Brain Research Bulletin 128 (2017) 34–39

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Effects of dimethyl sulfoxide on the morphology and viability of primary cultured neurons and astrocytes

Chen Zhang[a], Yuanying Deng[a], Hongmei Dai[a], Wenjuan Zhou[a], Jing Tian[a], Guoying Bing[b], Lingling Zhao[a,*]

[a] Department of Paediatrics, Third Xiangya Hospital, Central South University, Changsha, Hunan, China
[b] Department of Anatomy and Neurobiology, University of Kentucky, School of Medicine, Lexington, KY 40502, USA

ARTICLE INFO

Article history:
Received 10 August 2016
Accepted 3 November 2016
Available online 9 November 2016

Keywords:
Dimethyl sulfoxide
Primary culture
Neurons
Astrocytes
Morphology
Survival

ABSTRACT

Background: Dimethyl sulfoxide (DMSO) is a widely used solvent and vehicle for *in vivo* and *in vitro* administration of test compounds. Effects of DMSO independent of the test compound, such as in studies examining morphological plasticity or neurotoxic responses, may lead to spurious results.
Aim: To investigate effects of DMSO concentration ([DMSO]) on morphology and survival of primary cultured neurons and astrocytes.
Methods: Primary cultured neurons and astrocytes were treated with 0.25%–10.00% [DMSO] for 12–48 h. Viable cell number and morphology were compared to untreated cultures using the CCK-8 assay and phase-contrast microscopy. Expression levels of the neuronal marker NeuN and astrocyte marker glial fibrillary acidic protein (GFAP) were determined by immunofluorescence and western blotting.
Results: A [DMSO] ≤ 0.50% had no effect on neuronal number or NeuN expression up to 24 h, while ≥1.00% induced a progressive and dramatic loss of both viability and NeuN expression even after 12 h. Brief (12 h) exposure to ≤1.00% DMSO had no effect on astrocytes survival or GFAP expression, while ≥5.00% significantly reduced both at all exposure durations. In contrast to neurons, exposure to 0.50% and 1.00% DMSO for 24 or 48 h enhanced astrocytes proliferation and GFAP expression. Astrocytic processes were maintained at 0.50% and 1.00% DMSO, while neurons exhibited marked neurite retraction at ≥0.50%.
Conclusion: A [DMSO] ≥ 0.5% markedly disrupts neuronal morphology and reduces viability, even after brief exposure. In astrocytes, 0.50% and 1.00% DMSO appear to induce reactive gliosis. For treatment of neural cells, [DMSO] should be ≤0.25% to obviate spurious vehicle effects.

1. Introduction

Dimethyl sulfoxide (DMSO) is the most widely employed solvent for *in vitro* and *in vivo* administration of lipophilic compounds (Santos et al., 2003; Balakin et al., 2006) as well as for cryopreservation (Slichter et al., 2014). Good experimental design dictates that the drug-treated group be compared to a group treated with vehicle only. However, depending on concentration, DMSO alone can exert diverse and often contrary effects, including anti-inflammatory (Capriotti and Capriotti, 2012), anti-oxidant (Phillis et al., 1998a; Túnez et al., 2005), neuroprotective (Phillis et al., 1998a, 1998b; Túnez et al., 2005; Shimizu et al., 1997; Di Giorgio et al., 2008), and necrotic/apoptotic responses (Yuan et al., 2014; Hoyt and Szer, 2000; Junior and Arrais, 2008; Mueler and Theurich, 2007; Windrum and Morris, 2003; Hanslick and Lau, 2009). The toxicity of any drug depends on the ambient concentration, and indeed concentrations at or above ~1%–5% likely induce cell death by disrupting cell membranes (Yuan et al., 2014; Gurtovenko and Anwar, 2007; Notman et al., 2006; De menourval, 2010), while at lower concentrations, antioxidant and other beneficial effects may predominate (Phillis et al., 1998a; Túnez et al., 2005). These beneficial properties have been exploited clinically (Capriotti and Capriotti, 2012). Many of these effects may be outside the measurement parameters of a given experiment but still affect the process under study, so it is critical to establish threshold DMSO doses.

