammalian species (rats and rabbits) direct cardiac electrophysiological changes, conduction blocks and arrhythmias among GBH-mediated effects. Plasmatic (and urine) level determinations of G and electrocardiographic Holter monitoring seem warranted to ascertain whether cardiovascular risk among agro-alimentary workers might be defined.

Keywords Glyphosate-based herbicide · Intoxication · Cardiovascular system · Arrhythmias · Cardiac electrophysiology · Humans · Mammals

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Introduction

In a glyphosate (G)-based herbicide (GBH), the declared active principle G is mixed with several adjuvants that help it to penetrate the plants' cell membranes [1, 2], its stabilization and liposolubility. G is a glycine phosphonate, a small molecule close to the amino acid glycine (169 g/mol), with a basic secondary amino function and monobasic (carboxylic) and dibasic (phosphonic) acidic sites at both ends. G is hydrosoluble like amino acids and was originally patented in 1964 as a metal chelator (US Patent No. 3,160,632) like dications [3]. In Roundup (R), the most common GBH, proprietary and practically unknown adjuvants (Fig. 1) are supposed to be specific to plants, but nontarget effects are described in the literature [4–6]. There are different R formulations, each having G as

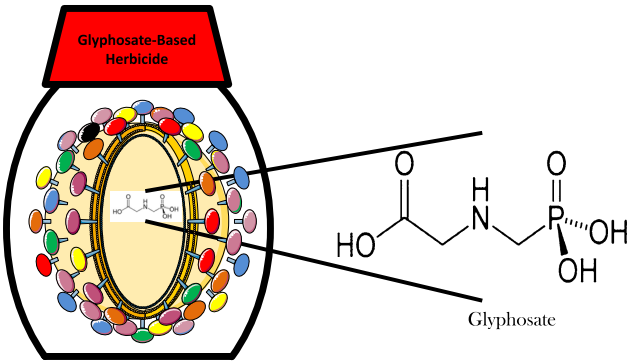

Fig. 1 Glyphosate-based herbicide formulation. Glyphosate, the active principle recognized to kill weeds, is surrounded by adjuvants whose proprietary formulae are unknown. Each glyphosate-based herbicide has different adjuvants

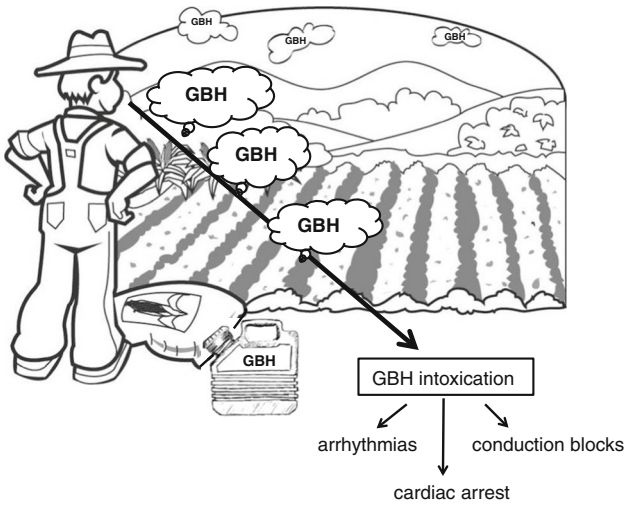

Fig. 2 Farmer spraying of GBH. Seasonal spraying of GBH may put farmers and their household at risk for intoxication since extremely high concentrations are used (typically from 10,000 to 20,000 ppm). Profession-related suicidal attempts in agro-alimentary personnel are a further risk element

declared active principle (between 36 and 48 %). R is known as the most used commercial formulation among pesticides worldwide; it is used by farmers and household to kill weeds, and its use is very important with genetically modified organisms (GMOs) that are engineered to tolerate it [7]. This property considerably increases R use and consequently the amount present in food and feed where regulation admits it [8].

