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Toxicity and behavioral effects of dimethylsulfoxide in planaria

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Abstract

In this work, we describe aspects of the toxicity and behavioral effects of dimethylsulfoxide (DMSO) in planaria. Planarian worms have traditionally been a favored animal model in developmental biology. More recently, this organism is being recognized as an animal model in neuropharmacology research. DMSO is often used in cell and tissue culture as a cryoprotectant agent and is also commonly used to enhance the solubility of hydrophobic drugs in aqueous solutions. This compound can elicit various physiological effects in both vertebrates and invertebrates. Many drugs and drug candidates are hydrophobic, needing solvents like DMSO to be able to reach their physiological targets. As planaria becomes increasingly popular in neuropharmacology research, a description of the DMSO effects in this organism is essential. We found that DMSO is toxic to planarians at concentrations above 5% (705 mM), with an LD_{50} of 10% (1.4 M) at exposure times above 5 min. At sub-toxic concentrations, DMSO decreases planarian exploratory behavior in a concentration-dependent manner. This reduction in locomotor behavior is reversible and preincubation-independent. DMSO at a concentration of 0.1% (14.1 mM), which is usually enough to solubilize hydrophobic substances in aqueous solutions, did not display any toxic or behavioral effects in planaria. Therefore, in this animal model, DMSO concentrations above 0.1% should be avoided in order to be able to reliably observe any behavioral or toxic effects of hydrophobic drugs.

Keywords: Planaria; DMSO; Dimethylsulfoxide; Motility; Toxicity; Artificial pond water

Dimethylsulfoxide (DMSO) is often used in cell and tissue culture as a cryoprotectant agent [9]. DMSO is also a radioprotective agent, a free radical scavenger [33] and an aprotic solvent, this is, a solvent unable to donate hydrogen atoms to form hydrogen bonds [9]. DMSO is also commonly used to enhance the solubility of hydrophobic drugs in aqueous solutions [3]. In vertebrates, DMSO can elicit various types of physiological effects, which have been described as analgesic- or anesthetic-like, and are usually associated to decreases in nerve conductance [7,6,14]. In bullfrogs, DMSO also potentiates the suppression of synaptic transmission by lidocaine, an established local anesthetic [27]. In contrast, DMSO seems to enhance nociceptive transmission in newborn rats [12]. Several types of DMSO effects have also been described in invertebrates. For example, in the insect *Locusta migratoria*, DMSO eliminates the response of sensory neurons linked to mechanoreceptors [29], decreases locomotor behavior in the nematode *Caenorhabditis elegans* [2] and has been used to immobilize filarial larvae [15]. At the molecular level, DMSO is known to perturb phospholipid bilayers [1]. DMSO also interacts with ion channels [16,17,24] and with sodium–potassium ATPases [31]. Since DMSO can display several types of pharmacological effects in a variety of organisms, baseline studies using DMSO alone must be done in order to properly assess any effect of experimental drugs in a given biological system.

Fresh water planarians, non-parasitic worms of the phylum Platyhelminthes, have traditionally been a favored animal model in developmental biology; their remarkable regenerative properties have been extensively described [18,23]. They have also been traditionally used in toxicological research [8,26]. Planarian worms are commercially available at very low cost and are very easily kept in the laboratory. More recently, this organism is being recognized as a very useful animal model in neuroscience research. In evolutionary terms, planarians are one of the earliest examples of organisms displaying cephalization [24]. They have well-defined nerve cords and bi-lobed cerebral ganglia [18,25], as well as photoreceptors and chemoreceptors located at the head region [18]. The planarian nervous system uses many of the neurotransmitters found in vertebrates, including humans [4,30], and it displays ultrastructural features mainly observed in the vertebrate nervous system such as multipolar neurons and

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dendritic spines [25]. These worms have also been used to study abused drugs such as cocaine, amphetamines, cannabinoids and opiates [5,13,19,20,22].

Many drugs and drug candidates are hydrophobic in nature, needing solvents like DMSO to dissolve in aqueous solvents in order to reach their physiological targets. To the best of our knowledge, the effects of DMSO in planaria have not been systematically studied. Thus, as planaria becomes increasingly popular in pharmacological research, a description of the effects of DMSO by itself in this organism is necessary. In this work, we describe aspects of the toxicity and behavioral effects of DMSO in planaria.

Planarian worms (*Dugesia* sp.) were purchased from Carolina Biological Company (Burlington, NC) or Ward's (Rochester, NY). Dimethylsulfoxide was purchased from Fisher Scientific (Suwanee, GA). General laboratory materials and supplies were purchased from Fisher Scientific (Suwanee, GA) or Sigma–Aldrich (St. Louis, MO). All the experiments described here were done at room temperature using artificial pond water (APW, NaCl, 6 mM; $NaHCO_3$, 0.1 mM; $CaCl_2$, 0.6 mM; pH 7.3) as the vehicle. All graphs, data fittings and statistical procedures were done using the GraphPad or Instat software packages (GraphPad Software, San Diego, CA).

