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Evaluation of the neurotoxicity of DMSO infused into the carotid artery of rat

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ABSTRACT

Introduction: Despite the explanations put forth in many studies regarding histopathological evidence of the inflammatory stage related with the infusion of dimethyl sulfoxide (DMSO) in the vessel wall and its lumen, there has been no research to evaluate its neural toxicity when it is infused via the intracarotid route. This study was designed to evaluate the possible neurotoxic effects of DMSO on the closer and distant brain tissue and carotid artery when it was slowly infused into the internal carotid arteries of the rats.

Methods: The right common carotid artery bifurcation was exposed through a midline neck incision, and then except those of the control group animals ($n = 5$), the experimental material (normal saline, $n = 5$ or anhydrous DMSO, $n = 10$) was infused into the internal carotid artery of the Wistar albino rats. After the experimental materials were administered intra-arterially, brain tissues were harvested for histopathological and biochemical studies at 72 h for investigation of the acute stage changes and on 10th day for investigation of the chronic stage changes. Internal carotid arteries of both sides were also removed for histopathological evaluation. During sacrification of the rats, whole body blood of them are collected for biochemical evaluation.

Results: There was no statistically significant difference between the groups regarding comparison of the mean values of the hippocampal neuronal cell counts and the carotid artery diameters in both acute and chronic stages. Also, mean values of the lipid peroxidation levels of harvested brain tissues and serums of the collected bloods were similar in control, saline and DMSO groups.

Conclusion: This experimental study suggested that DMSO has no toxic effect on the neural and arterial tissues of rats when it is slowly infused into the carotid artery.

Introduction

Dimethyl sulfoxide (DMSO) is an amphipathic molecule with a highly polar domain and two apolar groups, making it soluble in both aqueous and organic media. So, DMSO is a very efficient solvent for water soluble compounds and is a hydrogen-bound disrupter.[1] DMSO which is commonly used in several human therapeutic situations, such as drug-delivery systems, cryopreservation of autologous peripheral blood stem cells, and embolisation of cerebral aneurysms or arteriovenous malformations (AVM) has a variety of biological actions that have made it target of numerous studies.[2,3]

Although, it has occasionally been proposed to be neuroprotective, oxidative and induce behavioural alterations, its mechanisms of action are still unclear.[4] It has been reported to alter the permeability of cell wall and facilitate the transport of substances across membranes.[4] Besides all of the previously aforementioned pharmacological applications for the treatment of different pathologies, several systemic side effects of DMSO have also been reported.[1] Additionally, rapid intra-arterial injections presumably causing fatal solvent related adverse effects (such as vasospasm, angionecrosis, endothelial denuding, internal elastic lamina disruption, subarachnoid haemorrhage, stroke, and death) have also been documented.[2,5] In animal studies, Sampei et al. suggested that during the infusion of DMSO into the cerebral arteries (such as embolisation of the cerebral aneurysm or AVMs), care should be taken not to damage normal vessels and brain tissue around the lesion.[6] Chaloupka et al. showed that DMSO tends to be angiotoxic and neurotoxic.[5,7] Furthermore, recently published studies point to the neurotoxicity associated with DMSO-preserved hematopoietic progenitor cell infusion in human.[8–10] This issue may be explained by its high solubility and permeability.

Despite the explanations put forth in many studies regarding histopathological evidence of inflammatory changes in the vessel

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wall and the lumen,[2,5,6] to the best of our knowledge there has been no research on the neural toxicity of DMSO infused via intracarotid route. In our previous study, we speculated that DMSO used in intra-arterial embolisation procedures may also enter the circulation; and may rapidly move away from blood into cerebral tissue and/or cerebrospinal fluid circulating around these vessels. Our study demonstrated that DMSO produced some neurotoxic effects when it was infused into the subarachnoid space of the rabbit. Furthermore, these toxic effects were negatively correlated with the distance and concentration of the infused experimental material.[11] Regarding our previous study results, this study was designed to evaluate the possible neurotoxic effects of DMSO to the close and distant brain tissue and carotid artery when it was slowly infused into the internal carotid arteries of the rats.

Materials and methods

Materials

Anhydrous DMSO (DMSO, Micro Therapeutics Inc., Irvine, CA), and normal saline solution were used in this study. Anhydrous liquid DMSO density is approximately 1.10 mg/ml, its intravenous LD_{50} is 5.2–8.1 g/kg for the rat, and its half life in the serum is approximately 60–72 h.[12]

Anaesthesia was performed with intraperitoneal administration of 40 mg/kg ketamine HCl (Ketalar®; Pfizer Inc., USA), and 5 mg/kg xylazine HCl (Rompun® 2%; Bayer HealthCare AG, Germany).

Twenty Wistar albino rats of 250–350 mg weight were used, which were randomly divided into two main groups used for the acute stage investigation (72 h after the injections of experimental materials) and chronic stage investigation (ten days after the injection).