Farm or household users may underestimate GBH risks during occupational exposure. In a case study, we documented the agricultural practices in a farmer's family [9]. G was found in the farmer's urine after spraying (10 μg/ml or ppm) [9], and this was consistent with other studies, where occupational exposures resulted in G urinary up to 0.233 ppm [10, 11]. Due to its widespread use, G is also found in farm and non-farm homes [12] like in urine of non-occupationally exposed women, where G was found at a mean of 73.6 ppb [13]. Second ways of contamination with G are accidental and suicidal attempts by farmers. These generally punctual exposures are in the range of acute intoxication doses. Extreme exposure (around 100–200 ml of the pure formulation ingested) resulted mainly in respiratory and hepatorenal damages [14]. In intentional suicidal ingestions, up to 500 ml was ingested [15]. Death was strongly associated with older age, larger ingestions and high plasma G concentrations on admission (>734 ppm) [16]. On the other hand, many farmers using G have experienced an accidental intoxication [17]. G is not the sole problem of R; differential effects between R and G are observed in studies in various mammalian species in vivo [18–20]. The toxicity of adjuvants seems to be a real problem in toxicology [21]. Some of them have been identified in GBH and are even more toxic than G on human cells [19]. Residues of pesticide are found in tap water, groundwater [22], food and feed and result from the formulation (G plus adjuvants) commercially sold. The ecosystem is always exposed to the formulations of R [23].

Pesticides have been demonstrated to also cause severe circulatory failure in poisoned humans after acute intoxication [14], which is an important health problem arising from pesticides in the countries of the developing world [24]. Indeed, 131 cases of GBH intoxication were reviewed at a local teaching hospital in Taiwan from 1988 to 1995 [25]. In 1973, the World Health Organization (WHO) suggested that 500,000 cases of acute serious pesticide poisoning occurred annually and 3 million cases hospitalized with 220,000 deaths in 1985 [26]. In the developed countries, a small percentage of poisoning is related to suicide [27]. It is estimated that there could be as many as 25 million agricultural workers in the developing world suffering an episode of poisoning each year [27]. Notably, after acute intoxication due to GBH ingestion, cardiac arrhythmias are frequent and the common symptoms were those of cardiovascular shock with conduction blocks [28]. However, few reports concentrated on rhythmic consequences of GBH intoxication and the mechanisms whereby arrhythmogenesis might be increased. We focus here on G and R cardiovascular effects, especially addressing an hypothesis pointing to increased arrhythmogenic potential in mammals, knowing that their effects on different physiological systems were addressed by others [23, 29].

Cardiovascular Investigations with Glyphosate-Based Herbicides

An updated review of the literature presents scanty investigations performed in mammals to assess GBH cardiovascular effects. This contrasts with the severe impact of

the reported GBH toxic consequences as alluded above. Moreover, there is a relatively high probability that by the agro-alimentary chain, including the rising market penetrance of GMO, by definition tolerating GBH used for food and feed [7], high concentrations of GBH might affect humans as the chain end-effectors. Finally, potentially dangerous professional exposures (typically from 10,000 to 20,000 ppm of R during spraying; Fig. 2) or profession-related suicidal attempts in agro-alimentary personnel [27] may necessitate a closer scrutiny of these problems, aimed at reducing risks and death rate. Nevertheless, case reports and retrospective hospital reviews reporting cardiovascular effects of GBH are quite rare (Table 1) and just a few points to the presence of arrhythmias as a potential consequence of GBH toxicity and as a cause of death.

Investigations in Animals

In Vitro Studies

Song et al. [30] have shown that in H9C2, an heart rat cell line, the toxicity of an adjuvant called LN-10 (0.4–100 μM) is increased in the presence of G at low doses (0.066–17 ppm) after 72 h of exposure. Kim et al. [31] in the same cell line showed that TN-20 adjuvant (0.4–0.85 ppm) in mixture with G (0.8–1.7 ppm), after 72 h, aggravated mitochondrial damage and induced apoptosis and necrosis. These results were considered relevant to explain the cardiovascular instability observed in patients with acute GBH intoxication, including hypotension and arrhythmias [32].

Ex Vivo Studies

Chan et al. [33] continuously recorded for 20 min the impact of various formulations of agricultural chemicals on isolated rat aorta and heart. They showed a significant negative inotropic action by G, whereas the adjuvants completely inhibited contraction of the isolated hearts. Chan et al. observed that this is in accordance with the effects of acute oral pesticide BASTA^{TM} (glufosinate as declared active principle) poisoning in patients developing various clinical signs, including consciousness disturbance, convulsions, pyrexia, respiratory failure and, in severe cases, dying of refractory circulatory failure [34].

In Vivo Studies

Daruich et al. [35] showed a disruption of isocitrate dehydrogenase, glucose-6-phosphate dehydrogenase, and malic dehydrogenase activity in heart of female Wistar rats and fetuses after 21 days of G treatment (≥2,000 ppm) in water during gestation. On the other hand, Chan and Malher [36] in B6C3F1 male mice pointed to heart weight increase after 13 weeks of G treatment.

