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A potential role of mechano-gated potassium channels in meningioma-related seizures

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ABSTRACT

Every third patient with intracranial meningioma develops seizures of poorly understood etiology. Tumor and peritumoral edema may exert mechanical pressure on the cortex that may affect mechano-gated potassium channels - KCNK2 and KCNK4. These channels regulate neuron excitability and have been related to seizures in some other conditions. The objective of the present study was to explore a potential relation between the levels of these proteins in tumor tissue and adjacent cortex and seizures development. The study included 19 meningioma patients that presented one or more preoperative seizures and 24 patients with no seizures. Tissue samples were collected in the course of surgical removal of the meningioma. Postoperative seizure freedom was achieved in 11 out of 19 patients. The relative level of KCNK2 in the cortical tissue was lower in patients with preoperative seizures. On the other hand, cortical tissue level of KCNK4 was higher in patients that became seizure-free after the surgery. In addition, relative levels of KCNK4 in the cortical and tumor tissue appear to be lowered by the treatment with anti-seizure medication levetiracetam. These results imply that KCNK2 and KCNK4 may be involved in the development of meningioma-related seizures and may represent promising therapeutic targets.

1. Introduction

Meningiomas are the most frequent benign intracranial tumor [1]. They provoke seizures in approximately 30 % of patients. This may result in cognitive problems and decreased quality of life [2]. On the other hand, anti-seizure medications (ASM) may show different neurocognitive side-effects [3]. Therefore, prevention and improved treatment of meningioma-related seizures represent an important therapeutic target. However, mechanisms of development of these seizures are not fully understood. Epidemiological studies have found increased odds of seizures in patients with peritumoral edema, absence of headaches, non-skull based tumors, grade II and III tumors, and in male subjects [2,4]. Surgical removal of meningioma results in seizure freedom in about 70 % of patients that experienced preoperative seizures [2]. In addition to neuro-oncological perspective, the mechanisms of tumor-induced seizures may be important for the understanding the development and mechanisms of seizures and epilepsy in general. Comparisons of patients with and without preoperative seizures and postoperative seizure freedom appear to be a viable approach to elucidating the mechanisms of

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seizures development.

Meningiomas may interrupt morphologic and biochemical-molecular milieu of the cortex [4,5]. The pathophysiological changes that have been proposed to play a potential role in seizures development include altered connectivity and localization of synaptic vesicles, altered concentrations of voltage-dependent channels, calcium channels, and glutamate receptors, alterations in ionic balance, and others [4–6]. Mechanical interactions between meningioma and cortex have been largely overlooked. Such interactions may change the intrinsic excitability of cortical neurons via a potential impact on channels that are sensitive to mechanical stimuli – mechano-gated channels. It becomes recognized that mechano-gated channels are present in nearly all cell types, including neurons and tumor cells, and that they are involved in more aspects of (patho)physiology than previously appreciated [7]. Mechano-gated potassium channels may be of particular importance for seizure development, taking into account the central role of K^+ currents in hyperexcitability and epilepsy [8].

Herein, we analyzed the levels of mechano-gated channels – potassium two-pore domain channel subfamily K members 2 and 4 (KCNK2 and KCNK4; also known as TREK-1 and TRAAK) in tumor tissue, and in the adjacent cortical tissue that was located below the tumor and peritumoral edema. KCNK2 and KCNK4 are principal K^+ channels for rapid action potential conduction on afferent neurons [9]. In the nervous tissue, KCNK2 and KCNK4 channels are manly localized at the nodes of Ranvier in myelinated axons [7]. In the absence of mechanical pressure and increased membrane tension, KCNK2 and KCNK4 show low open probability. With the increase of membrane tension, these channels become activated, although with different dynamics [10]. Previous studies have implicated a relation between seizures development and decreased KCNK2 activity KCNK4 hyperactivity [11,12]. In addition to these mechano-gated channels, the level of potassium voltage-gated channel subfamily KQT member 2 (also known as $K_v7.2$; encoded by the gene *KCNQ2*) was established in the cortical tissue (the expression was not detectable in tumors). This channel is crucial for neuronal excitability in some inherited epilepsies [13]. KCNQ2 was used as an internal reference to establish whether the presence of meningioma has an impact on mechano-gated potassium channels or affects potassium transport and homeostasis in a more general manner.