The acute stage group was randomly divided into four groups listed as below:

- Control (no chemical material was infused) (*n*: 5)
- Saline (normal saline solution was infused in a volume of 0.1 ml) (*n*: 5)
- DMSO-A (anhydrous DMSO was infused in a volume of 0.1 ml; the right hemisphere of the brain) (*n*: 5)
- DMSO-AF (anhydrous DMSO was infused in a volume of 0.1 ml; the left hemisphere of the brain) (*n*: 5)

The chronic stage group was randomly divided into two groups listed as below:

- DMSO-C (anhydrous DMSO was infused in a volume of 0.1 ml; the right hemisphere of the brain) (*n*: 5)
- DMSO-CF (anhydrous DMSO was infused in a volume of 0.1 ml; the left hemisphere of the brain) (*n*: 5)

Methods

This experimental study was performed in accordance with the guidelines for the use of laboratory animal subjects in research set by the Ethical Committee of Kırıkkale University (Date: May 27th, 2009; number: 09/29).

All animals were placed under sedational anaesthesia with intraperitoneal ketamine HCl 40 mg/kg and xylazine HCl 6 mg/kg during spontaneous respiration at room temperature. Under an operating microscope, bifurcation of the right common carotid artery was exposed through a midline neck incision, and then except the control group, experimental material (normal saline or anhydrous DMSO) was administered slowly (within 30 s) at a volume of 0.1 ml through the right internal carotid artery (ICA) of the rats using a 26G needle.[7,13–18] After this procedure, all rats were removed from sedational anaesthesia spontaneously under the blanket. They were then kept at normal room temperature, and examined by a neurosurgeon twice a day for the development of any neurological deficit. Seventy two hours later, all animals except those of DMSO-C group; and ten days later all animals of the DMSO-C group were re-sedated with intraperitoneal ketamine HCl 40 mg/kg and xylazine HCl 6 mg/kg for sacrification. For sacrification, the whole body blood was collected from the vena cava inferior, and then the rats were decapitated (Fig. 1).

All rat brains were divided into two hemispheres with a cut from sulcus centralis and then the hippocampal formations of both hemispheres were dissected and stored in 10% buffered formaldehyde solution at room temperature for future histopathological examination. In addition, the ICA of the both sides was also resected for future histopathological evaluation of angiotoxicity.

For biochemical examination frontal regions samples dissected from both hemispheres and the serum of the collected whole body blood were immediately stored at −30 °C in dry air. The remaining right brain tissues were used for histopathological and biochemical evaluation of the possible neurotoxic effects of DMSO to the closer brain tissues whilst left brain tissues were used for the evaluation of its distant effects.

Histopathological analysis

For histopathological examination, all tissue samples were fixated in 10% buffered formaldehyde and processed according to the routine light microscopic tissue processing technique. Serial sections of 5 μm thickness stained with haematoxylene–eosin (H&E) were examined and photographed by using an Olympus

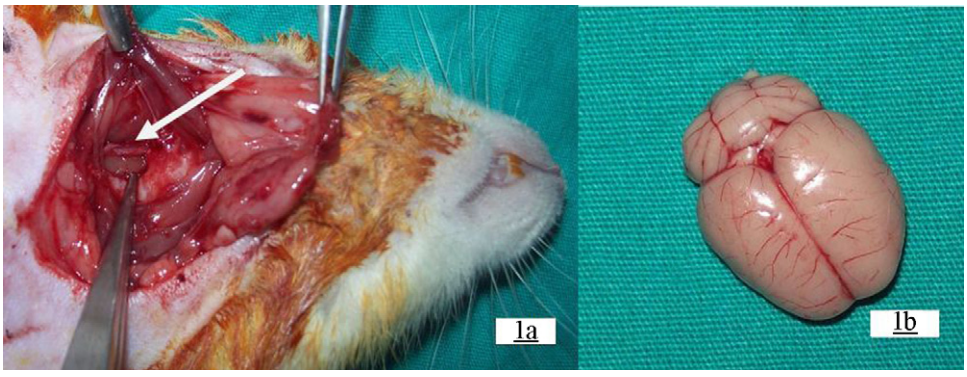

Fig. 1. Figures demonstrate: (a) the dissected right carotid artery of the rat, and (b) the macroscopic appearance of the whole cerebrum, cerebellum, and cervical spinal cord following administration of DMSO.

CX-41 Microscope. The number of cells in each group was counted and calculated separately in three areas per section of the hippocampal CA1 region. Then the number of the neurone cells was calculated as an average per rat. The vessel walls and lumens of the right and left internal carotid arteries were also measured for diameter and thickness by using a computer image analysis system (Aperio image scope, version 10.1.3.2028). The investigator who performed these measurements was blind to the study groups.

