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Investigation of the Effects of Dexpanthenol on Brain Tissue in Experimental Global Cerebral Ischemia-Reperfusion Injury

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ABSTRACT

AIM: To investigate the protective and therapeutic effects of dexpanthenol in experimental global cerebral ischemia-reperfusion injury.

MATERIAL and METHODS: Thirty-two female Wistar-Albino rats were used, and the rats were divided into four groups (sham, sschaemia reperfusion [IR], IR+dexpantol [IR+DXP] and DXP+IR), with eight animals in each group. At the end of 72 hours of reperfusion, the rats were decapitated after performing the rotarod and accelerrod tests, their brain tissues were removed and histopathologically examined, and superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and malonyldialdehyde (MDA) levels were evaluated.

RESULTS: In this study, motor skill functions deteriorated in the ischemia-reperfusion (IR) group compared to the sham group, while significant improvements were observed in both the IR+DXP and DXP+IR groups ($p<0.05$). There were no notable differences in CAT, SOD, and GPx enzyme levels among the groups ($p>0.05$); however, malondialdehyde (MDA) levels increased in the IR group and decreased significantly in the IR+DXP group ($p<0.05$). Similarly, glutathione (GSH) levels were lower in the IR group but higher in the IR+DXP group ($p<0.05$). Neuronal degeneration also significantly increased in the IR group but decreased in the IR+DXP group ($p<0.05$).

CONCLUSION: Overall, these findings suggest that dexpanthenol has a neuroprotective effect, particularly when administered during reperfusion, effectively improving motor skills and reducing neuronal damage.

KEYWORDS: Dexpanthenol, Cerebral ischemia, Reperfusion injury, Rat brain

ABBREVIATIONS: CAT: Catalase, **DXP:** Dexpanthenol, **GPx:** Glutathione peroxidase, **GSH:** Glutathione, **IR:** Ischemia-reperfusion, **MDA:** Malonyldialdehyde, **SOD:** Superoxide dismutase

INTRODUCTION

Cerebral ischaemia is a leading cause of disease-related deaths and disabilities globally (24). The World Health Organisation (WHO) defines stroke as a condition causing sudden onset, focal, or global cerebral dysfunction in the cerebrum, spinal cord, or retina, lasting 24 hours or more, leading to disability or death without any cause other than a vascular one (34). In Western societies, around 85% of strokes are ischaemic and 15% are haemorrhagic, making stroke the most common neurological disease and the leading cause of mortality (18).

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Stroke is the third leading cause of death worldwide, following coronary artery disease and malignant diseases. With the growing elderly population, stroke-related deaths and complications are increasing. Stroke not only causes neurological issues but also psychiatric and various medical complications, including cardiopulmonary and metabolic disorders, anxiety, depression, gastrointestinal bleeding, infections, venous thromboembolism, pressure sores, and malnutrition (31). The economic impact of stroke, including treatment, rehabilitation, and decreased productivity, is substantial, accounting for 2–4% of global health expenditures and over 4% of such expenditure in developed Western countries (33).

Cerebral ischaemia can be focal or global, depending on the underlying cause. Global cerebral ischaemia may result from acute brain oedema after cardiac arrest or subarachnoid haemorrhage, while focal ischaemia can occur due to embolic occlusion or local vasospasm following trauma or haemorrhage (36,37). It begins with the depletion of oxygen, ATP, and glucose due to decreased or ceased cerebral blood flow and ends with neural tissue destruction (36). Reperfusion damage, caused by the rapid entry of free oxygen and free oxygen radicals (FOR), is more severe than ischaemia-related damage (27,46). Antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT), help prevent damage from free oxygen radicals during oxidative metabolism (19).

Dexpanthenol (D-panthenol; [+]-2, 4-dihydroxy-N-3-hydroxypropyl 3, 3 dimethylthiamide) (DXP) is an alcoholic analogue of pantothenic acid (PA), also known as provitamin B5, which is oxidized to PA in tissues (9). PA and its derivatives enhance intracellular reduced glutathione (GSH), coenzyme A (Co A), and ATP synthesis, particularly in mitochondria (5). PA also increases epithelialisation, anti-inflammatory responses, and antioxidants, all of which play a key role in cellular defence and repair against oxidative stress and inflammation (43).

