ORIGINAL ARTICLE

Journal of Pre-Clinical and Clinical Research
www.jpccr.eu

No effect of the uraemic toxin indoxyl sulfate on memory consolidation in scopolamine-treated mice

Krzysztof Łukawski[1,A-D,F] ✉, **Grzegorz Raszewski**[2,B,E-F]

[1] *Department of Physiopathology, Institute of Rural Health, Lublin, Poland*
[2] *Department of Toxicology and Food Protection, Institute of Rural Health, Lublin, Poland*
A – Research concept and design, B – Collection and/or assembly of data, C – Data analysis and interpretation, D – Writing the article, E – Critical revision of the article, F – Final approval of the article

Łukawski K, Raszewski K. No effect of the uremic toxin indoxyl sulfate on memory consolidation in scopolamine-treated mice. J Pre-Clin Clin Res. doi:10.26444/jpccr/215396

Abstract

Introduction and Objective. Compared to the general population, patients with end-stage renal disease (ESRD) are more likely to have neurological conditions, such as memory impairments. It is thought that the cognitive impairment may affect 30% – 80% of patients with ESRD. Patients on haemodialysis are especially vulnerable to the biological effects of uraemic toxins. It has been suggested that one of the uraemic toxins most likely to affect cognitive function in patients with ESRD is indoxyl sulfate (IS). The aim of the study is to investigate the potential effect of IS on memory consolidation.

Materials and Method. Adult male Swiss mice were used in the study. Memory consolidation was assessed in the step-through passive avoidance test. Indoxyl sulfate potassium salt (IS) and scopolamine hydrobromide (SCO) were employed in the experiments. IS (200, 400 mg/kg) and SCO (1 mg/kg) were given intraperitoneally (i.p.). Each drug was administered as a single injection. Additionally, acetylcholinesterase (AChE) activity was determined in the brain following applied drug treatments.

Results. IS administration (200 and 400 mg/kg i.p.) alone and in combination with SCO (1 mg/kg i.p.) did not affect memory consolidation in the passive avoidance test. Exposure to IS did not significantly alter the activity of AChE in the brain tissue.

Conclusions. This study shows that acute exposure to IS does not impair memory consolidation in mice. Chronic exposure to IS in animals is necessary to obtain additional information concerning the toxin's effects on memory consolidation.

Key words

indoxyl sulfate, memory, acetylcholinesterase, mice

INTRODUCTION

A frequent complication of chronic kidney disease (CKD) is cognitive impairment, which can range from mild deficits in the early stages to more serious disorders, such as mild cognitive impairment and dementia in the later stages [1]. Contributing variables encompass uraemic toxins, structural alterations in the brain, disruption of the blood-brain barrier (BBB), anaemia, and comorbidities such as diabetes mellitus [1]. Thirty to eighty percent of patients with end-stage renal disease (ESRD) may have cognitive impairment [2]. The accumulation of uraemic toxins due to renal failure is believed to significantly contribute to cognitive impairment [3]. Indoxyl sulfate (IS) has been proposed as a uraemic toxin belonging to groups of the toxins which impair cognitive function in patients with CKD [3–5]. Poor executive skills in CKD patients have been linked to elevated serum IS levels [6]. It has also been suggested that higher levels of IS may cause more severe cerebrovascular lesions, which could impair patients' cognitive function [6]. A direct toxic effect on brain neuronal cells is another potential mechanism [6]. Preclinical investigations have already demonstrated that IS can induce direct neurotoxicity *in vitro* [7], and its administration correlated with impaired cognitive performance in a rat model of CKD [8, 9]. This cognitive impairment in rodents with CKD is associated with BBB disruption, linked to activation of aryl hydrocarbon receptor (AhR) by IS [8].