Biochemical analysis

The biochemical data was obtained from the concentrations of malonyldialdehyde (MDA) levels in brain tissue. The MDA (as an important indicator of lipid peroxidation) levels were measured according to a method of Mihara et al.. The principle of the method was based on the spectrophotometric measurement of the colour that occurred during the reaction of thiobarbituric acid with MDA. The concentration of thiobarbituric acid reactive substances (TBARS) was calculated by the absorbance coefficient of malondialdehyde–thiobarbituric acid complex.[19] In this study, all specimens were evaluated by an experienced biochemist blinded to the study groups, and experimental material. Biochemical analyses were performed by thiobarbituric acid application and then 532 nm, spectrophotometry (Shimadzu® UV-120-02 Spectrophotometer) was used for measuring the lipid peroxidation levels in nanomoles per gram of wet tissue.

Statistical analysis

The study results consisted of hippocampal neuronal cell counts, carotid artery diameters and serum and tissue lipid peroxidation levels. The values were not normally distributed and variations were not homogenous between all groups. Therefore, *ANOVA* (one-way analysis of variance) test was not performed and all results were statistically analysed by using the *Krusskal–Wallis Multiple Variant Analysis* (the non-parametric analogue of ANOVA) test, and p values less than 0.05 were considered to be significant. Furthermore, *Wilcoxon Signed Ranks Test* (the non-parametric analogue of the paired samples t test) was performed to determine the statistical differences between the results of right and left brain hemispheres for each group.

p values less than 0.05 were considered to be significant.[20,21]

Results

Neurological examination

Three animals displayed mild ataxia and fatigue after the injection of DMSO; however, they were included in the study. The other animals displayed normal neurological and physiological function.

Light microscopy

In the control and saline groups neuropil in-between the neurons with euchromatic nuclei showed a normal architecture. No oedema, infiltration or stasis was seen in the neural tissue of these groups (Fig. 2a and b).

The specimens of the DMSO-A and DMSO-AF groups showed no degeneration or destruction of the neural tissue, haemorrhage, oedema or inflammatory cell infiltration (Fig. 2c and d).

Also the DMSO-C and DMSO-CF groups had no degeneration of the neural tissue, haemorrhage, oedema or inflammatory cell infiltration (Fig. 3a and b).

Furthermore, all resected internal carotid arteries appeared grossly normal with no angionecrosis. Histopathologic signs of angionecrosis, fibrin deposition, haemorrhage, disruption of internal elastic lamina, or acute and/or chronic cellular inflammation were not observed in any of the specimens investigated (Figs. 4 and 5).

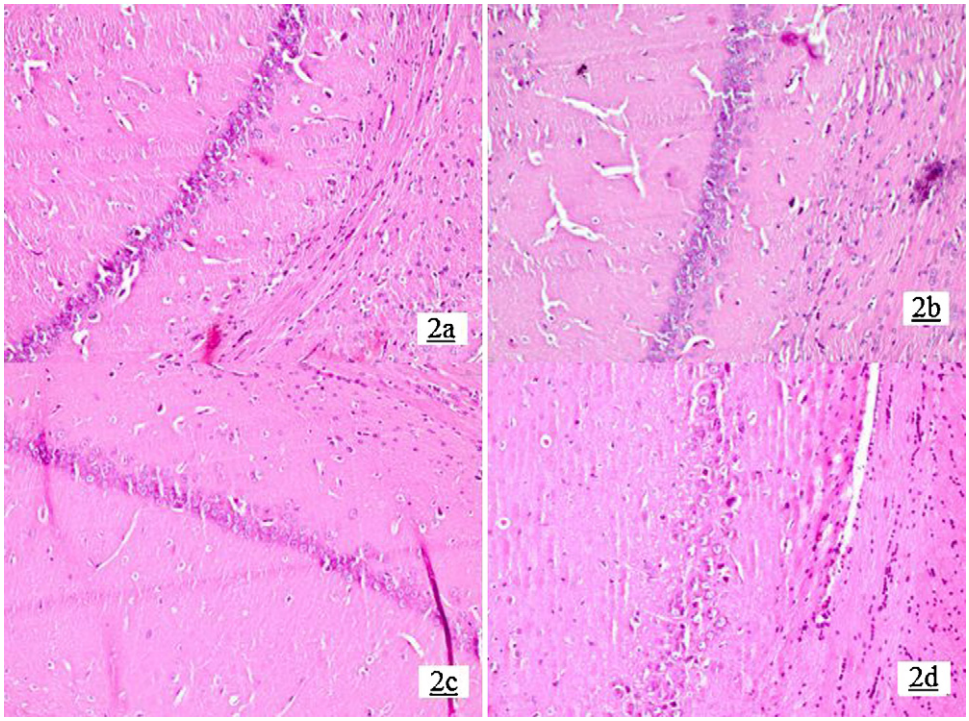

Fig. 2. (a and b) In the control and saline groups, neuropil in-between the neurons with euchromatic nuclei showed a normal architecture in the control and saline groups. No oedema, infiltration or stasis were seen in the neural tissue. (c and d) The specimens of the DMSO-A and DMSO-AF groups showed no degeneration or destruction of the neural tissue, haemorrhage, oedema or inflammatory cell infiltration, respectively (HEx40).