Investigations in Man

Case reports

In 1993, Marrs [37] was the first to discuss the structure of the anticholinesterase organophosphates (OPs), which are used predominantly as insecticides. OP poisoning can occur in a variety of situations and can be accidental or suicidal. The cholinergic syndrome caused by acetylcholinesterase inhibition and diagnosed based on the clinical signs and symptoms as well as on measuring erythrocyte acetylcholinesterase inhibition and/or plasma cholinesterase activity is a common consequence of OP poisoning. GBH, fire retardants and industrial intermediates were signaled as potential initiators of the cholinergic syndrome whose antidote is atropine. Cardiac arrhythmias, including *torsade de pointes,* were illustrated as being associated with OP poisoning [38, 39], sometimes after recovery from the acute syndrome. Histologic evidence of myocardial damage was also documented in rats [40].

Two rapid lethal intoxication cases by GBH-trimesium (Touchdown: now withdrawn from the market) were presented by Sorensen and Gregersen in 1999 [41]. A 6-year-old boy who accidentally ingested a mouthful of GBH died within minutes. The same happened to a 34-year-old woman who intentionally ingested approximately 150 ml of the same GBH formulation. Postmortem explorations showed gastrointestinal, pulmonary and cerebral edemas, and dilated right atrial and ventricular heart chambers. No arrhythmias were documented. It was pointed that the speed of which death occurred was much more rapid than lethal intoxications with R, and it was submitted that GBH-trimesium may have facilitated the absorption after oral ingestion. On the other hand, the 52-year-old man, found unconscious after intoxication with 300 ml GBH ingestion (containing 41 % G as an isopropylamine salt, 15 % polyoxyethyleneamine (POEA) surfactant, and water), had a Glasgow coma scale score of 11 (E3, V4, M4), but was discharged from the hospital after 1 week. In hospital, heart rate was 44 beats/min with a normal chest radiograph, and ECG showed junctional premature complexes [41].

Larger studies

Between 1980 and 1989, 93 cases of exposure to R were treated in a Taiwanese hospital [42]. The average amount of the 41 % solution of GBH ingested by non-survivors was 184 ± 70 ml (range 85–200 ml), but much larger

Table 1 Cardiovascular studies performed in mammals with GBH

Authors	Year	Species	Cell line	N^x	G	Adjuvants	Type	Administration	Arrhythmias	CV effects
Animals										
In vitro										
Song H.Y. [30]	2012	Rat	H9C2	–	+	+	M	–	–	Apoptosis
Kim Y. [31]	2013	Rat	H9C2	–	+	+	M	–	–	Apoptosis/Necrosis
Ex vivo										
Chan Y.C. [33]	2007	Rat	–	–	+	–	F	–	–	G: (−) inotropic
In vivo										
Chan P. [36]	1992	Mouse	–	–	+	–	–	O	–	Heart weight ↑
Daruich J. [35]	2001	Rat	–	–	+	–	–	O	–	Enzyme disruption
Humans										
Case reports										
Marrs T.C. [37]	1993	Man	–	–	–	–	OP	A/S	+	Cholinergic Syndrome
Sorensen F.W. [40]	1999	Man	–	$2^{100\ \%}$	+	+	GBH	A/S	–	Death within min
Larger reports										
Talbot A.R. [42]	1991	Man	–	$93^{7.5\ \%}$	+	+	GBH	A/S	–	ECG not performed
Loffredo C.A. [43]	2001	Man	–	66 TGA vs 771 C	–	–	P	A	–	↑ Risk of TGA
Kim Y.H. [45]	2013	Man	–	$153^{12.4\ \%}$	+	+	GBH	S	+	↑ QTc interval