To determine the toxicity of DMSO, planaria (1.0–1.5 cm long) were exposed to 1 or 0.5 mL of DMSO at various concentrations in 12- or 24-well dishes, respectively. Each well included only one worm in either DMSO-free APW or a single DMSO concentration in APW. Each worm was used only once. The worms were exposed to the experimental conditions for a period of 2–20 min and the fraction of live worms in each DMSO concentration was recorded. These data were plotted as the fraction of live worms *versus* DMSO concentration at different time periods (Fig. 1A) and fit to a Hill-type equation in the form:

$$F = \frac{LD_{50}^{n}}{LD_{50}^{n} + S^{n}} \quad (1)$$

where F is the fraction of live worms at each DMSO concentration, S the percentage of DMSO, LD_{50} is the DMSO concentration that kills 50% of the worms and n is the Hill coefficient. The Hill coefficient is an indication of the steepness of the curve and under very limited circumstances, can provide information about the types and modes of interaction of the binding sites for a given experimental compound [32]. The Hill coefficient, however, provides little or no information about the specific mechanism of any described physiological effects [32], and therefore will not be studied in this work.

DMSO begins to show toxicity at concentrations above 3% (423 mM) at exposure times longer than 2 min with LD_{50} values close to 10% at exposure times from 8 to 20 min (Fig. 1B, Table 1). At DMSO concentrations above toxic levels, the worms autolysed upon dying. The autolysis time is directly proportional to the DMSO concentration (data not shown).

DMSO elicited several types of behavioral responses in planaria, such as "corkscrew"-like movements, twitching and pharynx protrusion among others, which were more pronounced at higher concentrations of DMSO. These effects were very similar to behaviors in phenol-exposed planaria [8]. This series of behavioral effects seems to be a common stress-related response in this organism; these effects have also been reported with exposure to a variety of other substances

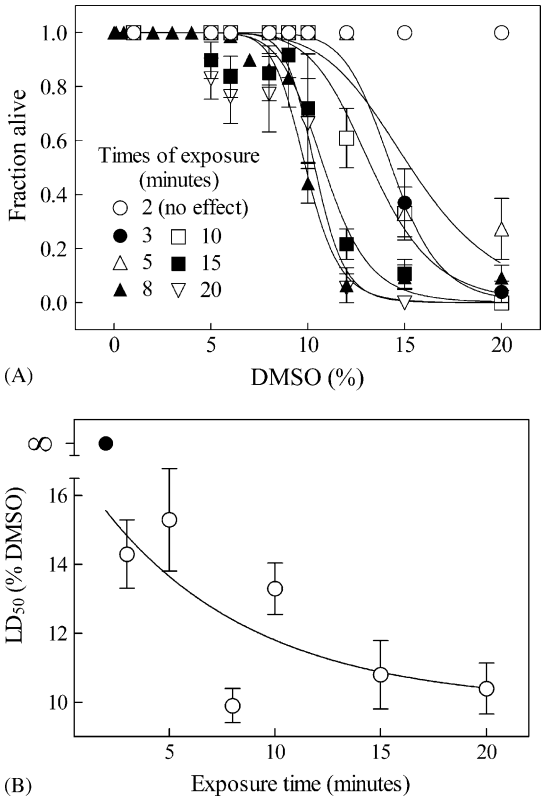

Fig. 1. (A) Planaria survival as a function of DMSO concentration at different exposure times, as indicated. Each data point includes the average of 12–18 worms. The error bars represent the standard error of the mean. The LD_{50} values are shown in Table 1. (B) LD_{50} values as a function of exposure time. The closed circle represents the experiments at an exposure time of 2 min. The error bars represent the 95% confidence interval.

Table 1
LD_{50} values for DMSO exposure at different time periods

Exposure time (min)	LD_{50} (%)
2	ND[a]
3	14.3 (13.6, 15.1)[b,c]
5	16.5 (15.2, 17.7)[c]
8	9.9 (9.6, 10.2)
10	13.3 (12.8, 13.8)[c]
15	10.8 (10.1, 11.5)
20	10.4 (9.7, 11.1)

The LD_{50} values were obtained by fitting the data from Fig. 1A to Eq. (1) (see text), with the exception of the 2-min time period, at which no toxicity was observed (Fig. 1A). These LD_{50} values are plotted as a function of exposure times in Fig. 1B.

[a] ND: not determined.

[b] Numbers in parenthesis represent the 95% confidence interval for the parameter.

[c] These values were significantly different from the value at the time period were behavioral responses to DMSO were observed (8 min; $P < 0.01$ by the Dunnett Multiple Comparisons Test).