This study aimed to investigate the neuroprotective effects of dexpanthenol on ischaemia reperfusion injury by inducing global brain ischaemia in rats, using motor skill tests (rotarod and accelerod), histopathology, and biochemical analyses (MDA, SOD, CAT, GPx, GSH). The simultaneous clipping of both arteria carotis communis and the reduction or cessation of cerebral flow is a common method to study cerebral ischaemia pathophysiology (14) and was chosen for its ease of application and lack of need for craniectomy.

MATERIAL and METHODS

This study was approved by the Inonu University Experimental Research Unit and Experimental Animals Ethics Committee on 09.01.2020 with protocol number 2020/01-3.

In the study, 32 female Wistar-Albino rats weighing between 200 and 250 grams and aged 3–4 months old, which had not previously been used in any experiment, were used. The rats were obtained from the Inonu University Experimental Animals Application and Research Centre laboratory. An accelerod device in the laboratory of Inonu University Faculty of Medicine, Department of Pharmacology was used in the study. Biochemical analyses were performed in the research laboratory of the Department of Biochemistry, and histopathological analyses were performed in the research laboratory of the Department of Histology and Embryology.

Rats were housed in polycarbonate cages with a maximum of four animals in each cage, at constant room temperature and humidity (22 ± 3°C temperature and 60 ± 7% humidity), with daily cage cleaning and nutrition (standard animal feed and sufficient water) and appropriate light during the study period.

The rats were divided into four main groups:

Group 1 - Sham Group (n=8): This group underwent only cervical midline incision and paratracheal dissection without cerebral ischemia, followed by surgical closure.

Group 2 - Ischemia-Reperfusion (IR) Group (n=8): In this group, the carotid arteries were bilaterally clamped to induce 30 minutes of cerebral ischemia, followed by 72 hours of reperfusion after surgical closure. No medication was administered.

Group 3 - Post-Ischemia Dexpanthenol Treatment (IR+DXP) Group (n=8): In this group, the carotid arteries were bilaterally clamped to induce 30 minutes of cerebral ischemia, followed by the administration of 500 mg/kg of dexpanthenol intraperitoneally (I.P) at the 1st hour, 2nd, and 3rd days. The group was then subjected to 72 hours of reperfusion.

Group 4 - Pre-Ischemia Dexpanthenol Treatment (DXP+IR) Group (n=8): Starting three days before cerebral ischemia, this group received 500 mg/kg of dexpanthenol intraperitoneally (I.P.) at 08:00–10:00 AM on the 1st, 2nd, and 3rd days. On the 3rd day, the carotid arteries were bilaterally clamped to induce 30 minutes of cerebral ischemia, followed by 72 hours of reperfusion.

The rats were decapitated 72 hours after the induction of ischemia following rotarod and accelerod tests, and the experiment was then concluded.

Anesthesia

Before the surgical procedure, all rats were administered 10 mg/kg of xylazine (Bayer) and 50 mg/kg of ketamine hydrochloride (Parke-Davis) intraperitoneally (I.P.). If needed, additional doses were given intermittently, not exceeding 20% of the initial doses.

Rotarod, Accelerod Test

This test, conducted to measure motor skills in experimental animals, consists of special rotating rod setups on which the animals (usually rats or mice) try to maintain their balance. The rat is forced to walk on a system programmed to rotate at predetermined speeds. The time until the rat loses its balance and falls off the rotating system is measured. The test provides insight into the extent of brain damage induced in the experiment, the effectiveness of the treatment given, and the rat's sense of fatigue (6). The device was adjusted according to the purpose of the experiment, with the rods rotating at a constant speed (RPM) or at varying speeds over a certain period. In this study, the Rotamex 4/8 system (Columbus Instruments) was used to detect balance and coordination loss

in experimental cerebral ischemia at speeds of 5, 10, 20, 30, and 40 RPM and within 4 and 10 minutes at 1-79 accelerating RPM, with a maximum duration of 300 seconds.

