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CORRESPONDENCE

Differential impact of pure glyphosate and glyphosate-based herbicide in a model of peripheral nervous system myelination

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Since its introduction to the market in 1974, glyphosate has become the world's most commonly used herbicide. Its herbicidal activity is believed to result from the inhibition of the shikimate enzymatic pathway required for the biosynthesis of aromatic amino acids in plants [2, 4]. While there is no relevant physiological role for the shikimate pathway in mammals, the potential health risks of exposure to glyphosate residues from food and environmental contamination continue to excite controversy [8, 9]. Although glyphosate has long been marketed as safe for humans and higher animals, several studies have implicated pure glyphosate or glyphosate-based herbicide (GBH) formulations in cytotoxicity, carcinogenicity, inflammation and endocrine disruption [5, 8, 10]. GBH formulations often contain the isopropylamine salt of glyphosate in combination with undisclosed auxiliary agents and surfactants which are supposed to enhance compound stability and penetrance into plant tissues. While manufacturers of GBH have claimed these auxiliary agents to be inert, several reports suggest that GBH formulations may be considerably more toxic than pure glyphosate [1, 3, 7, 8].

With regard to the neurotoxic potential of glyphosate or GBH, evidence is sparse and no studies have been conducted thus far to investigate the impact of glyphosate or GBH on the peripheral nervous system (PNS). One case report has linked exposure to GBH with the development of peripheral neuritis, but lacks causative evidence [6]. Therefore, we aimed at investigating the effects of pure glyphosate and a GBH formulation (containing glyphosate isopropylamine as active ingredient) on murine embryonic dorsal root ganglia (DRG) cultures.

Following cultivation of freshly explanted DRGs under neurite-stimulating conditions for 7 days, glyphosate or GBH was added to DRG cultures at the start of myelination-induction corresponding to 0.0005% and 0.005% glyphosate in the culture medium. After 10 days of incubation, the extent of myelination was assessed by determining the total number of internodes per neurons in individual culture wells stained with Sudan Black. We did not recognize any differences in myelination in cultures treated with pure glyphosate at either concentration compared to vehicle treatment (Fig. 1a–c; Suppl. Fig. 1). However, treatment with GBH significantly interfered with myelination, an effect that appeared to be concentration-dependent (Fig. 1d–f). Similarly, in cultures with pre-existing myelin, GBH treatment was associated with a concentration-dependent demyelinating effect, which was not observed after treatment with pure

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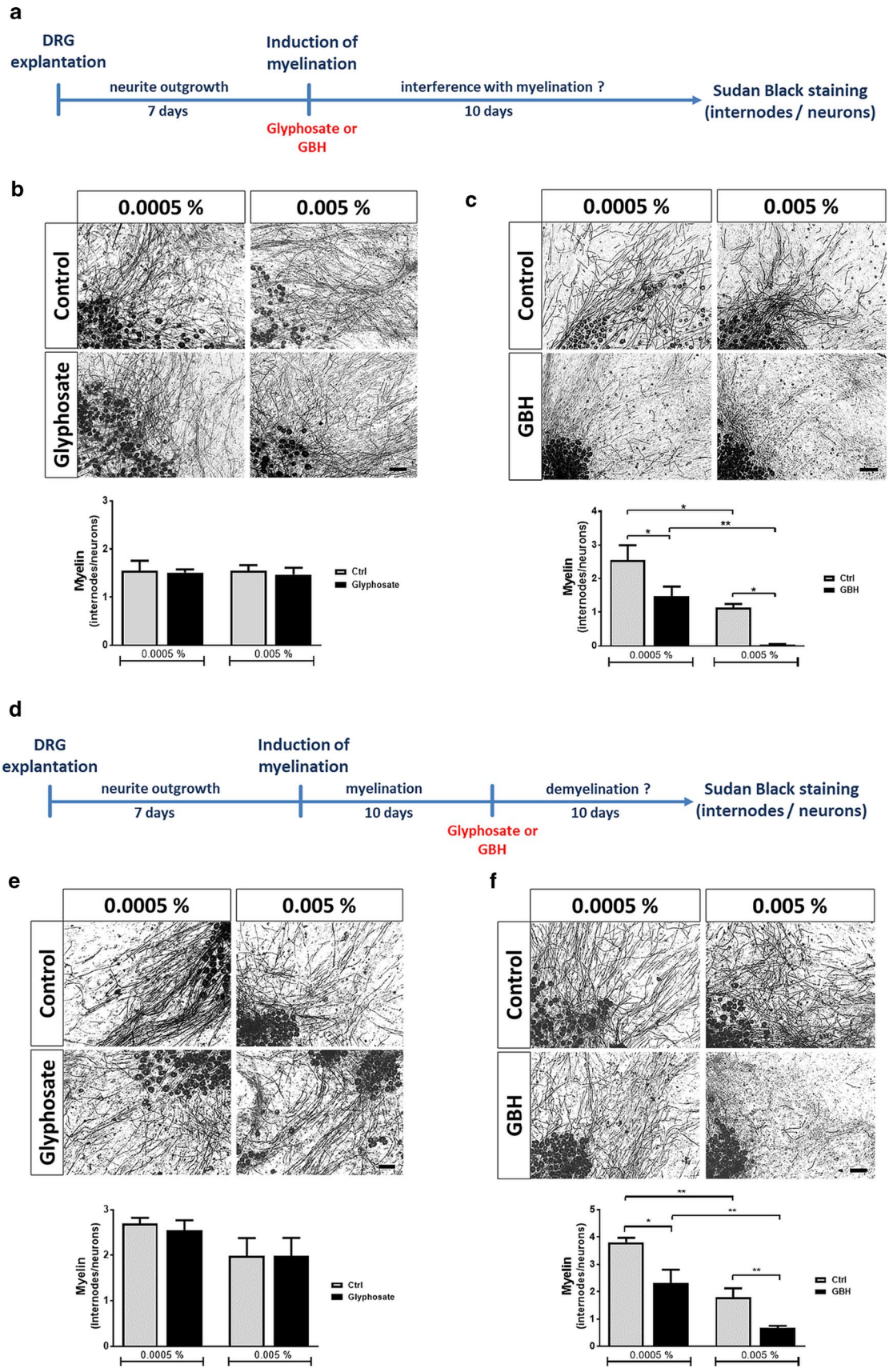
a
DRG explantation
Induction of myelination
neurite outgrowth
7 days
interference with myelination ?
10 days
Sudan Black staining (internodes / neurons)
Glyphosate or GBH
b
0.0005 %
0.005 %
Control
Glyphosate
Myelin (internodes/neurons)
Ctrl
Glyphosate
c
GBH
d
myelination
demyelination ?
e
f