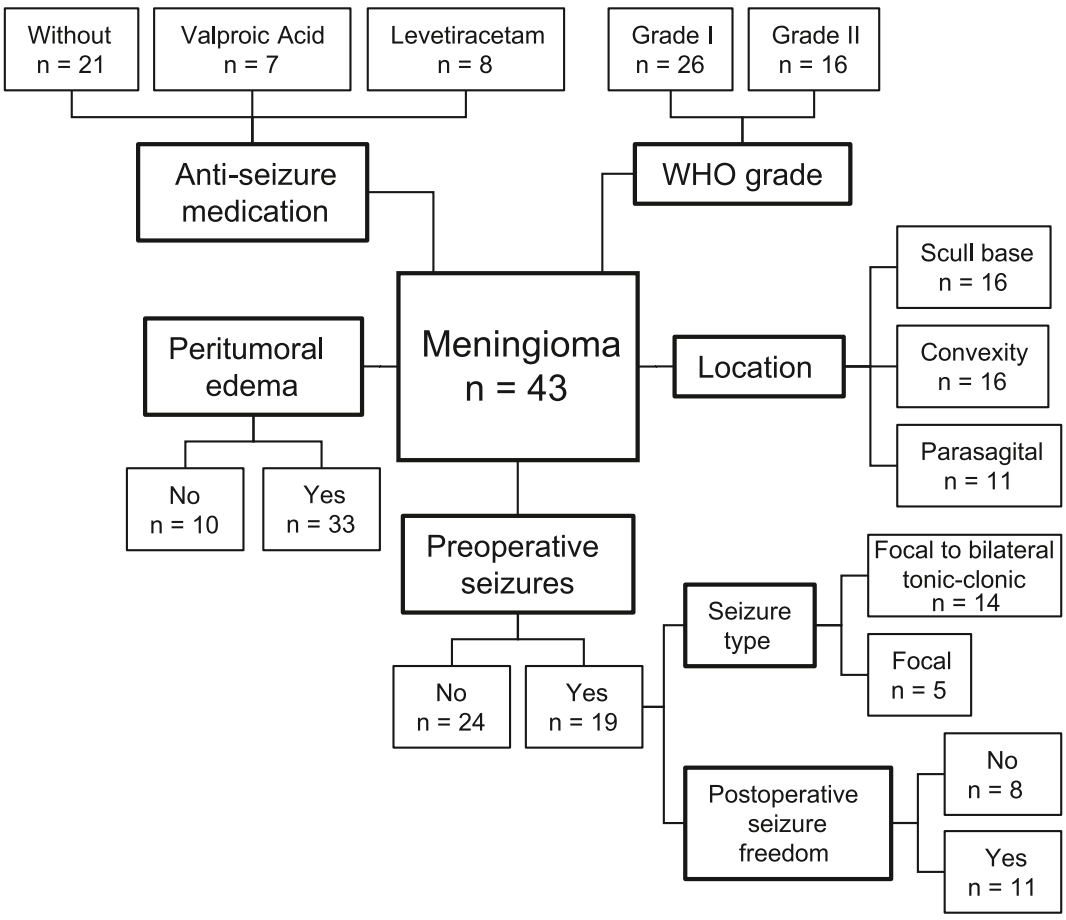

Fig. 1. Study design. n - the number of patients in each (sub)group.

2. Material and methods

2.1. Study design

The study involved 43 patients with intracranial meningioma. Exclusion criteria were: reoperation (second or third surgery), meningioma over the eloquent cortical zone and meningioma that did not penetrate the arachnoid membrane. At least one preoperative seizure was presented by 19 patients (mean age: 53.6 ± 3.0 years), whereas 24 did not show seizures (mean age: 64.8 ± 2.0 years). Study design and clinical data are presented in Fig. 1 and Supplementary Table S1. Samples of tumor tissue and adjacent cortical tissue (located beneath the tumor) were collected and snap frozen in liquid N_2 in the course of surgical treatment at the Neurosurgery Clinic, University Clinical Center of Serbia (from July 2020 to June 2021). All patients were informed in detail about their medical condition, type of treatment and further use and handling of their brain and tumor samples and signed the consent form. Institutional approval for this study was granted by Ethics Committee in accordance with internationally accepted ethical standards (The Helsinki Declaration of 1964, as revised in 1975, 1983 and 1989). The samples were stored at −80 °C until further analysis of the levels of KCNK2, KCNK4, and KCNQ2 proteins. Data were analyzed according to a set of different parameters: presence of preoperative seizures, presence of peritumoral edema, seizure type, postoperative seizure freedom, location, WHO grade, and the use of ASM. It is important ot note that the period of use of ASM prior to the surgery was between one week and four months, which prevented us from gathering sufficient data on refractoriness.

