Coumarins as potential supportive medication for the treatment of epilepsy

Jarosław Bryda¹,², Mirosław Zagaja², Aleksandra Szewczyk² and Marta Andres-Mach³*

¹ Department of Veterinary Hygiene, Voivodship Veterinary Inspectorate, Lublin, Poland,
² Isobolographic Analysis Laboratory, Institute of Rural Health, Lublin, Poland,
³ E-mail: mandres@wp.pl

INTRODUCTION

Coumarins are organic, biologically active compounds belonging to the benzopyrone family (1,2-benzopyrones or 2H-1-benzopyran-2-ones). They are divided into four subtypes based on their chemical structure. Simple coumarins are formed by benzene rings fused with α-pirons which are hydroxylated, alkoxylated or alkylated at the C7, C6 and C3 positions of benzopyrone. Isocoumarin derivatives are formed by two rings: benzene and α-isopirone with substituents in positions C3, C6, C7 and C8. Furanocoumarins, consisting of a five-membered furan ring fused with coumarin, are divided into two types – psoralen, at the C6-C7 positions, or angelicin, at the C7-C8 position. Pyranocoumarins, with substituents, condense a six-membered pyran ring with a coumarin ring at the C6-C7 position (Mead et al., 1958; Jain et al., 2012; Medina et al., 2015). Variable chemical structures and a diversity of substitutions in the skeleton affect the diverse pharmacological activities of coumarins (Kumar et al., 2015; Kubrak et al., 2017). Coumarins are mainly found in secondary plant metabolites, acting as growth regulators, controlling biochemical transformations and demonstrating defensive properties against infection (Chattha et al., 2018). They are also found in some bacteria, fungi or sponges and can be synthesized chemically (Matos et al., 2015).

Coumarins and coumarin-related compounds possess a wide range of pharmacological profiles. Their properties and their impact on the cardiovascular system (Najmanova et al., 2015), nervous system (Skalic-Ka-Woźniak et al., 2016), body immunity (Rohini et al., 2014) and digestive system (Popp et al., 2017) are currently being researched. New derivatives are still being discovered and synthesized due to their potential uses.

Recent studies have revealed the effective use of coumarins in cancer therapy as medicaments and for...
mitigating the effects of radiotherapy (Rohini et al., 2014). One of the most common coumarins, warfarin (4-hydroxycoumarin), is used in medicine as an oral anticoagulant (Kumar et al., 2015). Antitumor activity has been demonstrated for osthole in the suppression of the spread of breast cancer cells. Tests with bioluminescence have shown that osthole inhibits the promoter of metalloproteinase-2 (MMP-2) and indirectly inhibits the activity of this enzyme, which may lead to inhibition of tumor migration (Yang et al., 2010). Anticancer properties were also exhibited by imperatorin, esculetin, chartreusin and fraxetin with various mechanisms of action (Luo et al., 2011). Interestingly, coumarins have been shown to possess strong anti-inflammatory properties. This is due to their antioxidant activity and effect on reactive oxygen species. Esculetin exhibited protective effects on rat intestines in colitis (Witaicenis et al., 2010). In other studies, extracts used externally have been shown to reduce skin inflammation and edema (Kwon et al., 2011). Derivatives such as esculetin, fraxetin and daphnetin exhibit antioxidant activity, acting as inhibitors of the lipoxygenase and cyclooxygenase enzyme pathways (Kirsch et al., 2016). In vitro studies on coumarin compounds have served to demonstrate their antimicrobial and antifungal activity. Tests carried out on Staphylococcus aureus, Bacillus subtilis and Escherichia coli strains revealed a much stronger antibacterial effect of synthetic coumarin compounds compared to several conventional antibiotics (Vyas et al., 2012). Much research has been devoted to the effect of coumarins on the central nervous system (CNS). Therefore, numerous experimental studies focus on disorders such as epilepsy, schizophrenia, depressive and anxiety disorders or Alzheimer’s disease (Skalicka-Woźniak et al., 2016).

Possible anticonvulsant properties of natural coumarins

Epileptic seizures are the result of excessive abnormal neuronal activity in the brain. The primary treatment for epilepsy is the administration of antiepileptic drugs (AEDs). These drugs reduce the frequency of seizures and help patients control seizure occurrence. Unfortunately, a significant proportion of patients exhibit little or no improvement with current drug therapies. Moreover, the issue of chronic side effects due to the drugs is also significant (Sharma et al., 2013).

Experimental epileptic models are used to assess the activity of potential anticonvulsant drugs and enable an estimation of the clinical profile of a substance’s action on the CNS. One of the basic in vivo animal models for assessing potential anticonvulsant properties of drugs is the maximal electroshock seizure (MES) test in rodents (Castel-Branco et al., 2009). It allows for the modeling of specific pharmacodynamic effects required to protect against seizures. Additionally, it is possible to assess the bioavailability of a given substance based on the analysis of its concentration in specific brain structures in post-mortem tissue preparations (Rogawski et al., 2006).