◂**Fig. 1** GBH, rather than pure glyphosate, impedes myelination and acts as a demyelinating agent. DRG cultures were treated with pure glyphosate, GBH or pH-matched vehicle at the start of myelination-induction (**a**–**c**) or after an initial myelination period of 10 days (**d**–**f**). Cultures were then stained with Sudan Black and myelin content was assessed by determining the number of internodes per neurons in individual culture wells. Scale bars indicate 100 µm. Data represent mean ± SEM $N \geq 3$ for each column. $^{*}P \leq 0.05$, $^{**}P \leq 0.005$, $^{***}P \leq 0.0005$; *ctrl* control

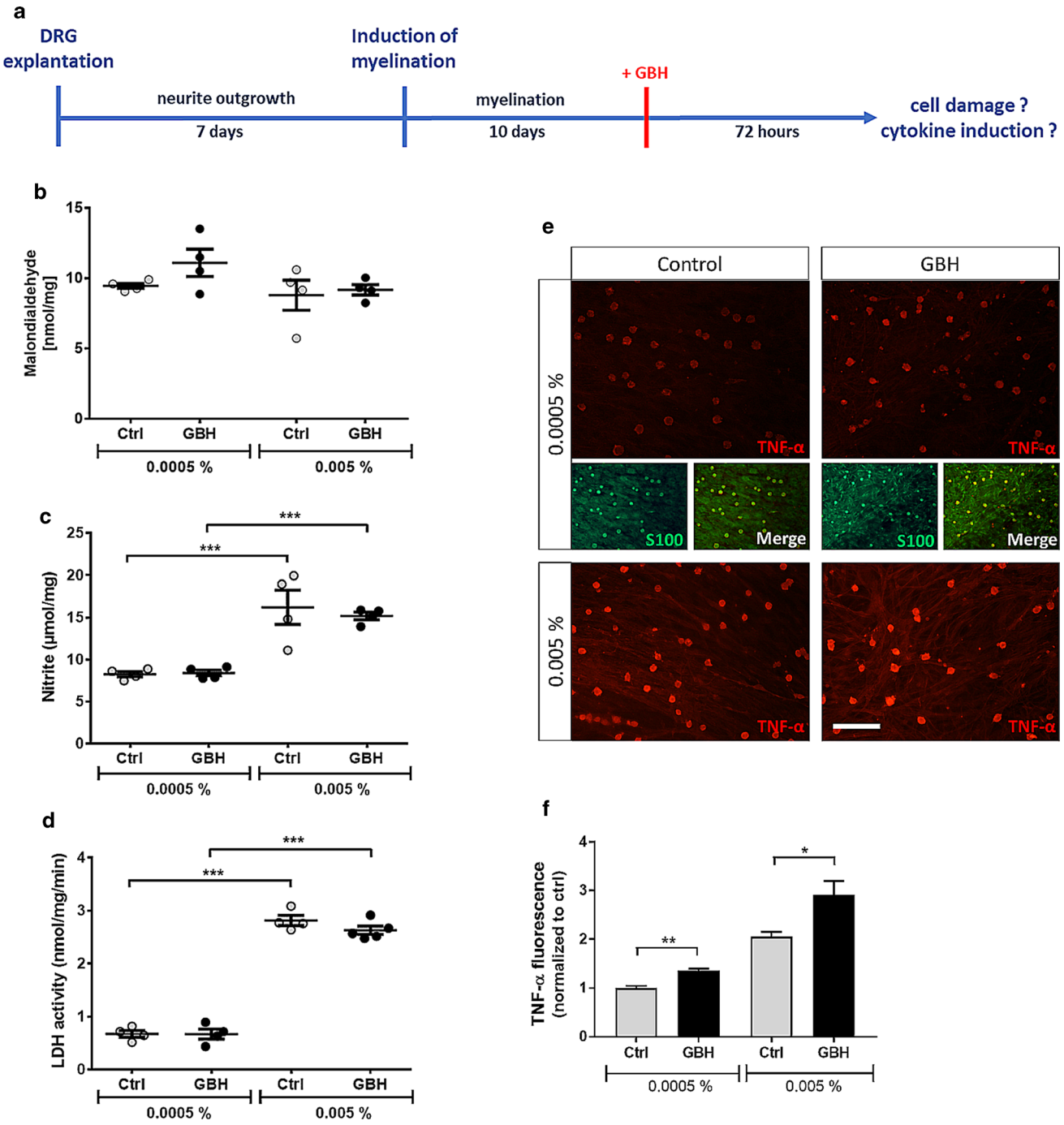

Fig. 2 GBH shows no specific impact on markers for cellular damage, but may contribute to inflammatory glial cell activation. Myelinated DRG cultures were treated with GBH or pH-matched vehicle at the indicated concentrations for 72 h. Each data point was pooled from four individual culture wells. Cultures were homogenized and the content of the lipid peroxidation marker malondialdehyde was determined via a thiobarbituric acid-based assay (**a**). Nitrite levels and lactase dehydrogenase (LDH) activity were determined in culture supernatant (**b**, **c**). TNF-alpha expression in S100-positive cells was determined by single-cell immunofluorescence analysis (**d**, **e**). Scale bar indicates 50 µm. $N \geq 3$ for each column. Data represent mean ± SEM $^{*}P \leq 0.05$, $^{**}P \leq 0.005$, $^{***}P \leq 0.0005$; *ctrl* control

glyphosate. As the GBH formulation contained the isopropylamine salt of glyphosate rather than pure glyphosate, we tested whether the demyelinating properties of GBH might be related to isopropylamine (Suppl. Fig. 2). However, the effect of glyphosate plus isopropylamine was not comparable to GBH treatment, suggesting that undisclosed additives might be responsible for its detrimental effect. Furthermore, the impact of GBH on DRG cultures appeared to be specifically related to myelin, but not to neurite integrity: in our findings neither glyphosate nor GBH impaired neurite outgrowth (Suppl. Figs. 3, 4).