A accidental, *C* controls, *CV effects* Cardiovascular effects, *D* death, *F* formulation commercial of GBH, *G* glyphosate, *GBH* glyphosate-based herbicides, *M* mixed of G and Adjuvant, *O* oral, *OP* organophosphate, *P* pesticides, *PR* pregnancy, *S* suicidal, *TGA* transposition of the Great Arteries, (+) yes, (−) no; ↑ increase, N^x number of men in the study with in exponent the rate of death (%)

amounts (500 ml) were reported to have been ingested by some patients and only resulted in mild to moderate symptoms. Accidental exposure was asymptomatic after dermal contact with spray (6 cases), while mild oral discomfort occurred after accidental ingestion (13 cases). Intentional ingestion (80 cases) resulted in erosion of the gastrointestinal tract (66 %), dysphagia (31 %) and gastrointestinal hemorrhage (8 %). Other organs were affected less often (lung 23 %, liver 19 %, cardiovascular system 18 %, kidney 14 % and central nervous system 12 %). There were seven deaths, all of which occurred within hours of ingestion; two before the patient arrived at the hospital. Deaths following R ingestion alone were due to a syndrome that involved hypotension, unresponsive to intravenous fluids or vasopressor drugs, and sometimes pulmonary edema, in the presence of normal central venous pressure. This was the first report where cardiovascular symptoms were reported after R ingestion. However, ECG was not performed, and arrhythmias were not documented [42].

The potential correlation between pesticide exposure and specific cardiovascular malformations was investigated by Loffredo et al. [43] in 2001. They found an association of transposition of the great arteries (TGA) in infants with maternal exposures to herbicides and rodenticides. The Baltimore-Washington Infant Study was a case–control study of congenital heart defects in liveborn infants, which interviewed parents about a wide range of environmental exposures, including pesticides, that occurred during and before pregnancy. An association of maternal exposure to any pesticides during the first trimester with TGA in their infants ($n = 66$) was observed, relative to 771 control infants, with an odds ratio of 2.0. No other heart defects were associated with pesticides. When analyzed by type of pesticide and adjusted for covariates, there were associations of TGA with maternal exposures to herbicides (odds ratio = 2.8) and to rodenticidal chemicals (odds ratio = 4.7), but not to insecticides. Unfortunately, no data were collected on specific chemicals or brand names. In 2013 [44], a review about the evaluation of G toxicity studies in rats and rabbits concentrating on cardiovascular development concluded that existing evidence in the literature does not support risks for increased cardiovascular defects as a result of G exposure during pregnancy. Further epidemiological investigations in humans are therefore urgently needed. It should be essential to run longitudinal studies and to evaluate specifically all chemicals involved in pesticide actions.

The most important clinical retrospective study, concentrating on cardiovascular problems after acute GBH ingestion, included 153 patients (19–93 years) and was published by Kim et al. [45] initially in 2013 from South Korea. Gastric lavage was performed on all subjects observed within 2 h after ingestion. There were 122 patients (80 %) with at least 1 abnormal ECG rhythm in the initial standard ECG. Prolonged QTc interval followed by intraventricular conduction delay and first-degree atrioventricular block was the most common changes. Non-survivors had a significantly more prolonged QTc interval when compared to survivors (542.0 ± 32.0 vs. 453.4 ± 33.6 ms) but similar heart rates. There were 19 deaths (12.4 %): two late complications (one pneumonia and sepsis and one multiple-organ failure) and 17 direct cardiac causes including refractory shock (14 patients) and ventricular tachycardia (three patients). Interestingly, more recently, Kim et al. [46], noting that the occupation of farming has been reported to be associated with a high suicide rate, and suicidal ideation as an important risk factor for suicide in farmers, explored the association between occupational pesticide exposure or poisoning history and suicidal ideation among male farmers in South Korea. They concluded that risk of suicidal ideation was related to occupational pesticide poisoning among male farmers. Among all farmers, 4.7 % ($n = 92$) reported suicidal ideation. After controlling for potential confounders, lifetime hospitalization due to pesticide poisoning showed a 2.48-fold increase in risk. Those with multiple poisonings showed more significant associations with suicidal ideation (odds ratios = 2.33 for once, 3.02 for more than once). Moderate- or severe-symptom severity of acute pesticide poisoning cases (odds ratio = 2.23) also showed increased risks of suicidal ideation than the milder classes did. However, no significant association was identified with cumulative lifetime pesticide application and suicidal ideation, and no relationship with cardiovascular risk factors or signs and symptoms was looked for [46].