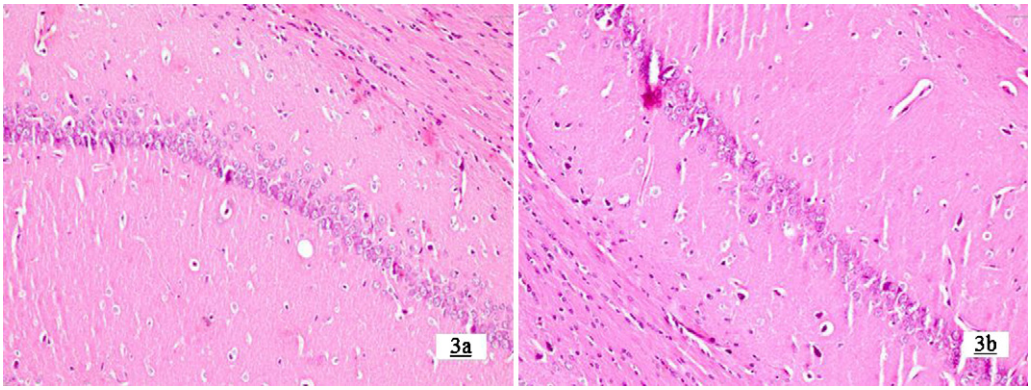

Fig. 3. (a and b) The DMSO-C and DMSO-CF groups had no degeneration of the neural tissue, haemorrhage, oedema or inflammatory cell infiltration (HEx40).

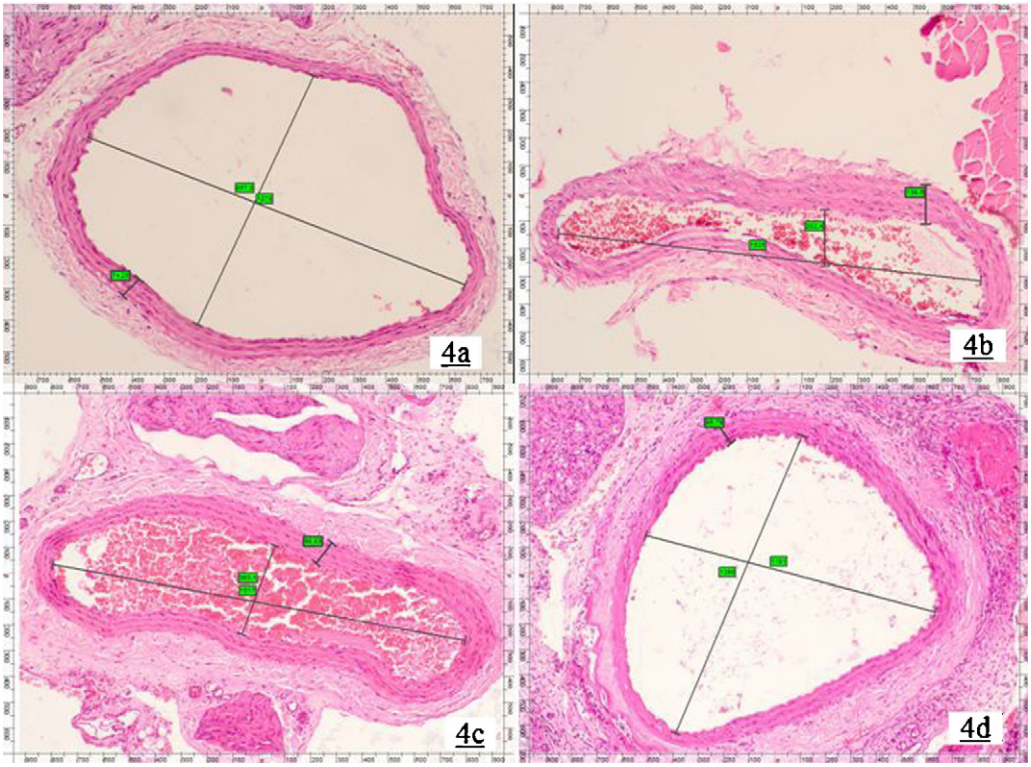

Fig. 4. All resected carotid arteries of the control (a), saline (b), DMSO-A (c), and DMSO-AF (d) group appeared grossly normal with no angionecrosis (HEx40).