CKD and Alzheimer's disease (AD) are two common and devastating disorders that frequently coexist [10]. Although epidemiological and clinical studies show that CKD is a significant and independent risk factor for cognitive impairment [11], its involvement in the progression of Alzheimer's disease (AD) remains uncertain. The first evidence in this respect was provided by the work of Nakagawa et al. [12]. In a mouse model of AD, they demonstrated that CKD itself considerably accelerates the cognitive decline. In general, it has been proposed that CKD contributes to the development of cognitive impairment and AD through uraemic toxin accumulation, blood pressure elevation, renin-angiotensin system over-activation, erythropoietin deficiency, disruption of 1,25-dihydroxyvitamin D production, and microvascular dysfunction [13].

OBJECTIVE

It is still unclear how uraemic toxins could contribute to the progression of AD. The aim of the study is to examine the effect of IS in scopolamine (SCO)-induced amnesia, which is a well-established animal model for investigating the underlying processes and therapy of cognitive decline

✉ Address for correspondence: Krzysztof Łukawski, Department of Physiopathology, Institute of Rural Health in Lublin, Jaczewskiego 2, 20-090 Lublin, Poland
E-mail: lukaw@mp.pl

Received: 15.10.2026; accepted: 09.12.2025; first published: 29.12.2025

in neurodegenerative illnesses [14]. As an animal model of AD, the SCO-evoked memory impairment in the passive avoidance task was used [15]. SCO is an anticholinergic drug that can cause impairments in the processes of memory acquisition and consolidation in the passive avoidance test by blocking the muscarinic cholinergic receptors (M1 and M2) [15]. The present study focuses on evaluating memory consolidation in this test.

MATERIALS AND METHOD

Animals. The study used adult male Swiss mice weighing 22–30 g, obtained from a licensed dealer (J. Kolacz, Warsaw, Poland). Animals were housed in laboratory cages under standardized laboratory settings, including a 12-hour light-dark cycle, room temperature of 22 ± 2 °C, and relative humidity of 50–60%. Food pellets and tap water were freely available. The animals were divided into experimental groups of eight at random, and each mouse was utilised once during the tests. Every experimental procedure conformed with EU Directive 2010/63/EU for animal studies and was approved by the Local Ethics Committee (License No. 46/2019).

Drugs. Scopolamine hydrobromide (SCO) and indoxyl sulfate potassium salt (IS) were acquired from Sigma-Aldrich. IS (200–400 mg/kg) was dissolved in phosphate buffered saline (PBS) and administered intraperitoneally (i.p.) as single injections at the volume of 10 ml/kg body weight. Similarly, SCO (1 mg/kg) was administered i.p. acutely. It was dissolved in regular saline (0.9% NaCl) and injected at the volume of 10 ml/kg body wt. IS was given in doses that did not cause histopathological alterations in the brains of the mice [7].

Passive avoidance task. To evaluate the impact of IS and SCO on memory consolidation in animals, a single-trial step-through passive avoidance test was employed. On the first day (training day), mice were placed separately in a lighted box (12 × 20 × 15 cm) adjacent to a darkened compartment (24 × 20 × 15 cm) that had a generator-connected electric grid floor. At floor level, a 4 × 7 cm doorway was positioned in the middle of a shared wall. Each mouse was subjected to an electric shock of 0.3 mA administered to a foot for 3 s after entering the darkened compartmen. During this trial, latency each mouse (time to enter the dark box) was recorded. After receiving the electric foot shock, animals were removed from the dark box and administered with drugs. IS and SCO were injected either separately or in combination. SCO (1 mg/kg) was given immediately after the shock while IS (200–400 mg/kg) was administered 20 min later. Twenty-four hours later, the same mice (without treatment this time) were brought to the illuminated box in order to perform a retention test (the latency of entering the darkened compartment was measured again). The experiment ended when the mouse entered the darkened compartment or after 300 s of observation. The mice that stayed out of the dark box for 300 s were thought to remember the task.