Glyphosate Metabolism and Plasma-Level Determinations

G inhibits plant growth through interference with the production of essential aromatic amino acids by inhibition of the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme. This enzyme catalyzes the formation of 5-enolpyruvylshikimate-3-phosphate from phosphoenolpyruvate (PEP) and shikimate-3-phosphate, which are responsible for the biosynthesis of chorismate [47], an intermediate in phenylalanine, tyrosine and tryptophan biosynthesis, the absence of which in mammals may explain the relatively low systemic toxicity of G [oral median lethal dose (LD50) for rats 4,320 mg/kg, rabbits 3,800 mg/kg] [48]. In the terrestrial environment, G is mainly biodegraded to aminomethylphosphonic acid (AMPA) when metabolized by bacterial in soils [49]. According to the animal study in Sprague–Dawley rats, approximately 35–40 % of the

administered dose was absorbed from the gastrointestinal tract, and urine and feces were equally important routes of elimination after one oral dose (10 mg/kg) [50]. Animal studies indicated that virtually no toxic metabolites of G were produced when it was administrated orally and that there was little evidence of metabolic activity with essentially 100 % of the body G burden related to the parent compound [51].

The importance of measuring G plasma concentrations has not been fully assessed. It obviously can confirm exposure for forensic purposes, but it might also have a role with quantifying or predicting the severity of poisoning [52]. G is considered to be of low toxicity, so the rationale for undertaking its plasma-level determinations is that it is a reasonable surrogate measure of exposure to unmeasurable adjuvants, which are supposed to be more stable and lipophilic. Concentrations between 734 and >1,000 μg/ml (or ppm) were reported in patients with severe poisoning, although death occurred with a concentration as low as 734 μg/ml [16]. In that investigation, Roberts et al. aimed at describing the clinical outcomes, dose–response and G kinetics following self-poisoning with GBH. This prospective observational case series was conducted in two hospitals in Sri Lanka between 2002 and 2007. They included patients with a history of acute poisoning. Clinical observations were recorded until discharge or death. During a specified time period admission ($n = 216$, including five deaths) and serial ($n = 26$), blood samples were collected. There were 601 patients identified; the majority ingested a concentrated formulation (36 % w/v G): 27.6 % were asymptomatic, 64 % had minor poisoning and 5.5 % had moderate to severe poisoning. There were 19 deaths (case fatality 3.2 %) with a median time to death of 20 h. The apparent elimination half life of G was 3.1 h.

A method to determine G in human plasma by ion chromatography was established in 2012 by Wang et al. [53]. The protein in heart blood from a corpse was precipitated with acetonitrile. The large molecules and Cl^- in the supernatant were removed by a Dionex OnGuard II RP column and a Dionex OnGuard II Ag column, respectively. The filtrate was separated on an IonPac AS-19 column with KOH solution as eluent produced online by an eluent generator (EG). A suppressor with external water mode and a conductivity detector for the detection were used. The linear range of this method was 10–100 mg/l with a correlation coefficient (r^2) of 0.9999. The limits of detection (LOD, S/N = 3) and quantification (LOQ, S/N = 10) of G in blood were 0.12 and 0.39 mg/l, respectively. The G content in a heart blood sample from a corpse in an actual case was 508 mg/l detected by this method. This was a simple, sensitive, accurate method that can rapidly provide reliable clues and evidences for G poisoning cases, meeting the needs of public security work although it is unclear whether any large investigation was performed using it.

Zouaoui et al. [28] performed the first investigation whereby plasma G concentrations and cardiovascular symptoms or signs were co-reported in 2013 among 13 cases of acute GBH intoxication. Plasma G concentrations were determined by another ion chromatographic method inspired by Tomita and Okuyama with slight modifications [54]. The method is based on a derivatization with Para-Toluene Sulfonyl Chloride (PTSCl) followed by a liquid–liquid extraction in acidic conditions in order to concentrate the derivative products. Dibutylphosphate is used as internal standard. Prior to the serum analysis, 2 ml of sample was deproteinized by adding 0.5 ml of acetonitrile. Briefly, derivatization was performed in 2 ml of urine or in 2 ml deproteinized serum by adding 0.5 ml of PTSCl (10 g/l in acetonitrile) and 1 ml of phosphate buffer at pH 11 for 15 min in an ultrasonic water bath then for 15 min at 50 °C. PTSCl derivatives of G were extracted with 8 ml of ethyl acetate after adding 1 ml of 6 M hydrochloric acid. In total, 2 ml of the dry residue reconstituted with 80 ml of 20 mM formate buffer (pH 3.0) and methanol (90/10, v/v) was injected into a high-performance liquid chromatography coupled with tandem mass spectrometry system. The chromatographic system consisted of a Perkin-Elmer Series 200LC pumping system and a Series 200 auto-sampler. An API 2000 triple quadrupole mass spectrometer (AB Sciex, Courtaboeuf France) was used for detection with negative electrospray ionization mode. The intra-assay precision and accuracy were assessed at low and high concentrations relative to calibration range: analysis of five spiked hair samples at each concentration. This method exhibits a LOD of 20 mg/l for G. Intra-assay precision CVs and relative bias are less than 20 % over the calibrating range. Due to the very high concentration found in the real samples, high dilution of samples in deionized water was used. Using this method, G mean values varied between 61 mg/l (range 0.6–150 mg/l) and 4,146 mg/l (range 690–7,480 mg/l), respectively, in mild–moderate intoxication and fatal cases. In the severe intoxication case for which blood has been sampled, the blood G concentration was found at 838 mg/l. Death was in general associated with larger taken dose (500 ml in one patient) and high blood G concentrations. In fatalities, the common symptoms were cardiorespiratory arrest, cardiovascular shock, hemodynamic disturbances, intravascular disseminated coagulation and multiple-organ failure. To predict clinical outcomes and to guide treatment support in patients who ingested G, blood concentrations were indeed useful, confirming that a large amount of GBH ingested (>190 ml) and G blood levels > 734 mg/l were predictors of the risk of dying as previously described [16, 52].