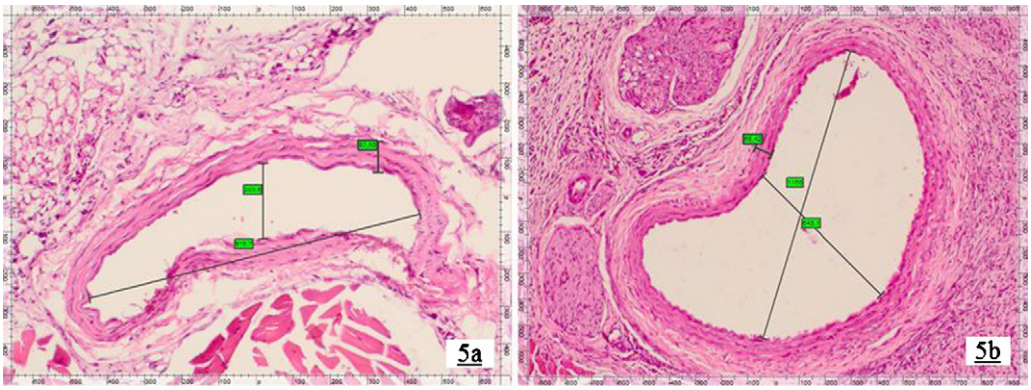

Fig. 5. Histopathologic signs of angionecrosis, fibrin deposition, haemorrhage, disruption of internal elastic lamina, or chronic cellular inflammation were not observed in any of the specimens of the DMSO-C (a) and DMSO-CF (b) groups (HEx40).

Table 1
This descriptive table shows the mean values of the neuronal cell counts, the wall thickness of the carotid artery, and serum and tissue lipid peroxidation levels of the DMSO-A, DMSO-AF, DMSO-C, DMSO-CF, saline, and control groups.

Group		*N*	Minimum	Maximum	Mean	Std. deviation
Control	HNC	5	40.66	64.33	57.32	9.58
	CAT	5	74.26	138.60	93.74	26.03
	BLPO	5	0.330	0.416	0.388	0.03
	SLPO	5	0.044	0.070	0.054	0.01
Saline	HNC	5	59.66	72.33	64.59	5.04
	CAT	5	69.51	95.24	83.19	10.69
	BLPO	5	0.372	0.450	0.412	0.02
	SLPO	5	0.045	0.075	0.059	0.01
DMSO-A	HNC	5	50.66	82.00	62.06	12.79
	CAT	5	70.34	104.20	89.59	13.11
	BLPO	5	0.320	0.434	0.393	0.04
	SLPO	5	0.033	0.066	0.054	0.01
DMSO-AF	HNC	5	30.00	66.33	56.19	15.19
	CAT	5	81.28	98.59	88.91	6.26
	BLPO	5	0.320	0.570	0.410	0.09
DMSO-C	HNC	5	63.33	69.00	67.06	2.25
	CAT	5	55.07	93.71	81.07	15.73
	BLPO	5	0.350	0.406	0.372	0.02
	SLPO	5	0.032	0.062	0.045	0.01
DMSO-CF	HNC	5	61.33	71.33	66.53	3.59
	CAT	5	68.42	100.00	86.01	11.35
	BLPO	5	0.284	0.410	0.373	0.05

CAT: the wall thickness of the carotid artery; HNC: hippocampal neuronal cell count; SLPO: serum lipid peroxidation level; BLPO: lipid peroxidation level of the brain tissue; *N*: number of animal.

Hippocampal neuronal cell count results

There was no statistically significant difference between the groups which were evaluated at the acute stage and between the groups which were evaluated at the chronic stage regarding the mean values of the neuronal cell counts. Furthermore, mean values of the acute stage groups were not different from those of the chronic stage groups ($X^2 = 9.575$; $p = 0.088$) (Table 1).

In addition, when mean values of the neuronal cell count were compared for each group, results of the right hemispheres were not significantly different from those of the left hemispheres regarding the acute ($Z = -0.135$; $p = 0.893$) or chronic stage ($Z = -0.271$; $p = 0.786$).

In conclusion, it can be said that anhydrous DMSO given intra-arterially did not produce any neurotoxic effect in the rat brain either at the acute or chronic stage (Fig. 6).

Carotid artery diameters

There was no statistically significant difference between the groups which were evaluated at the acute stage and between the groups which were evaluated at the chronic stage regarding the mean values of vessel wall thickness. Furthermore, mean values of the acute stage groups were not different from those of the chronic stage groups ($X^2 = 1.573$; $p = 0.905$) (Table 1).

Additionally, when mean values of the vessel wall thickness were compared for each group, results of the right carotid artery were not significantly different from those of the left carotid artery at acute ($Z = -0.135$; $p = 0.893$) or chronic stage ($Z = -1.753$; $p = 0.080$).

Eventually, it can be said that anhydrous DMSO given intra-arterially did not produce any vasospasm or angiotoxic effect in the internal carotid artery of rat both at the acute and chronic stage (Fig. 7).