Cerebral Ischemia Model and Application

The rats were fasted for 24 hours before surgery, with only water provided. To induce anesthesia, 10 mg/kg of xylazine hydrochloride and 50 mg/kg of ketamine hydrochloride were administered intraperitoneally (I.P.). The anesthetized rats were placed in the supine position, and the surgical area on the cervical region was shaved and painted with a 10% povidone-iodine solution to ensure asepsis. After a midline cervical skin incision, a retractor was placed, and the bilateral paratracheal areas were bluntly dissected. The common carotid arteries were exposed and separated from the vagus nerve, and Yasargil aneurysm clips were placed on both arteries. After 30 minutes, the clips were removed, arterial blood flow was checked, and the incision site was sutured closed once blood flow was observed to be sustained. All subjects were then allowed free access to food and fluids.

Collection of Tissue Samples for Histopathological and Biochemical Analyses

Xylazine and ketamine hydrochloride were used to induce deep anaesthesia in all groups of rats following a predetermined procedure. After deep anaesthesia had been induced, the brain tissue of the rats was removed without any damage. For histological and biochemical analysis of brain tissue, the brain was divided into two hemispheres and one of the hemispheres was placed in 10% formol solution for histological examination. The other hemisphere was placed in previously prepared and numbered containers for each subject and kept at -70ºC for biochemical analysis.

Tissue Reduced Glutathione (GSH) Measurement

GSH levels of tissue samples were measured according to the method described by Ellman (15).

Tissue Malondialdehyde (MDA) Measurement

MDA levels of tissue samples were measured according to the method described by Ohkawa (28).

Measurement of Tissue Superoxide Dismutase (SOD) Activity

SOD enzyme activity levels of tissue samples were measured according to the method described by Sun and Oberley (42).

Measurement of Tissue Catalase (CAT) Activity

CAT enzyme activity levels in tissue samples were measured according to the method of analysis described by Aebi (1).

Measurement of Tissue Glutathione Peroxidase (GPx) Activity

Measurement of GPx enzyme activity levels in tissue samples was performed according to the method of analysis described by Pagli et al. (30).

Statistical Analysis

Statistical analyses were performed with the SPSS package program version 20.0. The Kruskal-Wallis H Test, a non-parametric test, was used for general comparison of groups in terms of all variables, and the Mann-Whitney U test was used for pairwise comparisons between groups. $p<0.05$ was considered statistically significant.

RESULTS

Since there was no statistically significant difference since all groups completed the course at 5 rpm by remaining in balance for 300 s, the balance times at 5 rpm were not included in the comparison. Comparisons were made between the balance times at 10, 20, 30, and 40 rpm. When compared with the IR group, the sham group completed the course by staying in balance for a longer time at all minute cycle speeds (10, 20, 30, 40 rpm), and the differences between the balance times at all rpm speeds were found to be statistically significant ($p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$). Although the sham group completed the course by staying in balance for a longer time at all rotation speeds (10, 20, 30, 40 rpm) compared to the IR+DXP group, the results were not statistically significant ($p=0.214$, $p=0.172$, $p=0.103$, $p=0.082$). The sham group completed the course by staying in balance for a longer time at all rotation speeds (10, 20, 30, 40 rpm) compared to DXP+IR group and the balance times at 20, 30,4 0 rpm were statistically significant ($p=0.084$, $p=0.001$, $p=0.002$, $p=0.003$). Compared to the IR+DXP group, the IR group completed the course with a shorter balance time at all minute rotation speeds (10, 20, 30, 40 rpm), and the times at all rpm speeds were statistically significant ($p=0.001$, $p=0.001$, $p=0.001$, $p=0.001$). Compared to the DXP+IR group, the IR group completed the course by staying in balance for a shorter time at all minute rotation speeds (10, 20, 30, 40 rpm), and the balance times at 10, 30, 40 rpm were statistically significant ($p=0.005$, $p=0.031$, $p=0.004$, $p=0.002$). The IR+DXP group completed the course by staying in balance for a longer time at all minute rotation speeds (10, 20, 30, 40 rpm) and the balance times at 20, 30 rpm were statistically significant when compared with the DXP+IR group ($p=0.452$, $p=0.001$, $p=0.009$, $p=0.130$) (Table I).