Acetylcholinesterase (AChE) activity in the brain. The modified version of Ellman's colorimetric approach was used to detect AChE activity [16]. After completing the passive avoidance test, the brains of the decapitated animals were removed and put in a deep freezer set at -80 °C. The next day, after taking the mouse brains out of the deep freezer, they were washed, rinsed with cooled phosphate buffer (pH 7.8), dried, and weighed. The fresh, unfrozen tissue was homogenised in ice-cold 0.05 M sodium phosphate buffer (pH 7.8) at a ratio of 1 g of tissue to 5 ml. After adding an aliquot of 50 µl homogenate to a 20 ml buffer containing 5.5 dithiobis-2-nitro-benzoic acid-DTNB (10 mg/100 ml), 4 ml of the sample was utilised to assess AChE activity. At the beginning of the test, 50 µl of 20 mM propionylthiocholine iodide (PTC) was added to the samples. PTC was broken down by AChE to produce thiocholine, which subsequently combined with DTNB to generate yellow 5-thio-2-nitrobenzoate. The materials were then centrifuged for 5 min at 1,850 g. A Bio-Tek ELx800 microplate reader with a wavelength of 412 nm was used to measure changes in absorbance that were directly related to AChE activity.

Statistics. The results from the training day of the passive avoidance task were analyzed with one-way ANOVA. The retention test data were compared using Kruskal-Wallis non-parametric ANOVA. The activity of AChE was compared with the use of one-way ANOVA. P-value less than 0.05 was employed to ascertain statistical significance between groups.

RESULTS

Passive avoidance task. There were no differences in latency between experimental groups on the training day in the passive avoidance test, as revealed by one-way ANOVA ($F_{(5,42)} = 1.030$; $p = 0.4125$) (Fig. 1A). Similarly, the Kruskal-Wallis test revealed a non-significant overall group effect in the retention test ($H = 3.087$; $p = 0.6866$). There were no differences between groups under study for memory consolidation (Fig. 1B).

AChE. The ANOVA revealed a non-significant overall group effect for AChE activity in the brain tissue ($F_{(3,26)} = 1.673$; $p = 0.1972$) (Fig. 2).

DISCUSSION

The study indicated that IS had no significant effect on memory consolidation, either administered solely or in conjunction with SCO. Previous research suggests that IS may constitute a potential treatment target for CKD-related cognitive impairment [17]. The effect of IS on memory processes has been extensively studied in animal models. Rats subjected to chronic treatment with the toxin (200 mg/kg of body wt per day) demonstrated reduced accurate responses and an increase in latency to complete the performance of the T maze test, indicating a deterioration in spatial memory [9]. Bobot et al. [8] examined cognitive deficits in the novel object recognition test, the object location task, and social memory tests in animals with renal failure. For the purpose, models of CKD were used in which rats were subjected to an adenine-rich diet or 5/6 nephrectomy. Additionally, after the injection of 99mTc-DTPA (imaging marker), the BBB disruption assessed with the use of SPECT/CT imaging. The discrimination index in the novel object recognition test and the levels of IS in serum were shown to be significantly

