

Thermal Sensitivity and Dimethyl Sulfoxide (DMSO)

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Abstract

Dimethyl sulfoxide (DMSO) is commonly used as a solvent for hydrophobic substances, but the compound's innate bioactivity is an area of limited understanding. In this investigation we seek to determine the analgesic potential of DMSO. We addressed the issue by assessing the perception of thermal pain stimulus, using a 55 °C hotplate design, in conscious mice. The latency of withdrawal behaviors over a range of incremental accumulative intraperitoneal DMSO doses (0.5–15.5 g/kg) in the same mouse was taken as a measure of thermal endurance. The findings were that the latency, on average, amounted to 15–30 s and it differed inappreciably between the sequential DMSO conditions. Nor was it different from the pre-DMSO control conditions. Thus, DMSO did not influence the cutaneous thermal pain perception. The findings do not lend support to those literature reports that point to the plausible antinociceptive potential of DMSO as one of a plethora of its innate bioactivities. However, the findings concern the mouse's footpad nociceptors which have specific morphology and stimulus transduction pathways, which cannot exclude DMSO's antinociceptive influence on other types of pain or in other types of skin. Complex and as yet unresolved neural mechanisms of perception of cutaneous noxious heat stimulus should be further explored with alternative experimental designs.

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Keywords

Analgesia • Dimethyl sulfoxide • Nociception • Noxious heat • Pain • Perception • Thermal stimulus

1 Introduction

Dimethyl sulfoxide (DMSO) is best known for its capability of dissolving hydrophobic substances in neurobiological research. The compound is widely used in both *in vitro* and *in vivo* experimental routines. DMSO is of low toxicity, but it appears not neutral biologically *per se*. A recent pursuit for DMSO's properties has unraveled multifarious bioactivities on the detrimental-beneficial continuum, with the predominance of advantageous effects. These effects include neuroprotection, anti-inflammatory and anti-oxidant role (Kelava et al. 2011), with clinically proven benefits in genitourinary ailments (Shirley et al. 1978). DMSO is also reported to have topical analgesic effects, in particular in the skin-related nociception, which is due likely to its ease of permeation through biological barriers. Potentiation of the antinociceptive effect of topical analgesic medications has been noted in case of DMSO admixture (Stanos and Galluzzi 2013; Kumar et al. 2011).

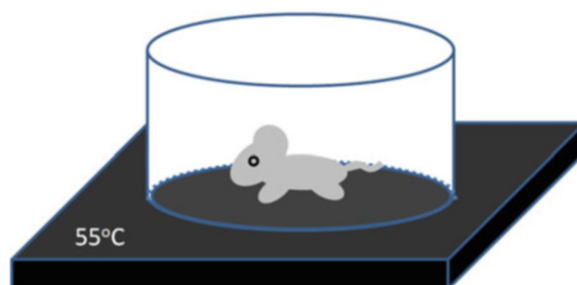
High temperature is an archetype nociceptive stimulus in experimental investigations. Thermal skin sensitivity, particularly assessed in the hotplate routine, in which the animal displays reflex pain-behaviors, such as paw licking or body jerks, is believed to involve supraspinal structures (Gregory et al. 2013). Perception of the thermal stimulus is widely used as a measure of the analgesic power of a compound. Therefore, in the current report we set out to assess the potential of DMSO to subdue thermal pain perception. We addressed the issue by examining the response to a hotplate-applied thermal stimulus in the conscious mouse.

2 Methods

The study was approved by the Ethics Committee for Animal Care and Use of the National Hospital Organization at the Murayama Medical Center in Musashimurayama, Tokyo. Experiments were carried out in seven male C57BL/6 mice (aged 7.1 ± 1.8 weeks, weighing 22.3 ± 3.6 g) that were housed at 12/12 light-dark cycle, with the light on at 7:00 a.m., and at controlled temperature of 25 °C. The current study on the influence of DMSO on perception of thermal pain expands on the previous investigation concerning the interaction of DMSO with respiratory function and it was carried out in the same set of conscious mice (Takeda et al. 2016). Sensitivity to thermal pain was considered an independent research ramification of the DMSO bioactivity and, therefore, was herein described as a separate entity.