The sham group, compared to the IR group, completed the course by staying in balance for a longer time in both accelerations from 0 to 79 rpm, lasting 10 min and 4 min, and the difference between the time to stay in balance during the acceleration in both groups was statistically significant ($p=0.001$ and $p=0.001$). When compared with the IR+DXP group, the sham group completed the course by remaining in balance for a longer time in both acceleration tests lasting 10 min and 4 min, from 0 to 79 rpm, but the difference between the time to remain in balance during the acceleration test lasting only 4 min was found to be statistically significant ($p=0.462$ and $p=0.011$). When compared with the DXP+IR group, the sham group completed the course by remaining in balance for a longer time in both acceleration tests, lasting 10 min and 4 min from 0 to 79 rpm, but only the acceleration test lasting 4 min was found to be statistically significant ($p=0.248$ and $p=0.023$). Compared to the IR+DXP group, the IR group completed the course for a shorter time in both acceleration tests lasting 10 min and 4 min, from 0 to 79 rpm and both acceler-

Table I: Rota-Rod Test Results Between Groups

	5rpm Med. ± SD (Min-Max)	10rpm Med. ± SD (Min-Max)	20rpm Med. ± SD (Min-Max)	30rpm Med. ± SD (Min-Max)	40rpm Med. ± SD (Min-Max)
Sham	**300 ± 0** (300-300)	**293.75 ± 17,6** (250-300)	**198.88 ± 59,7** (123-300)	**156.75 ± 73,3** (85-300)	**46 ± 40,7** (15-140)
IR	**300 ± 0** (300-300)	**131.63 ± 30,8** (85-80)	**45.75 ± 25,3** (12-85)	**11,75 ± 4,9** (5-17)	**6.25 ± 2,3** (5-10)
IR+DXP	**300 ± 0** (300-300)	**273.75 ± 38,8** (200-300)	**162,88 ± 37,3** (122-240)	**108,38 ± 41,5** (62-180)	**23,12 ± 16,7** (10-58)
DXP+IR	**300 ± 0** (300-300)	**246.25 ± 68,2** (120-300)	**78.38 ± 25,6** (35-110)	**51,12 ± 29,9** (10-105)	**13.13 ± 4,5** (10-23)

Table II: Accelerod- Test Results Between Groups

	0-79 rpm (10 min.) Med. ± SD (Min-Max)	0-79 rpm (4 min.) Med. ± SD (Min-Max)
Sham	**169.88 ± 72,8**	**86.5 ± 29,8**
	(94-274)	(65-140)
IR	**66,87 ± 19,4**	**34.25 ± 9,2**
	(39-90)	(20-51)
IR+DXP	**128.25 ± 27,9**	**59.38 ± 10,2**
	(84-162)	(45-73)
DXP+IR	**116.50 ± 58,3**	**57.50 ± 14,5**
	(39-188)	(30-72)

ation tests were found to be statistically significant (p=0.002 and p=0.002). Compared to the DXP+IR group, the IR group completed the course by remaining in balance for a shorter time in both acceleration tests lasting 10 min and 4 min, from 0 to 79 rpm, but only the acceleration test lasting 4 min was found to be statistically significant (p=0.128 and p=0.010). Compared to the DXP+IR group, the IR+DXP group completed the course by remaining in balance for a longer time in both acceleration tests, lasting 10 min and 4 min from 0 to 79 rpm, but no statistically significant difference was found between the groups in both tests (p=0.753 and p=0.958) (Table II).