2.2. Tissue preparation

Upon thawing on ice, samples were homogenized in ice-cold RIPA buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1 % Triton X-100, 0.5 % SDS, 1 mM EDTA, 2 mM DTT, 0.1 mM PMSF and a protease inhibitor cocktail) in 1:10 mass ratio. All chemicals were obtained from Merck (Darmstadt, Germany). Homogenates were gently shaken on ice for 30 min with 5 s vortex every 30 s, and centrifuged at 16000 g/30 min/4 °C. Protein-containing supernatants were collected and analyzed.

2.3. SDS-polyacrylamide gel electrophoresis (PAGE) and Western blot

Supernatants were incubated with Laemmli buffer for 10 min at 95 °C, resolved on 10 % SDS-polyacrylamide gel and transferred to a PVDF membrane. This was followed by blocking in 5 % bovine serum albumin and incubation with antibodies. Abcam (Trumpington, Cambridge, UK) primary antibodies ab90855, ab22897 and ab8227 were used to detect KCNK2, KCNQ2 and β-actin, respectively, whereas Thermo Fisher Scientific (Waltham, MA, USA) primary antibody PA5-109267 was used to detect KCNK4. Subsequently, blots were incubated with secondary goat anti-rabbit antibody conjugated with horseradish peroxidase (ab6721, Abcam). Immunopositive bands were then visualized using the method of enhanced chemifluorescence with an iBright™ FL1500 Imager (Thermo Fisher