Biochemical analysis

There was no statistically significant difference either between the groups which were evaluated at the acute stage or between the groups which were evaluated at the chronic stage regarding the comparison of mean values of the serum and tissue lipid peroxidation levels. Furthermore, mean values of the acute stage groups were not different from those of the chronic stage groups ($X^2 = 4.529$; $p = 0.476$ and $X^2 = 7.004$; $p = 0.220$, respectively for lipid peroxidation levels of the brain tissue and serum) (Table 1).

Additionally, when the mean values of the tissue lipid peroxidation levels were compared for each group at different stages, results of the right hemispheres were not significantly different from those of the left hemispheres ($Z = -0.674$; $p = 0.500$ and $Z = -0.405$; $p = 0.686$ for the acute and chronic stage groups, respectively)

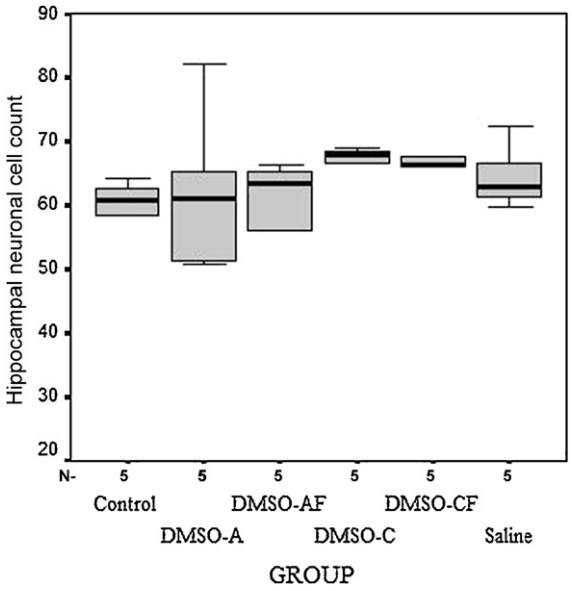

Fig. 6. The mean values of the neuronal cell counts of the DMSO-A, DMSO-AF, DMSO-C, DMSO-CF, saline, and control groups. Each error bar shows the minimum and maximum of the cell count values.

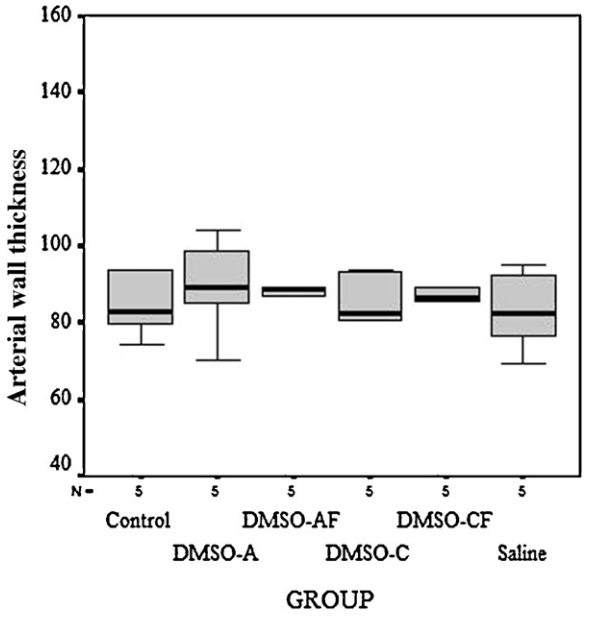

Fig. 7. The mean values of arterial wall thickness of the DMSO-A, DMSO-AF, DMSO-C, DMSO-CF, saline, and control groups. Each error bar shows the minimum and maximum of the thickness values.

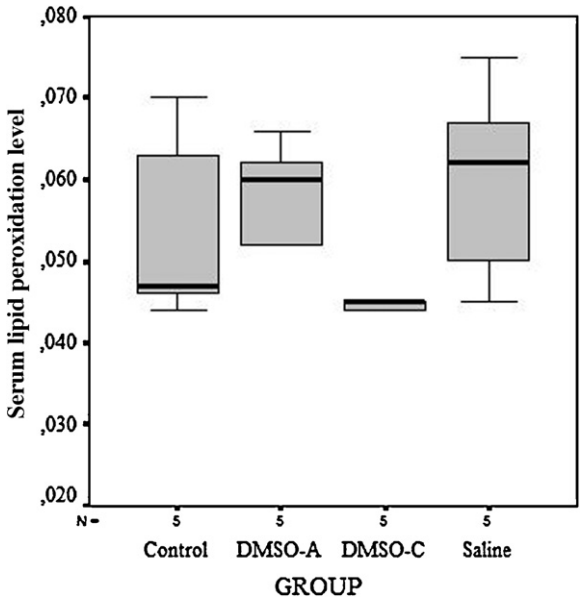

Fig. 9. The mean values of the serum lipid peroxidation levels of the DMSO-A, DMSO-C, saline, and control groups. Each error bar shows the minimum and maximum of the lipid peroxidation level values.