In neuroscience research, DMSO is often used to dissolve pesticides, but the concentration must be limited to obviate vehicle-mediated neurotoxicity (Julien et al., 2012). It has become a general rule in biology that concentrations ≤0.1% (v/v) DMSO are innocuous, although this assumption may not have solid experimental support. For example, 14 studies on the *in vitro*

* Corresponding author at: Third Xiangya Hospital of Central South University, Department of Paediatrics, 138, Tong Zipo, Changsha, Hunan 410013, China.
E-mail address: llzhao2011@qq.com (L. Zhao).

http://dx.doi.org/10.1016/j.brainresbull.2016.11.004
0361-9230/

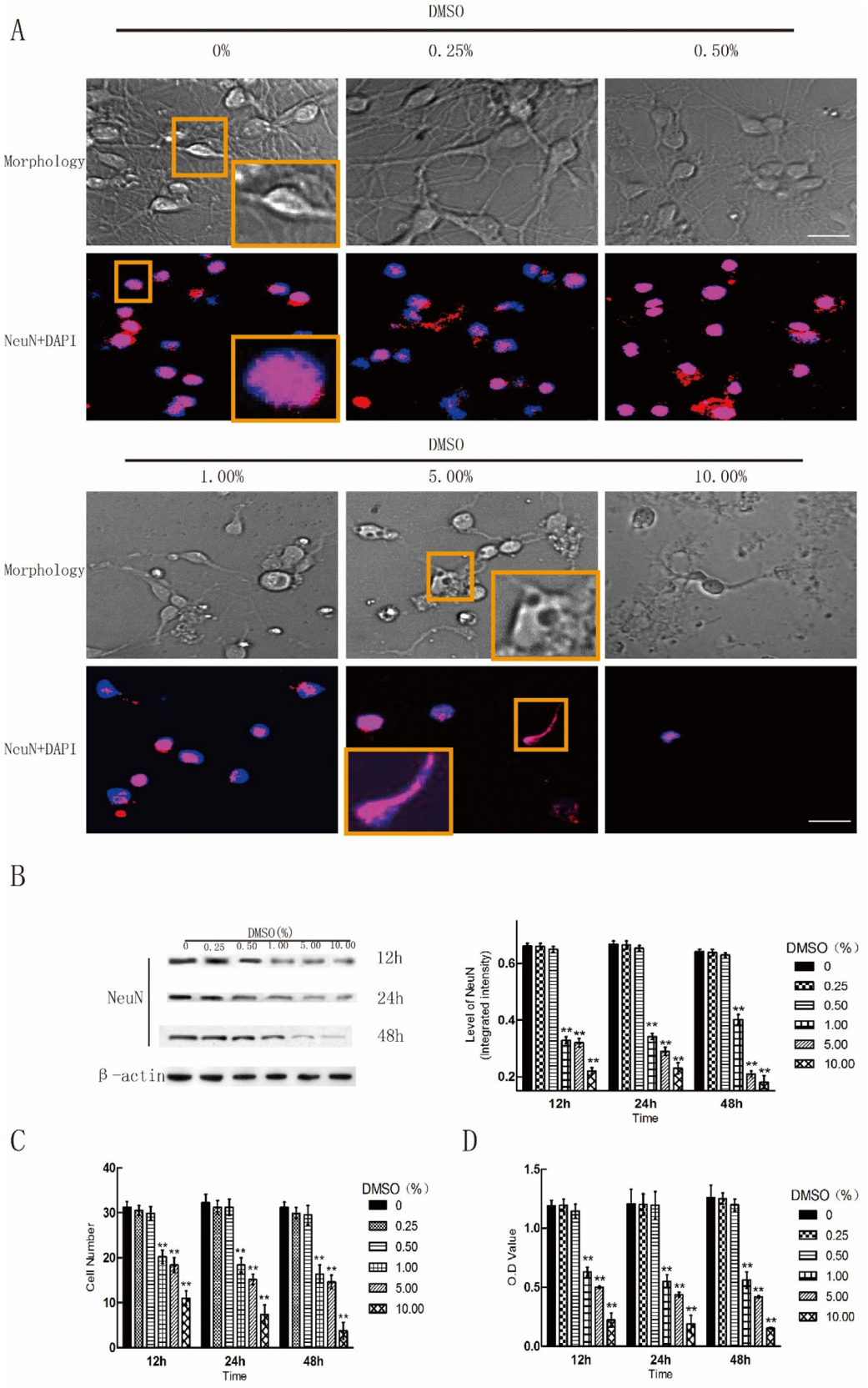

Fig. 1. Effects of dimethyl sulfoxide (DMSO) concentration and exposure duration on neuronal NeuN expression, morphology and survival. Primary cultured fetal rat cortical neurons were treated with 0% (control), 0.25%, 0.5%, 1%, 5%, and 10% DMSO for 12, 24, and 48 h. (A) After 24 h DMSO treatment, Neuronal morphology was observed under light microscope (magnification: × 400), and the NeuN level was observed under fluorescence microscopy (magnification: ×400), Red: NeuN; Blue: DAPI. 5%DMSO resulted in nuclear deformation and fragmentation (square frame). (B) NeuN protein expression was determined by western blot. β-actin was used as an internal gel loading control. (C) Cell number was counted under light microscopy (magnification: ×400). (D) Neuronal survival was determined by CCK-8 assay. Data are shown as mean ± standard error of the mean (SEM) of three independent experiments performed in quintuplicate $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* 0% DMSO at the same time point. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)