To better understand the mechanism underlying the demyelinating effect of GBH, we next investigated markers for cellular damage (Fig. 2a–d). However, we only recognized an unspecific increase in some markers for cell damage with increasing concentrations of both GBH and vehicle. Since these findings did not adequately explain the observed differences, we considered inflammatory glial cell activation as a possible mechanism. Therefore, we measured the expression of the inflammatory cytokine TNF-alpha via single-cell immunofluorescence analysis in S100-positive cells (Fig. 2e, f), indicative of Schwann and satellite cells. We found an elevation of TNF-alpha expression with increasing concentrations of both GBH and vehicle, which was more pronounced under GBH treatment. The induction of TNF-alpha expression in response to GBH could also be confirmed in pure Schwann cell culture lysates via ELISA, which was paralleled by increased nitric oxide release (Suppl. Fig. 5). These results suggest that inflammatory activation of glial cells may indeed underlie the observed demyelinating effect of GBH.

As the exact composition of the GBH used in this study remains unknown, we could not conclusively identify the compound responsible for demyelination. However, the very fact that the ingredients are not fully declared is a topic that merits further discussion. Further studies are warranted to identify and characterize the neurotoxic potential of auxiliary agents in widely used herbicide products. It cannot be ruled out that individuals chronically exposed to GBH or related chemicals might be at increased risk of developing diseases of the PNS, including demyelinating neuropathies. As such, further epidemiological studies are urgently required to corroborate or refute these assumptions.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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