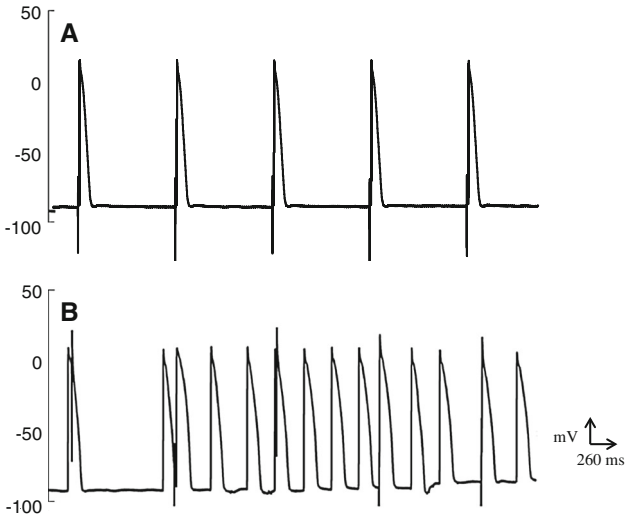

Fig. 3 Representative example of arrhythmias occurring in ventricular tissues after Roundup superfusion. Rabbit experiments are illustrated. **a** Control period; **b** Frequent premature ventricular beats after 30 min of Roundup 50 ppm superfusion [2014, unpublished observations]

Potential Mechanisms Relating GBH to Arrhythmias

It was recently shown in rat testis and Sertoli cells that R disrupts male reproductive functions by triggering L-type voltage-dependent Ca^{++}-channel-mediated cell death [55]. In particular, it was shown that R at 360 ppm led to an important decrease in $^{45}Ca^{++}$ influx, whereas R at 36 ppm induced an increase in $^{45}Ca^{++}$ uptake, an effect ascribed to G and partially prevented by the L-type voltage-dependent Ca^{++}-channel antagonist nifedipine 10 µmol/l, thus indicating that disruption in Ca^{++} homeostasis plays a critical role in the toxic effects of GBH [55]. More than 20 years ago, it was demonstrated that G increased mitochondrial membrane permeability to protons and Ca^{++}, suggesting early on a mechanism for the toxic effect of GBH by a cationic chelator action [56]. We addressed specifically these hypotheses to investigate the potential mechanism whereby R might be arrhythmogenic in mammalian heart tissues.

In rat and rabbit ventricular tissues, we studied the effects of R and G on in vitro electrophysiology [2014, unpublished observations]. Moreover, a complementing study was performed in rat ventricular tissues, using pharmacological agents to increase L-type voltage-dependent Ca^{++}-current by BAY K 8644 [57] or Ca^{++} intracellular content by ouabain, a Na^+/K^+-ATPase inhibitor [58], before R superfusion. Our results fit the hypothesis of decreased Ca^{++} uptake as the possible consequence of a cationic chelator action [55, 56] to explain R cardiotoxic effects, and we led some electrophysiological evidence to further support it.