No statistically significant difference was found between the groups in terms of tissue CAT, GPx, and SOD results; therefore, pairwise comparisons were made only between GSH and MDA. When the sham group was compared with the IR group, GSH levels were higher in the sham group than in the IR group (p=0.007), MDA levels were higher in the IR group than in the sham group (p=0.001), and the difference between both results was statistically significant. When the sham group was compared with the IR+DXP group, it was found that GSH levels were lower in the IR+DXP group than in the sham group (p=0.598) and MDA levelx were higher in the IR+DXP group than in the sham group (p=0.527), although the difference between both results was not statistically significant. When the sham group was compared with the DXP+IR group, GSH levels were found to be lower in the DXP+IR group than in the sham group; however, the difference between the results was not found to be significant (p=0.156). MDA levels were found to be higher in the DXP+IR group than in the sham group (p=0.001) and the difference between the results was found to be statistically significant. When the IR group was compared with the IR+DXP group, GSH levels were higher in the IR+DXP group than in the IR group (p=0.031), and MDA levels were lower in the IR+DXP group than in the IR group (p=0.015). When the IR group was compared with the DXP+IR group, it was found that GSH levels were higher in the DXP+IR group than in the IR group and MDA levels were higher in the DXP+IR group than in the IR group, although the difference between both results was not significant (p>0.05). When the IR+DXP group was compared with the DXP+IR group, it was found that GSH levels were higher in the IR+DXP group than in the DXP+IR group (p=0.248) and the difference between these values was not statistically significant, while MDA levels were lower in the IR+DXP group than in the DXP+IR group (p=0.013) (Table III).

Brain tissue samples taken for histopathological examination were fixed with 10% formaldehyde for 48 hours. After fixation, brain tissue samples were subjected to routine histological tissue follow-up procedures and embedded in paraffin blocks. Sections of 6 µm thickness were prepared from the paraffin blocks using a microtome. The sections taken on slides were stained with haematoxylin-eosin (H-E) and examined and photographed with a Leica DFC 280 light microscope and the Leica QWin image analysis system (Leica Microsystems Imaging Solutions, Cambridge, UK). Cerebrum tissues removed at the end of the experiment were fixed in 10% formaldehyde. After tissue tracing, 4–5 µm thick sections were taken from the prepared paraffin blocks. The sections were stained with the haematoxylin-eosin staining method to determine their general morphological structure. The cerebral cortex was evaluated for neuronal degeneration. The severity of neuronal degeneration was ascertained by determining the number of degenerated neurons (neurons with shrunken hypereosinophilic cy-

toplasm and pyknotic nuclei) in 10 randomly selected areas under x40 magnification (35).

In the sections belonging to the sham group, neurons with normal morphological characteristics with round, large, and euchromatic nuclei and a small number of degenerated neurons were found (Figure 1A). There was a significant increase in the number of degenerated neurons with shrunken, hypereosinophilic cytoplasm and pyknotic nuclei in the IR group, and this increase was statistically significant when compared with the sham group (p=0.0006) (Figure 1B). It was observed that the number of degenerated neurons decreased with DXP application before and after IR. However, the decrease in the number of degenerated neurons in the DXP+IR group was not statistically significant when compared with the IR group (Figure 1C). On the other hand, the decrease in the number of degenerated neurons in the IR+DXP group was statistically significant compared to both IR and DXP+IR groups (p=0.0006) (Figure 1D). The number of degenerated neurons in each group is detailed in Table IV.

Table III: Tissue GSH, SOD, MDA, GPx and CAT Results Between Groups

	CAT (K/g Protein) Med. ± SD (Min-Max)	GPx (U/g protein) Med. ± SD (Min-Max)	SOD (U/g protein) Med. ± SD (Min-Max)	GSH (nmol/g wet tissue) Med. ± SD (Min-Max)	MDA (nmol/g wet tissue) Med. ± SD (Min-Max)
Sham	**56.7 ± 16,8** (25.7-87.1)	**33.43 ± 9,6** (17.15-45.4)	**250.2 ± 33** (234.5-336.6)	**744.6 ± 60** (673-836)	**135 ± 33,9** (101,3-202,6)
IR	**37.7 ± 26,3** (27.3-90.6)	**26.2 ± 13,7** (19.1-60.2)	**251.5 ± 19,2** (221.5-280.4)	**657.9 ± 37,7** (622-734)	**229.16 ± 18,4** (206.7-269.2)
IR+DXP	**54.8 ± 18,9** (38.2-91.6)	**32.4 ± 27,4** (21.07-96.87)	**246.9 ± 45,2** (216.9-348.4)	**724.2 ± 83** (633-887)	**159,2 ± 51,4** (110-225)
DXP+IR	**59.6 ± 17,1** (36.5-86.08)	**34.7 ± 20,4** (22.5-86.2)	**278.4 ± 41,9** (224.3-362.2)	**708.9 ± 54,2** (622.2-775.2)	**234.9 ± 42,8** (206-327.7)