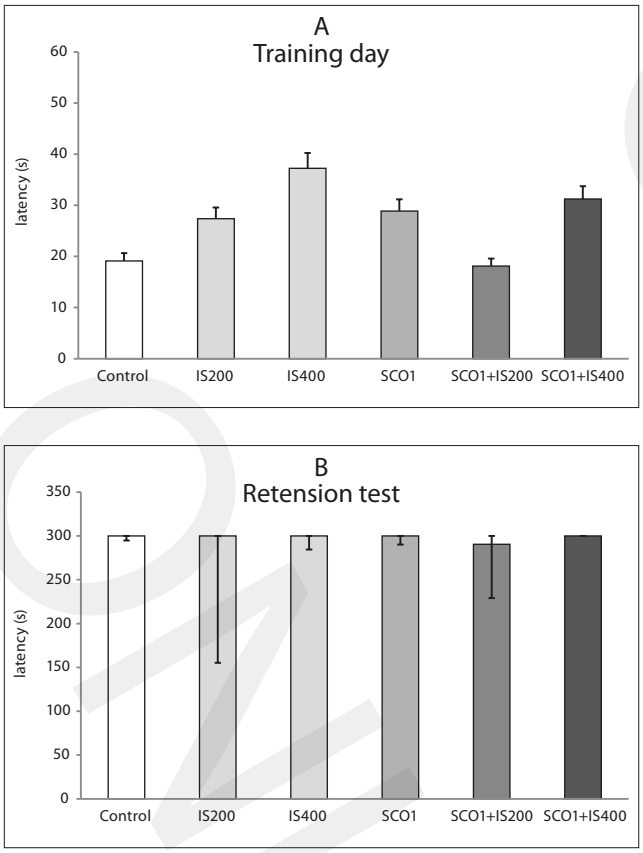

Figure 1. The effect of the combined administration of indoxyl sulfate (IS) and scopolamine (SCO) on memory consolidation in the passive avoidance test. SCO (1 mg/kg i.p.) was given immediately after the electric foot shock, and IS (200 or 400 mg/kg i.p.) was administered 20 min after SCO injection. Latency (in seconds – s) to enter the dark box was recorded on the training day and 24 h later (retention test for the evaluation of memory consolidation). Results are shown as mean ± SEM values (training day) (A) and median values with the 25th and 75th percentiles (retention test) (B). The number of mice in each group was 8. Not significant *vs* respective control groups (ANOVA or Kruskal–Wallis test)

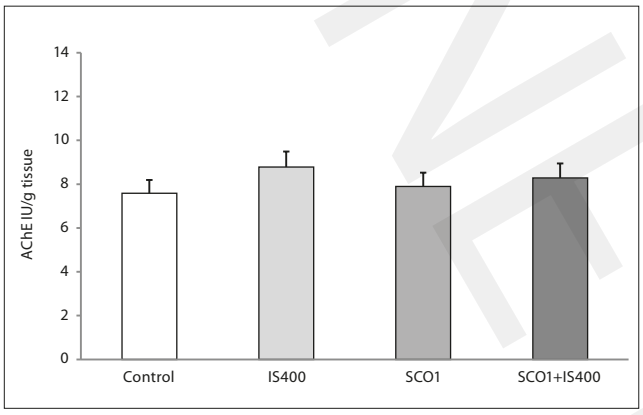

Figure 2. The effect of the combined administration of indoxyl sulfate (IS) and scopolamine (SCO) on brain acetylcholinesterase (AChE) activity in mice. Results are shown as mean ± SD of eight determinations and expressed in IU/g of wet brain tissue. Not significant *vs* control group (ANOVA)

correlated with the brain's ^{99m}Tc-DTPA content. When IS was added to the drinking water of rats fed the adenine-rich diet, it was discovered that the rats' increased serum IS concentrations were linked to a more severe cognitive impairment and increased BBB permeability. Sun et al. [18] reported that mice exposed to IS administration (chronically given a dosage of 100 mg/kg i.p.) exhibited a greater escape latency distance, as well as the latency time during the acquisition phase of a Morris water maze test in a study that utilised unilaterally nephrectomised animals.

While the outcomes of behavioural studies in animals necessitate confirmation in clinical trials, it is important to highlight that the intraperitoneal doses of IS (200–400 mg/kg) administered in the current study produced blood toxin concentrations in mice comparable to those observed in patients with ESRD. As previously shown in mice, after IS treatment (400 mg/kg i.p.), the mean IS plasma concentration was 168.19 μg/ml (mg/l) [16]. In patients with uraemia, high mean serum IS concentrations are around 44 mg/l, while IS levels in some individuals as high as 236 mg/l have been documented [19].

The observed memory impairments in rodents occurred mainly after long-term exposure to IS. Therefore, the acute administration of the uraemic toxin applied in the current study may partially explain the absence of the impact of IS on memory consolidation in the passive avoidance task. Additionally, subsequent to the acute treatment of the toxin, the activity of brain AChE remained unchanged, which may also account for the lacking effect of IS on mice performance in the passive avoidance task. It was reported earlier that a variety of psychomotor behavioural impairments were linked to a widespread decrease of AChE in mice with chronic CKD [20].

In the current study, IS combined with SCO had no impact on the performance of mice in the passive avoidance test. Furthermore, no retention deficits were observed after administering SCO alone. This result, concerning SCO, is not surprising, despite the use of a dose of SCO (1 mg/kg), which in studies by some authors caused memory consolidation impairment in a passive avoidance test in mice [21]. In contrast to this, there are reports indicating a lack of effect of SCO in doses around 1 mg/kg on memory consolidation [21]. Therefore, to definitively confirm that IS does not affect consolidation impairment induced by SCO administration, studies with a higher dose of SCO are needed.