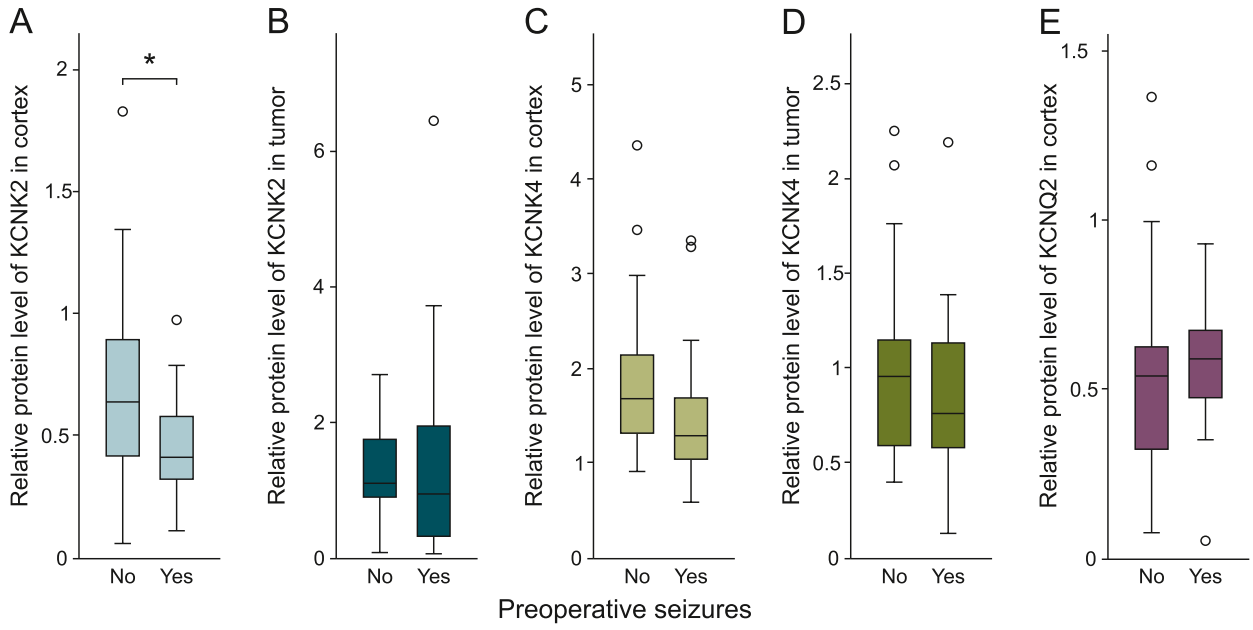

Fig. 2. Relative protein levels of potassium channels in tumor and adjacent cortical tissue from meningioma patients with (n = 19) and without seizures (n = 24). (A) KCNK2 in the cortical tissue. (B) KCNK2 in tumor tissue. (C) KCNK4 in the cortical tissue. (D) KCNK4 in tumor. (E) KCNQ2 in the cortical tissue. Boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range. Outliers (circles) are defined as data point values that are more than 1.5 × IQR outside the box). * - statistically significant ($p < 0.05$).

Scientific). For optimal comparison, all experimental samples, primary antibody positive control samples (as recommended by the manufacturer), and protein ladder were run on the same gel/blot. Densitometric quantification of the immunopositive bands was performed in ImageJ (NIH). Band intensities were normalized to loading control (β-actin).

2.4. Statistical analysis and data representation

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25.0 software (IBM Corp., Armonk, NY, USA). Data were represented as box plots (boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range; circles represent outliers, defined as data point values that are more than 1.5 × IQR outside the box). Extremes (defined as data point values that are more than 3 × IQR outside the box) were excluded from the analysis. Levene's test was used for the assessment of homogeneity of variance. For comparing two groups, *t*-test for equality of means was used on data with homogenous variance or ANOVA for multiple group comparisons. Otherwise, non-parametric tests Mann-Whitney or Kruskal–Wallis were used. Data were considered statistically significant at $p < 0.05$.

3. Results and discussion

The level of KCNK2 in the cortical tissue that was adjacent to tumor was lower in patients that suffered preoperative seizures than patients without seizures (Fig. 2A). No differences were observed for KCNK2 in tumor tissue, KCNK4 in cortex and tumor tissue, and KCNQ2 in the cortical tissue (Fig. 2). Representative blots are given in Supplementary Fig. S1. No reliable blotting of KCNQ2 could be observed for tumor samples. The meningioma/edema-induced mechanical pressure may lead to the opening of KCNK2 and KCNK4 and further to increased uncontrolled neuronal activity and high-frequency bursts of action potential. Although both KCNK2 and KCNK4 respond to membrane tension by activation/opening, there are some essential differences. KCNK4 shows a lower threshold of pressure activation [14], whereas KCNK2 appears to be closed at higher pressures unless some other activating stimulus is present [15]. The down-regulation of KCNK2 may represent a compensatory response to prolonged exposure to mechanical pressure by tumor and peritumoral edema. Pertinent to this, previous studies imply that lower activity of KCNK2 may lead to seizures development. KCNK2 knock-out model animals are highly susceptible to seizures [16]. In addition, pharmacological blockade of KCNK2 has been linked to side-effects that include seizures [17]. On the other hand, it has been shown that increased expression of KCNK2 silences the hyperexcitable neurons in the brain of epileptic rats and treats acute seizures. KCNK2 appears to inhibit neuronal firing by hyperpolarizing the resting membrane potential and decreasing input resistance [11]. Previous meta-analysis of epidemiological data has identified a number of factors that are related to increased odds ratio of seizures development [2,4]. We compared KCNK2 levels in the cortical tissue in the groups created according to those factors which include peritumoral edema, sex, headaches, tumor location, and WHO grade There were no significant differences in relation to the factors, other than the lower level of KCNK2 in the cortical tissue in