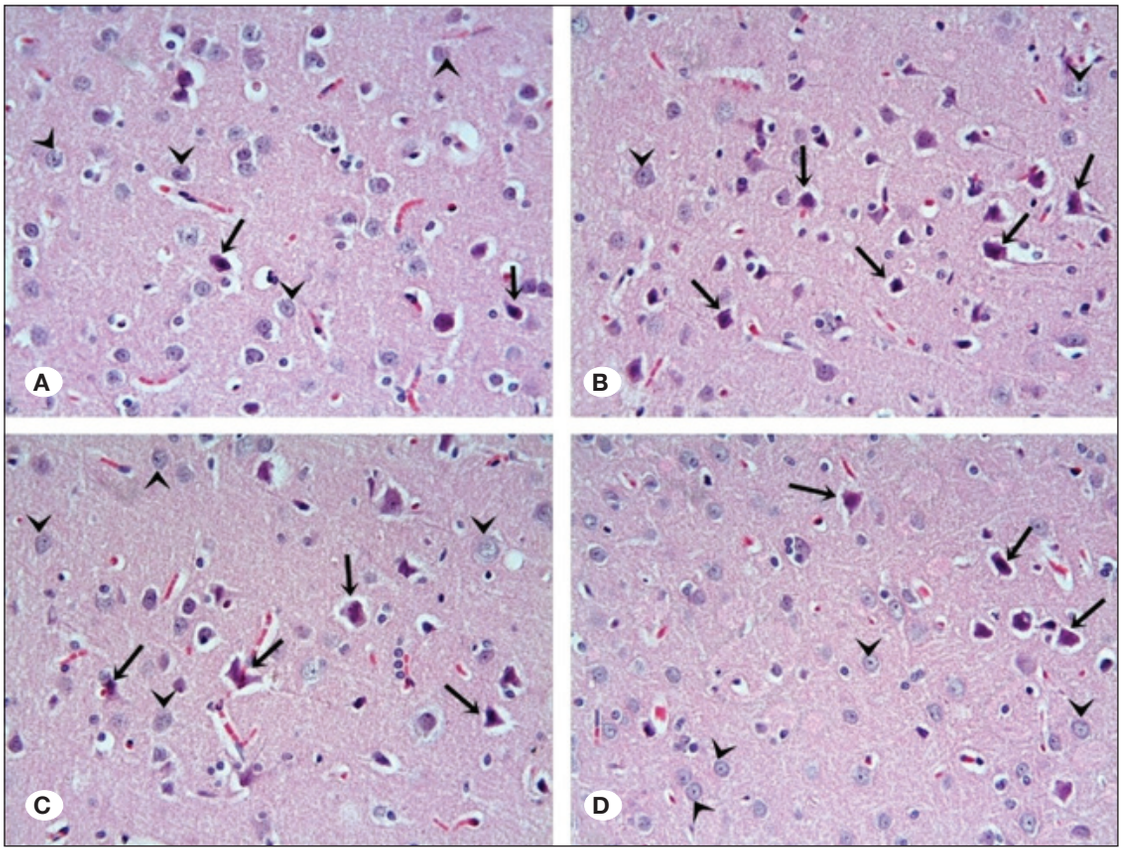

Figure 1: Appearance of the cerebral cortex according to the groups. **A)** Sham group; **B)** IR; **C)** DXP+IR; **D)** IR+DXP. Long arrow heads indicate normal neurons with large, round, and euchromatic nuclei; short arrow heads indicate degenerated neurons with shrunken, hypereosinophilic cytoplasm and pyknotic nuclei. H-E; x40.