CONCLUSIONS

The presented results demonstrate that acute exposure to IS does not impair memory consolidation in mice, including those additionally treated with SCO. Although exposure to single, high doses of IS in animals may, to some extent, mimic clinical exposure to high concentrations of the toxin in patients between dialysis sessions, the use of chronic administration of IS in mice is required to provide additional scientific insights on memory consolidation.

Acknowledgements
The study was supported by a grant from Institute of Rural Health in Lublin, Poland (Grant No. DS 19070).

REFERENCES

1. Kanbay M, Ozbek L, Guldan M, Abdel-Rahman SM, Sisman U, Mallamaci F, Zoccali C. Nutrition, cognition and chronic kidney disease: A comprehensive review of interactions and interventions. Eur J Clin Invest. 2025;55(6):e70045. https://doi.org/10.1111/eci.70045
2. Bobot M, Guedj E, Resseguier N, Faraut J, Garrigue P, Nail V, Hache G, Gonzalez S, McKay N, Vial R, Bouchouareb D, Lano G, Jourde-Chiche N, Duval-Sabatier A, Guilaume F, Guillet B, Burtey S. Increased Blood-

Brain Barrier Permeability and Cognitive Impairment in Patients With ESKD. Kidney Int Rep. 2024;9(10):2988–2995. https://doi.org/10.1016/j.ekir.2024.07.021
3. Liabeuf S, Pepin M, Franssen CFM, Viggiano D, Carriazo S, Gansevoort RT, Gesualdo L, Hafez G, Malyszko J, Mayer C, Nitsch D, Ortiz A, Pešić V, Wiecek A, Massy ZA; CONNECT Action (Cognitive Decline in Nephro-Neurology European Cooperative Target). Chronic kidney disease and neurological disorders: are uraemic toxins the missing piece of the puzzle? Nephrol Dial Transplant. 2021;37(Suppl 2):ii33-ii44. https://doi.org/10.1093/ndt/gfab223
4. Franco ÁO, Starosta RT, Roriz-Cruz M. The specific impact of uremic toxins upon cognitive domains: a review. J Bras Nefrol. 2019;41(1):103–111. https://doi.org/10.1590/2175-8239-JBN-2018-0033
5. Watanabe K, Watanabe T, Nakayama M. Cerebro-renal interactions: impact of uremic toxins on cognitive function. Neurotoxicology. 2014;44:184–193. https://doi.org/10.1016/j.neuro.2014.06.014
6. Yeh YC, Huang MF, Liang SS, Hwang SJ, Tsai JC, Liu TL, Wu PH, Yang YH, Kuo KC, Kuo MC, Chen CS. Indoxyl sulfate, not p-cresyl sulfate, is associated with cognitive impairment in early-stage chronic kidney disease. Neurotoxicology. 2016;53:148–152. https://doi.org/10.1016/j.neuro.2016.01.006
7. Adesso S, Magnus T, Cuzzocrea S, Campolo M, Rissiek B, Paciello O, Autore G, Pinto A, Marzocco S. Indoxyl Sulfate Affects Glial Function Increasing Oxidative Stress and Neuroinflammation in Chronic Kidney Disease: Interaction between Astrocytes and Microglia. Front Pharmacol. 2017;8:370. https://doi.org/10.3389/fphar.2017.00370
8. Bobot M, Thomas L, Moyon A, Fernandez S, McKay N, Balasse L, Garrigue P, Brige P, Chopinet S, Poitevin S, Cérini C, Brunet P, Dignat-George F, Burtey S, Guillet B, Hache G. Uremic Toxic Blood-Brain Barrier Disruption Mediated by AhR Activation Leads to Cognitive Impairment during Experimental Renal Dysfunction. J Am Soc Nephrol. 2020;31(7):1509–1521. https://doi.org/10.1681/ASN.2019070728