Thermal sensitivity to acute hotplate stimulation was studied according to the method of Anthony and Annika (2007). Briefly, the unrestrained conscious mouse was placed on a water hotplate, covered with a transparent acrylic glass cylinder to prevent from leaving the platform, preheated to 55 °C (Fig. 1). The latency time from the animal placement on the hotplate to the first instance of upright standing or jerky jump on the hind limbs was taken as a measure of thermal pain endurance. The test was discontinued at the latency time measured or at 60 s in case of lack of response. Hotplate examination was carried out in the untreated mice, physiological saline-treated mice, both considered basic controls, and then was repeated 50 min after each intraperitoneal injection of

Fig. 1 Schematic representation of the hotplate experiment



incremental doses of DMSO according to the scheme outlined below:

- Untreated
- Physiological saline 1.82 mL/kg
- DMSO 0.46 mL/kg + saline 1.36 mL/kg (DMSO dose: 0.5 g/kg)
- DMSO 0.91 mL/kg + saline 0.91 mL/kg (cumulative dose: 1.5 g/kg)
- DMSO 1.82 mL/kg (cumulative dose: 3.5 g/kg)
- DMSO 3.64 mL/kg (cumulative dose: 7.5 g/kg)
- DMSO 7.28 mL/kg (cumulative dose: 15.5 g/kg)

The dose of DMSO was titrated to the range of LD₅₀ dose level (Caujolle et al. 1964; Farrant 1964). DMSO has a long-term bioactivity, persisting for hours (DMSO 2007). Therefore, the most rational way to perform the experiment was to treat the incremental doses as a cumulative dose. The cumulative dose regimen had also the advantage of each mouse being its own control along the trajectory of responses, optimizing the study outcome. DMSO administration was in each mouse preceded by physiological saline injection in a volume of 1.82 mL/kg, used as control. The mean \pm SE values of response latency were compared with those before DMSO injection using Dunnett's *t*-test. The significance level was set at $p < 0.05$.

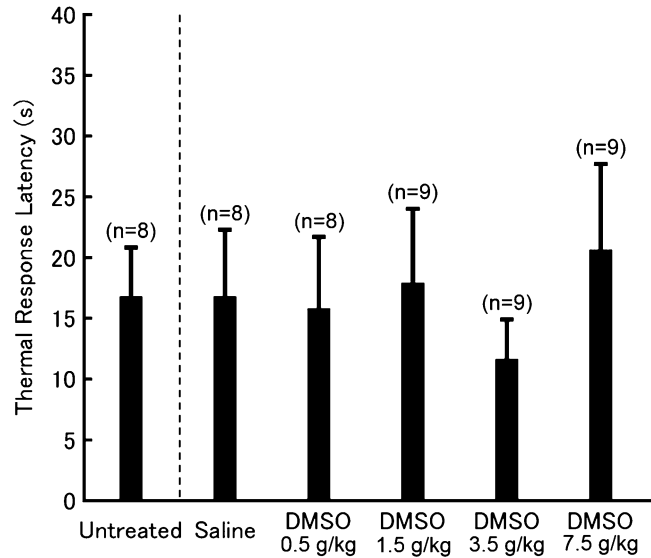
3 Results and Discussion

In all experimental conditions, except for the 15.5 g/kg DMSO, eight or nine out of the nine mice showed a paw licking, standing or jumping

response to thermal stimulation. The response latency, on average, ranged between 15 and 30 s in the majority of instances, with a fairly substantial interindividual scatter of values. Overall, there were inappreciable differences in the latency between the sequential DMSO conditions and the untreated or physiological saline-treated control level. Nor were there any appreciable differences between individual DMSO conditions (Fig. 2). At the 15.5 g/kg DMSO, which borders the LD₅₀ concentration (DMSO 2007), eight out of the nine mice were immobilized, so that the latency data for this condition could not be tallied and thus are not provided in the figure. The immobilization could likely be due to the central toxic effect of a near lethal dose of a compound that easily permeates through the brain-blood barrier. In fact, DMSO neurotoxic effects attributed to the barrier disruption, with brain edema or infarct, have been observed at much smaller doses (Kleindienst et al. 2006; Windrum and Morris 2003).