Table IV: Number of Degenerated Neurons Between Groups

	Degenerated neurons Med.(Min-max)
Sham	**1.5** (0.0-8.0)
IR	**5.0** (0.0-31.0)
IR+DXP	**2.0** (0.0-11.0)
DXP+IR	**3.5** (0.0-19.0)

DISCUSSION

Some amount of free oxygen radicals (FORs) is continuously produced in tissues, but these can be neutralised by antioxidant enzyme systems such as CAT, SOD, and GPx (2). The molecules that initiate pathophysiological events in cerebral ischemia are activated by the energy deficit resulting from decreased cerebral blood flow. When glucose metabolism is affected, a state of metabolic imbalance occurs in which ATP and phosphocreatine levels decrease and lactate levels increase. Decreased ATP causes an increase in intracellular calcium, sodium, and chlorine and extracellular potassium levels by disrupting membrane permeability and depolarisation (11). In tissue damage resulting from ischaemia, the duration and depth of ischaemia are the two most important determinant factors (10). In the case of re-oxygenation of ischaemic tissue as a result of reperfusion, a high amount of FOR is formed by the reduction of molecular oxygen by oxidative enzymes in the cell, and these free oxygen radicals play the most important role in the formation of ischaemia reperfusion injury. Superoxide radical (O_2^-), hydroxyl ion (OH^-) and hydrogen peroxide (H_2O_2) are the most important free oxygen radicals. Free oxygen radicals cause damage in the cell membrane by causing lipid peroxidation, and disruption of membrane permeability leads to excessive calcium (Ca^{+2}) accumulation in the cell (25). Increased intracellular calcium activates the apoptosis pathway, resulting in cell death (23). In addition, increased free oxygen radicals and lipid peroxides exert chemotactic effects on neutrophils and cause inflammation and cell damage in reperfused tissue (11).

Dexpanthenol (provitamin B5) is a synthetic alcohol form of pantothenic acid with antioxidant and anti-inflammatory effects (29). It enhances reduced glutathione (GSH), coenzyme A (CoA), and ATP synthesis in cells (12). GSH and glutathione-dependent peroxidases protect against oxidative stress and lipid peroxidation in ischaemia-reperfusion injury and are used in various conditions, including hepatotoxicity, wound healing, and nephropathy, without significant side effects (22,40). To date, although dexpanthenol is a very cheap and easily obtainable substance, there are not many studies in the literature showing its effects on cerebral ischaemia-reperfusion injury.

This study aimed to investigate the protective effect of dexpanthenol in experimental cerebral ischaemia-reperfusion injury. While ischaemia-reperfusion durations vary, studies show 10 minutes is sufficient for cerebral ischaemic damage and 15 minutes for reperfusion damage (20). Here, 30 minutes of bilateral carotid artery clamping followed by 72 hours of reperfusion induced cerebral tissue damage. Dexpanthenol was administered prophylactically (DXP+IR group) for 3 days, with 1x500 mg/kg being therapeutically administered before ischaemia (IR+DXP group) for 3 days and 1x500 mg/kg being administered immediately after ischaemia during reperfusion.

In the rotarod test, the sham group balanced the longest at all rpm speeds, while the IR group balanced the shortest. The IR+DXP group outperformed the DXP+IR and IR groups but was shorter than the sham group, and the DXP+IR group balanced longer than the IR group (all comparisons were statistically significant at all rpm speeds). These results reflect brain damage and exhaustion levels in the rats (6). Similarly, in the accelerod test, the balance times were ranked as sham, IR+DXP, DXP+IR, and IR groups (the accelerod 4 min test was significant, while the 10 min test was not in many comparisons). A study by Korkmaz et al. (21) showed that dexpanthenol improved rotarod and accelerod results in sciatic nerve damage. In this experiment, dexpanthenol administered before or after cerebral ischaemia significantly prolonged balance times compared to the untreated ischaemia group.