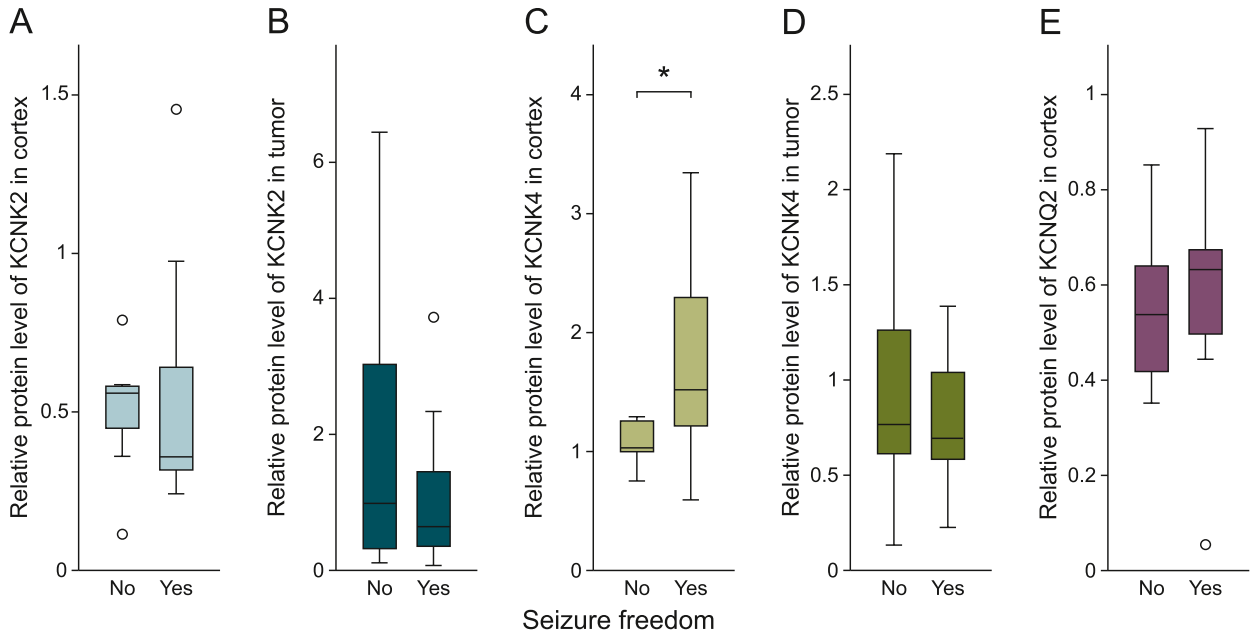

Fig. 3. Protein level of potassium channels in meningioma patients that showed preoperative seizures and were or were not seizure-free following the surgery. (A) KCNK2 in the cortical tissue. (B) KCNK2 in tumor tissue. (C) KCNK4 in the cortical tissue. (D) KCNK4 in tumor tissue. (E) KCNQ2 in the cortical tissue. Boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range. Outliers (circles) are defined as data point values that are more than 1.5 × IQR outside the box). * - statistically significant ($p < 0.05$).

meningioma grade I compared to meningioma grade II patients (data not shown). This may be related to a slower growth of meningioma grade I [2]. A slow growth of meningioma has been proposed to induce more profound peritumoral changes that may lead to epileptogenicity [4].

Fig. 3 shows that the level of KCNK4 was significantly higher in the cortical tissue of seizure-free patients than in patients that did not achieve postoperative seizure freedom. Other protein levels were not related to postoperative seizure freedom. It is worth noting that previous studies have linked increased incidence of postoperative seizures with larger tumor volumes and higher tumor grade [18, 19]. However, novel prediction factors are needed. Our data imply that KCNK4 may play a role in the development of seizures and may represent a prediction factor for the postoperative seizure freedom. Further, it appears that the treatment with ASM levetiracetam results in the down-regulation of KCNK4 in the cortical and tumor tissue (Fig. 4). The effects of levetiracetam on the reduction of voltage-gated potassium currents have been demonstrated previously [20]. Present results imply that the mechanisms of anti-seizure effects of this drug may involve the regulation of KCNK4 channel level as well. The hyperactivation of KCNK4 channel has been related to seizures development in recently identified rare genetic disorder FHEIG [12]. It is noteworthy that levetiracetam down-regulates the expression of KCNK3, a mechano-gated channel from the same family as KCNK4. The decreased expression results in the alleviation of severity and seizure frequency severity in chronic epilepsy animal model [21]. In addition to the use in epilepsy treatment, levetiracetam is applied as a prophylaxis for migraine [22,23]. Mechano-gated potassium channels are involved in migraine development [22], so the suppressing effects of levetiracetam against KCNK4 channel may be involved in the anti-migraine effects as well [24]. Finally, it is important to point out that no significant changes in the level of KCNQ2 were observed for any of the analyzed parameters. This implies that meningioma most likely do not affect potassium homeostasis in the cortex.