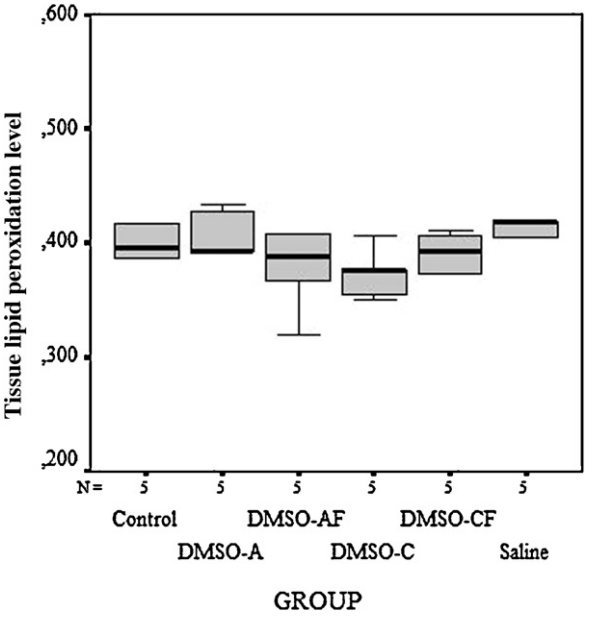

Fig. 8. The mean values of the tissue lipid peroxidation levels of the DMSO-A, DMSO-AF, DMSO-C, DMSO-CF, saline, and control groups. Each error bar shows the minimum and maximum of the lipid peroxidation level values.

These findings show that anhydrous DMSO did not affect the lipid peroxidation levels at both stages (Figs. 8 and 9).

Discussion

DMSO has been used for many years mainly as a solvent and tissue preservative. Recently, many clinical reports pointed out a variety of dose dependent adverse effects such as nausea, vomiting, flushing, fever, chills, dyspnoea, cardiac symptoms, transient hypertension, hypotension, anaphylaxis, encephalopathy, amnesia and seizures.[9] However, Mueller et al. pointed out in their clinical study that severe adverse reactions in general and neurotoxicity in particular upon infusion of DMSO-preserved autologous peripheral blood stem cells (DMSO-PBSC) occurred with a low incidence (2%). They also discussed that DMSO seems not to enrich in brain tissue, but affect the vascular system resulting in vasospasm which then may lead to altered neuronal function (such as a reversible leukoencephalopathy syndrome). Finally, they concluded that patients with cerebral disease were at higher risk for such neurotoxicity but a possible pathogenetic role was not supported by the literature; and they said that infusion of DMSO-PBSC can be performed safely in patients with preexisting cerebral disease despite the rare occurrence of neurotoxicity.[22]

Denko et al. reported in their animal model that DMSO administered via intraperitoneal route rapidly lodged in soft and hard tissues. In that study, higher levels of DMSO were found in soft tissues (such as spleen, stomach, lung, vitreus humour, brain, kidney, heart and aorta, respectively) than in cartilage or bone.[23] Kaye et al. observed in their study that DMSO was rapidly and extensively distributed through the tissues and brain in mice when it was infused via intravenous route, but they deduced that brain tissues contained the lowest concentration of DMSO amongst the tissues studied. They also reported that intravenous injection of DMSO had not produced any histological evidence of central nervous system changes.[24] Sampei et al. demonstrated that when it was administered via the intracarotid route, DMSO led to irreversible degenerative changes including vacuolisation of the endothelial cells and partial necrosis of vessel wall smooth muscle. On light microscopy, they also observed scarty areas of background and pale staining in areas corresponding to brain tissues due to exudated Evans blue in acute stage of the study (5 min). On the other hand, they observed no obvious abnormality and no exudation of Evans blue in any group of the chronic stage (10 days). However, they did not evaluate in detail the neurotoxicity of the DMSO. At the end of their study, they suggested that catheterisation should be strictly superselective when DMSO was used at high concentrations.[6] Moreover, Chaloupka et al. showed in their animal model that DMSO produced a mononuclear and neutrophil preponderant inflammatory cell infiltration in the vessel wall and contiguous neural tissue. They also concluded that rapid infusion (approximately 15 s or less) of the anhydrous DMSO into the swine rete mirable was associated with subarachnoid

haemorrhage, severe vasospasm resulted in cerebral infarction and death.[5] Furthermore, they pointed out in 1999 that lower dose rates of slower (approximately 30 s or more) superselectively infused anhydrous DMSO was associated with no significant clinical haemodynamic or neurologic sequale.[7,25]