In ischaemia-reperfusion injury, cells activate antioxidant enzymes like SOD, CAT, and GPx to protect against excessive free oxygen radicals (3). Ucar et al. (45) found lower SOD levels in the ischaemia-reperfusion group compared to the sham group, while Soylu Karapinar (41) observed lower GPx and CAT levels in ovarian ischaemia-reperfusion. Many studies show that ischaemia-reperfusion increases free radical levels, depleting antioxidant enzymes. Both studies reported increased SOD, GPx, and CAT levels in dexpanthenol-treated groups. Slyshenkov et al. (38) showed that dexpanthenol scavenges free radicals and boosts antioxidant enzyme synthesis. In our study, CAT and GPx were lower in the IR group compared to the sham group, while SOD levels were similar in both groups. In the IR+DXP and DXP+IR groups, all three enzymes were higher than the IR group, but the increase was not statistically significant ($p>0.05$).

Malondialdehyde (MDA) is a lipid peroxidation product and an indicator of oxidative damage in tissues, causing cell damage by interacting with membrane lipids (13). Previous studies show increased MDA levels due to ischaemia-reperfusion injury (8), and our study also found a significant increase in MDA levels in the IR group compared to the sham group. Dexpanthenol is thought to reduce lipid peroxidation-induced cell damage by boosting glutathione (GSH) synthesis and GPx enzyme activity (17). Ermiş et al. (16) and Tutun et al. (44) reported that dexpanthenol treatment decreased MDA levels in various injury models. In our study, the MDA level was significantly lower in the IR+DXP group compared to the IR group ($p<0.05$), but no decrease was observed in the DXP+IR group.

Glutathione, an essential tripeptide, is an important endogenous antioxidant involved in the final detoxification of free oxygen radicals (32). Besides working as a scavenger for free oxygen radicals, glutathione is known to be involved in DNA repair, activation of transcription factors, regulation of cell cycle, calcium homeostasis, and enzymatic activities. In oxidative stress, reduced glutathione (GSH) acts as a substrate for free oxygen radicals and GSH levels in tissue decrease (4). In

a study conducted by Mukherjee et al. (26), it was shown that the level of GSH in brain tissue decreased significantly as a result of 24 hours of cerebral reperfusion. Previous studies have shown that dexpanthenol increases GSH activity and balances endogenous antioxidants. In a study by Uçar et al. (45) investigating the effects of dexpanthenol in renal ischaemia-reperfusion injury, it was reported that GSH decreased significantly in the ischaemia group compared to the sham group, and GSH levels increased significantly in the dexpanthenol-treated groups compared to the IR group (45). Slyshenkov et al. (39) showed that pantothenic acid supplementation increased hepatic GSH and made liver cells more resistant to radiation. In a study by Çağın et al. (7) investigating the protective effects of dexpanthenol in experimentally induced mesentery ischaemia-reperfusion injury in rats, GSH levels were significantly higher in the dexpanthenol-treated group. In our study, results parallel to the studies in the literature were obtained and GSH levels were found to be significantly lower in the IR group compared to the sham group (p=0.007). A statistically insignificant increase in GSH level was found in the DXP+IR group in which dexpanthenol was administered before ischaemia-reperfusion compared to the IR group (p=0.187), and GSH levels were found to be significantly higher in the IR+DXP group treated with dexpanthenol after ischaemia-reperfusion compared to the IR group (p=0.031).

CONCLUSION

Considering that dexpanthenol is a good oxygen free radical scavenger and its antioxidant effects are have been well documented in previous studies, when the results of histopathological, motor skill tests, and biochemical parameters of cerebral tissue obtained in our study were evaluated together, it was thought that dexpanthenol may have a neuroprotective effect in the IR+DXP group treated with dexpanthenol after ischemia and during reperfusion. Although the DXP+IR group, in which dexpanthenol treatment was administered before cerebral ischaemia, caused a relative improvement in the results, this improvement was not statistically significant, and it was concluded that dexpanthenol given before cerebral ischaemia had no neuroprotective effect. Additional experimental and clinical studies are needed to clarify the role of dexpanthenol in the treatment of cerebral ischaemic stroke.

Declarations

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AUTHORSHIP CONTRIBUTION

Study conception and design: SS, SCO
Data collection: RE, SS, DEK
Analysis and interpretation of results: SS, RE, II
Draft manuscript preparation: SS, II, DEK
Critical revision of the article: RE, SCO
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