In close, it appears that mechano-gated potassium channels KCNK2 and KCNK4 are involved in the development of meningioma-related seizures and may represent promising therapeutic targets. However, the main drawback of our study – a small cohort, calls for further more detailed examination of the relations implicated here.

Data availability statement

Data will be made available on request.

Ethics statement

Institutional approval for this study was granted by Ethics Committee in accordance with internationally accepted ethical standards (number 1322/III-23 Ethical Committee Medical Faculty University of Belgrade).

CRediT authorship contribution statement

Ivan Bogdanović: Conceptualization, Data curation, Formal analysis, Writing – original draft. **Miloš Opačić:** Data curation, Formal analysis, Methodology, Writing – original draft. **Vladimir Baščarević:** Data curation, Formal analysis, Investigation. **Savo**

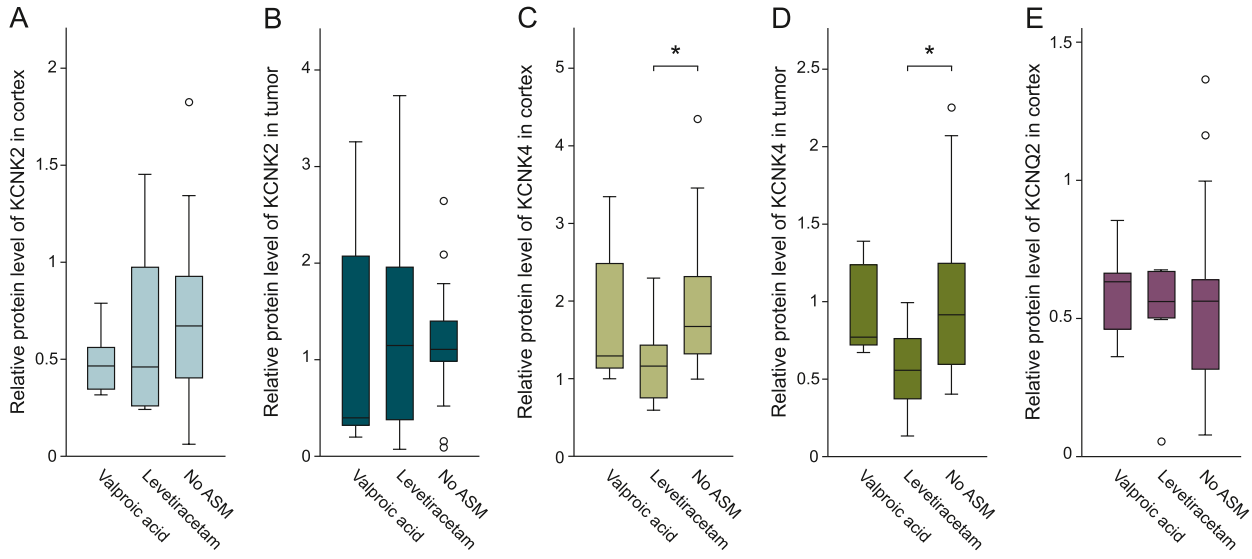

Fig. 4. Protein level of potassium channels in meningioma patients that were treated with valproic acid or levetiracetam or that received no anti-seizure medication. (A) KCNK2 in the cortical tissue. (B) KCNK2 in tumor tissue. (C) KCNK4 in the cortical tissue. (D) KCNK4 in tumor tissue. (E) KCNQ2 in the cortical tissue. Boxes represent the median and the 25th and 75th percentiles; whiskers represent the non-outlier range. Outliers (circles) are defined as data point values that are more than 1.5 × IQR outside the box). * - statistically significant ($p < 0.05$).

Raičević: Conceptualization, Data curation, Methodology, Writing – original draft. **Rosanda Ilić:** Data curation, Formal analysis, Investigation, Writing – original draft. **Danica Grujičić:** Conceptualization, Investigation, Supervision, Writing – original draft. **Ivan Spasojević:** Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. **Aleksandar J. Ristić:** Conceptualization, Data curation, Formal analysis, Methodology, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e20761.

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