In our study, we did not observe any early or late vasospasm, angionecrosis, arterial wall rupture, granulomatous obliterative angiitis or intimal hyperplasia in any histopathological slice of the carotid artery. So, we agree with the authors who advocate slow infusion of DMSO in order to avoid its angiotoxic effects during intra-arterial administration. Additionally, histopathological and biochemical results of our study revealed that DMSO destroyed the architecture of neither brain parenchyma nor vessel wall of the carotid artery. There was no inflammatory cell infiltration in any slice of the brain or carotid artery, and the neuronal cell count results of all groups were almost similar. These findings suggested that DMSO does not have any angiotoxicity or neurotoxicity when it is slowly administered via the intracarotid route. Also, it can be suggested that DMSO cannot alter the blood–brain barrier permeability when slowly infused via the intra-arterial route since it dissolves rapidly in the water content of the blood and moved away from the neural tissue by arterial circulation.[6,24] Although the blood–brain barrier disruption developed by the DMSO was not evaluated by injection of the Evans blue, it may be reasonably hypothesised that DMSO could pass through the blood–brain barrier with difficulty. Therefore, it could not reach the concentration of its toxic dose in neural tissue. Keane et al. and Broadwell et al. reported in their studies that DMSO did not alter the morphology of endothelial cells or brain parenchyma, and the permeability of both blood–brain barrier and skeletal muscle.[26,27] Moreover, Murayama et al. showed in a swine model that the brain slices of the rapid (0.5 ml/5 s) DMSO injection group disclosed low grade encephalitis with a mixed infiltrate of acute and chronic inflammatory cells, acute subarachnoid haemorrhage, and focal acute necrosis in subpial locations. However, they observed minimum or no angiographic vasospasm, minimal adventitial inflammatory response, and no clinical complications in the slow (0.5 ml/30–120 s) injection DMSO group.[25] As a conclusion, these data discussed above also support our aforementioned speculation about advantages of the slow infusion rates of DMSO.

DMSO was previously described as a free radical scavenger and antioxidant.[3,28] In this study, the serum and tissue lipid peroxidation levels were found similar in all study groups. This result may be explained with the hypothesis of DMSO as not causing free radical production from the membrane phospholipids of neural tissue. Another explanation may be related with the high permeability of DMSO. It can be suggested that DMSO could not show its toxic effects on neural tissue and vascular wall of the carotid artery since it was rapidly moved away from the cerebral tissue by the arterial circulation. On the other hand, growing clinical experience in the treatment of cerebral aneurysms and arteriovenous malformations is gained with Onyx (Micro Therapeutics, Irvine, California) which is currently the only commercially available nonadhesive liquid embolic agent using DMSO as the carrier solvent.[2] During such intra-arterial embolisation procedures DMSO may also enter into cerebral tissue from the circulating blood; and may lead to toxic effects.[11] To the best of our knowledge, there is still no investigation in order to demonstrate any localised neural tissue toxicity of DMSO when infused into a selected cerebral artery. We suggest that the toxicity profile of this solvent should be evaluated in future studies in which DMSO is infused into selected cerebral arteries (such as middle cerebral artery, anterior cerebral artery).

This preliminary study has a few pitfalls. *First*, although this evaluation may pave the way for future studies on this subject, it does not contain results of more specific biochemical analyses for other cytotoxic pathways of DMSO in the acute and/or chronic stages. Additionally, this study should be supported with electron microscopic findings which can show whether there are any ultrastructural findings of an inflammatory response and/or neuronal necrosis. *Second*, this preliminary study is far from explaining the toxic effects of DMSO in the very acute stage because of the selected time period. Also, it is far from demonstrating the toxic effects of rapid infusion of DMSO on carotid artery and cerebral tissue either histopathologically or biochemically. *Third*, we should evaluate the possible neurotoxic effects of the DMSO in a rat model with cerebral disease (e.g. subarachnoid haemorrhage model, hypoxia/reperfusion model etc.). But, in literature, many reports pointed out that a variety of dose dependent adverse effects (such as nausea, vomiting, flushing, fever, chills, dyspnoea, cardiac symptoms, transient hypertension, hypotension, anaphylaxis, encephalopathy, amnesia and seizures) may appear when DMSO used as a solvent and tissue preservative is given through intravenous route. And, we have expected that we could also explain this confused situation with this study by using healthy rat model.[9] Additionally, we thought that the possible neurotoxic effects of the DMSO may be confused with the primer cerebral disease findings when such an animal model is used. So, we did not prefer such an animal model. *Forth*, this study could not demonstrate the direct toxic effects of DMSO on neural tissues because it has not been directly injected into the animal brain parenchyma. *Fifth*, since the internal carotid artery is used for infusion, this study is far from demonstrating the localised neural tissue toxicity of DMSO when infused into a selected cerebral artery. *Sixth*, this study could be supported by injection of Evans blue which would demonstrate the blood–brain barrier disruption developed by DMSO.

Conclusion

This study demonstrated that DMSO is not toxic on the carotid artery and neural tissue of rats in the acute and chronic stage in rat when it is slowly administered via the intra-carotid route.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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