Indeed, conduction blocks, dose-dependent inexcitability and proarrhythmia (Fig. 3) may all follow to I_{Ca} decrease, thus accounting for lower R effects when intracellular Ca^{++} is increased by the pharmacological approach tested. Interestingly, we have also observed that APD_{90} (action potential duration at 90 % of repolarization) prolongation (an experimental correlate of QTc prolongation at the level of ECG in humans, also seen after R intoxication [45]) was prevented by the administration of a L-type calcium-channel agonist, which stimulates the I_{Ca}. Moreover, considering the cationic chelator action of R [55, 56], it is also possible that other direct electrophysiological actions are involved, and among these, I_{Na} and I_K blocking properties deserve special attention for they might as well be implicated in QTc prolongation [45]. When the exact chemical nature of the unknown adjuvant will be disclosed [19], an investigation will be possible of its electrophysiological effects in myocardial tissues, which should be done to elucidate the toxic effects of R, relevant for human poisoning [45].

Discussion

Whereas in developing countries GBH and pesticide intoxications are definitely a big health problem with millions of workers suffering an episode of poisoning each year [17, 24, 27] and deaths in the order of hundreds of thousand each year [26], developed countries either experience occupational exposures or relatives in the household are affected [9, 17], although the most frequent toxic consequence there is around suicide attempts [46], anyway making GBH toxicity a worldwide issue of concern. It is therefore essential that widespread hospital facilities may exist for plasma determination of G, at least as a means of approaching the GBH real concentrations [52], including their unmeasurable or undetermined adjuvants contents that probably will never be measured being proprietary formulations [21]. However, comparative investigations to define the most accurate ion chromatographic method were not performed, and limit of detection varies from as low as 0.12 mg/l [53] to 20 mg/l [28] with G content in a heart blood sample from a corpse of 508 mg/l [53] to values between 0.6 and 7,480 mg/l in other studies [16] [28]. That plasma determination is an essential prerequisite for risk assessment in GBH intoxication is well illustrated by the close relationship between the clinical case presentation or severity of the intoxication. Indeed, severe intoxications or death in cases where blood had been sampled presented with plasma G concentration of a relatively short range of 734–1,000 mg/l [16, 18].

It is impressive that only when standard ECGs were performed, at least one abnormal ECG was detected in 80 % of patients in a large retrospective series, and both

QTc prolongation and arrhythmias along with first-degree atrioventricular block were observed after GBH intoxication [45]. Moreover, non-survivors had a significantly more prolonged QTc interval when compared to survivors [45]. In another study where ECG was not measured, deaths following R ingestion were due to a syndrome that involved hypotension [42], again pointing to cardiovascular involvement. On the other hand, GBH intoxication case reports where cardiovascular signs and symptoms were measured in due time were few [28, 38, 39, 42, 45]. In any case, death rate was high after GBH intoxication (ranging from 3.2 [52] to 12.4 % [45]) with a quite rapid median time to death of 20 h [46] although the apparent elimination half life of G was relatively short (3.1 h. [52]). All this critically points to the possibility that life-threatening arrhythmias are the actual cause of death in GBH intoxication. Interestingly, in the context of both acute and chronic ischemic heart disease, QTc prolongation was a predictor of sudden death due to ventricular fibrillation [59–61].

The general belief is that R is an inert component when animals or humans are exposed acutely. However, we observed in in vitro rat and rabbit ventricular tissues APD_{90} changes after short superfusion and a high incidence of severe arrhythmias and of conduction blocks at the highest concentrations [2014, unpublished observations]. These arrhythmias and conduction blocks may follow to significant I_{Na} and I_K blocking properties [62, 63], possibly related to the cation chelator ability [56] of G and to decreased $^{45}Ca^{++}$ influx through L-type voltage-dependent Ca^{++}-channels shown by both R and G [55]. Thus, our experimental observations, strongly suggesting that in rat and rabbit ventricular myocardium electrophysiological changes are seen, including conduction blocks and arrhythmias among GBH-mediated effects, link animal and human risks [45] after acute intoxication.

It is for further studies to ascertain whether cardiovascular risk among agro-alimentary workers might be defined by both plasma (and urine)-level determinations of G and electrocardiographic Holter monitoring aimed at measuring arrhythmia and heart block incidences along with at considering QTc changes over long periods or on discrete, but comparative occasions around seasonal spreading of product times or to control for potentially chronic or acute intoxications. More data from biomonitoring studies underscore the importance of exposure assessment in epidemiologic studies and indicate that studies should incorporate not only duration and frequency of pesticide use, but also type of pesticide formulation [64].

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