9. Karbowska M, Hermanowicz JM, Tankiewicz-Kwedlo A, Kalaska B, Kaminski TW, Nosek K, Wisniewska RJ, Pawlak D. Neurobehavioral effects of uremic toxin-indoxyl sulfate in the rat model. Sci Rep. 2020;10(1):9483. https://doi.org/10.1038/s41598-020-66421-y
10. Roy A, Roy R, Bhattacharya P, Borah A. The Vicious Consequences of Chronic Kidney Disease on Cognitive Impairment and Alzheimer's Disease. ACS Chem Neurosci. 2025;16(10):1847–1859. https://doi.org/10.1021/acschemneuro.5c00050
11. Bugnicourt JM, Godefroy O, Chillon JM, Choukroun G, Massy ZA. Cognitive disorders and dementia in CKD: the neglected kidney-brain axis. J Am Soc Nephrol. 2013;24(3):353–363. https://doi.org/10.1681/ASN.2012050536
12. Nakagawa T, Hasegawa Y, Uekawa K, Kim-Mitsuyama S. Chronic kidney disease accelerates cognitive impairment in a mouse model of Alzheimer's disease, through angiotensin II. Exp Gerontol. 2017;87(Pt A):108–112. https://doi.org/10.1016/j.exger.2016.11.012
13. Zhang CY, He FF, Su H, Zhang C, Meng XF. Association between chronic kidney disease and Alzheimer's disease: an update. Metab Brain Dis. 2020;35(6):883–894. https://doi.org/ 10.1007/s11011-020-00561-y
14. Lu C, Wang Y, Xu T, Li Q, Wang D, Zhang L, Fan B, Wang F, Liu X. Genistein Ameliorates Scopolamine-Induced Amnesia in Mice Through the Regulation of the Cholinergic Neurotransmission, Antioxidant System and the ERK/CREB/BDNF Signaling. Front Pharmacol. 2018;9:1153. https://doi.org/10.3389/fphar.2018.01153
15. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Skalicka-Wozniak K, Michalak A, Musik I, Biala G. Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. Psychopharmacology (Berl). 2015;232(5):931–942. https://doi.org/10.1007/s00213-014-3728-6
16. Łukawski K, Raszewski G, Czuczwar SJ. Effects of the uremic toxin indoxyl sulfate on seizure activity, learning and brain oxidative stress parameters in mice. Neurosci Lett. 2024;820:137594. https://doi.org/10.1016/j.neulet.2023.137594
17. Hsieh CC, Lu KC, Huang CL, Wang JJ, Yeh TY, Lin SM, Chung YL, Hou YC, Huang YS. Indoxyl sulfate is associated with cognitive impairment in ESRD patients by activating the extrinsic apoptosis in the neuronal cells during differentiating process. Int J Med Sci. 2025;22(8):1736–1749. https://doi.org/10.7150/ijms.109245
18. Sun CY, Li JR, Wang YY, Lin SY, Ou YC, Lin CJ, Wang JD, Liao SL, Chen CJ. Indoxyl sulfate caused behavioral abnormality and neurodegeneration in mice with unilateral nephrectomy. Aging (Albany NY). 2021;13(5):6681–6701. https://doi.org/10.18632/aging.202523
19. Vanholder R, Schepers E, Pletinck A, Nagler EV, Glorieux G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: a systematic review. J Am Soc Nephrol. 2014;25(9):1897–1907. https://doi.org/10.1681/ASN.2013101062
20. Mazumder MK, Paul R, Bhattacharya P, Borah A. Neurological sequel of chronic kidney disease: From diminished Acetylcholinesterase activity to mitochondrial dysfunctions, oxidative stress and inflammation in mice brain. Sci Rep. 2019;9(1):3097. https://doi.org/10.1038/s41598-018-37935-3
21. Popović M, Giménez de Béjar V, Popović N, Caballero-Bleda M. Time course of scopolamine effect on memory consolidation and forgetting in rats. Neurobiol Learn Mem. 2015;118:49–54. https://doi.org/10.1016/j.nlm.2014.11.006