The finding of the current study was that DMSO over a broad spectrum of concentration did not interfere with the perception of thermal pain. DMSO did not affect pain perception either at the low end of the dose spectrum used of 0.5–1.5 g/kg, the concentration reportedly having distinct central neurotoxicity such as causing brain ischemic episodes and encephalopathy observed during transplantation of autologous stem cells cryoprotected with DMSO in hematologic cancers (Caselli et al. 2009; Windrum and Morris 2003), or at the high end of 7.5 g/kg, seldom if ever used in biological experiments, when distorted respiratory regulation is observed (Takeda et al. 2016). The detrimental bioactivities above outlined have to

Fig. 2 Response latency to thermal stimulation of 55 °C in the untreated, physiological saline-treated, and with incremental DMSO dose-treated mice. Differences between the presented conditions were inappreciable (Dunnett's *t*-test; $p > 0.05$)



do, in all likelihood, with disruptive DMSO effect on the blood-brain barrier.

The lack of DMSO effect on thermal pain perception was a rather unexpected finding in light of its antinociceptive properties reported in some studies. DMSO has an antipain effect longer than morphine, although seemingly unrelated to opioid transmission as it is not contingent on naloxone antagonism (Haigler 1983; Haigler and Spring 1981). It also displays skin-related antinociception in topical preparations in conjunction with morphine or lidocaine (Kumar et al. 2011; Kolesnikov et al. 2000), which goes beyond that presented by either archetype analgesic agent. These antinociceptive effects might have to do with DMSO-induced dampening of peripheral C-fiber activity (Shealy 1966) or with its easy penetration into the stratum corneum when applied to the skin surface (Sulzberger et al. 1967), which makes DMSO a carrier of the accompanying analgesic compounds into deeper skin layers. DMSO applied alone, however, seems to lose its analgesic power (Kolesnikov et al. 2000).

Footpad epidermis in the mouse belongs to glabrous (non-hairy skin) which is equipped with myelinated fiber endings and unmyelinated C-fiber endings terminating freely or as Meissner's-like corpuscles among the epidermal

keratinocytes (Abraira and Ginty 2013; Lindfors et al. 2006). Cutaneous nociceptors that mediate temperature sensation and pain are mostly unmyelinated endings of primary afferent neurons. Thermal sensation is transmitted *via* the dorsal root ganglions to the thalamus, where the pathway crosses to the contralateral sensory cortex (Abraira and Ginty 2013). The primary neurons are usually polymodal, responding to various sensory stimuli and containing various neuropeptides, with the predominance of calcitonin gene-related peptide and substance-P expression (Navarro et al. 1995). Thus, the neural perception pathways of thermal pain and other sensory stimuli intertwine.

The research on thermal transduction has recently focused on the transient receptor potential (TRP) channels subfamily V1 and A1. Both TRPA1 and TRPV1 colocalize in neurons of the dorsal root ganglia at the lumbar level which innervate the hindpaw in the mouse (Hoffmann et al. 2013), and both are expressed in unmyelinated peripheral nerve fibers (Weller et al. 2011). Coordinated and apparently not cross-dependent action of both channel types underlies the response to suprathreshold heat stimulation, which in the mouse corresponds to >42 °C. Recent evidence gathered from TRPA1 knock-out mouse studies indicates that TRPA1 has a critical role in

suprathreshold pain responsiveness specifically concerning the stimuli applied to the plantar surface of a hindpaw in the mouse (Minett et al. 2014), which is exactly what the current hotplate investigation was about. However, even in TRPA1/V1 double-knockout mice there still remains a substantial component of pain-reflex behavior (Hoffmann et al. 2013), which underscores the complexity of as yet unresolved mechanisms of noxious heat stimulation. How exactly the plantar nerve endings determine the summation of temperature intensity and duration into the above pain threshold level is by far unclear. Nonetheless, lack of thermal antinociceptive effect of DMSO suggests the compound is devoid of interaction with the specific nociceptor transducers outlined above which are responsible for transmission of the footpad thermal pain stimulus.

In conclusion, we believe we have shown that the paw-transduced behavioral withdrawal responses to noxious heat remains, on average, unchanged in DMSO-treated mice. Therefore, DMSO does not influence the cutaneous thermal pain perception. That does not exclude DMSO's antinociceptive influence on other types of pain or in other types of skin, such as hairy skin, or in other body locations; the factors that involve different neural transduction pathways and which, therefore, ought to be pursued with alternative designs of behavioral pain study.

Acknowledgements This work was supported by JSPS KAKENHI Grants 25540130, 26460311, 26670676, 15K00417, 15K12611, Health and Labor Sciences Research Grants of Japan, and JSPS Japan-Poland Researcher Exchange Program Grant.

Conflict of Interests None declared.

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