Anticonvulsant effects of coumarins are likely related to their influence on the ionotropic receptor for γ-aminobutyric acid (GABA). This was demonstrated by studies in which furanocoumarins were found to be partial benzodiazepine receptor antagonists, inhibiting the binding [3H] of diazepam to these receptors (Singhuber et al., 2011). In this study, which examined the effects of eighteen furanocoumarins on GABA-induced chloride currents (I\textsubscript{GABA}), seven compounds showed a greater than 20% enhancement of I\textsubscript{GABA}. Similar results were obtained in studies of coumarins isolated from Angelica pubescens (L.) that described their GABA-A\textsubscript{γ} receptor-modulating activity (Zaugg et al., 2011). In vivo analysis of the anticonvulsive activity of coumarins suggests they may indirectly act to increase GABA concentration in the CNS by affecting the activity of glutamic acid decarboxylase (Luszczki et al., 2007a; Simghuber et al., 2011; Zaugg et al., 2011).

Recent research provides evidence of positive supporting effects for coumarin compounds on the conventional AEDs (Table I, Table II). The promising pharmacological activity was demonstrated by the simple coumarins osthole and umbelliferone and also by several furanocoumarins – xanthotoxin and imperatorin (Luszczki et al., 2007a; 2009; 2010; 2011).

Osthole

Osthole (7-methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one) is a simple natural origin coumarin, which occurs in several medicinal plants such as Cnidium monnieri (L.) or Angelica pubescens (L.). Both in vitro and in vivo studies have revealed that osthole demonstrates neuroprotective (Liu et al., 2010), osteogenic (Ming et al., 2011), immunomodulatory (Liao et al., 2010), anticancer (Yang et al., 2010; Kao et al., 2012), hepatoprotective (Zhang et al., 2011), anticoagulant and antimicrobial properties (Rosselli et al., 2007).

The neuroprotective effects of osthole are related to an increase in neuronal conduction in the hippocampus. Osthole affects the membrane receptors by increasing the release of glutamate from rat hippocampal nerve terminals. It facilitated 4-aminopyridine (4-AP)–evoked glutamate release by activating N- and
P/Q-type Ca\(^{2+}\) channels through a signaling cascade involving protein kinase C (Wang et al., 2008).

In addition to the above-mentioned mechanism, and based on the intensification of glutamatergic transmission, the anticonvulsant activity of osthole has also been associated with modulation of membrane channel activity via blocking L-type Ca\(^{2+}\) channels and Na\(^+\) channels in mouse neuronal cells (Wu et al., 2002; Leung et al., 2010). Osthole was found to inhibit voltage-gated Na\(^+\) currents dependent upon its intracellular concentration and the current functional potential of the cell membrane in an N2A mouse neuroblastoma cell line (Leung et al., 2010). Moreover, osthole was identified as an activator of the GABA\(_A\) receptor in vitro (Zaugg et al., 2011; Singhuber et al., 2011).

Research conducted by Luszczki et al. (2009) showed that osthole reduced seizures induced by the MES test in mice. The experiment was performed by a systematic administration of the compound 15, 30, 60 and 120 minutes before the test. The maximal anticonvulsant effect was obtained after intraperitoneal (i.p.) administration of osthole between 15 and 30 minutes. In addition, chimney test results showed no disturbances in motor coordination.

Subsequent studies conducted by Luszczki et al. (2010) for various combinations of osthole with classic AEDs showed that osthole at a dose of 200 mg/kg significantly increased the electroconvulsive threshold in mice. Osthole administered with carbamazepine (CBZ), phenobarbital (PB), phenytoin (PHT) and valproate (VPA) did not have a significant effect on the anticonvulsant activity of the studied drugs (at test doses for osthole 50, 100 and 150 mg/kg). Assessment of the motor performance of the mice treated with the combinations of osthole and the above-mentioned classical AEDs showed no side effects (Luszczki et al., 2010; 2011).

### Umbelliferone

Umbelliferone (7-hydroxycoumarin) is a commonly occurring coumarin plant widely occurring in the Apiaceae (Umbelliferae) and Rutaceae families. Umbelliferone exhibits various pharmacological activities including antihiperglycemic (Ramesh et al., 2006), bronchodilating (Vasconcelos et al., 2009), antiedematogenic (Tayama et al., 2009), neuroprotective (Subramaniam et al., 2013), antinociceptive (Barros et al., 2010) and anticonvulsant (Zagaja et al., 2015a) properties. Due to its simple structure, it is used as a parent compound in the synthesis of various coumarins and heterocyclic compounds (Mazimba et al., 2017).

Subramaniam and Ellis (2013) identified a neuroprotective effect for umbelliferone, where this coumarin

---

### Table I. Effect of coumarins on the anticonvulsant activity of conventional antiepileptic drugs against maximal electroshock-induced seizures in mice.

<table>
<thead>
<tr>
<th>Coumarins</th>
<th>CBZ ED(_{50}) (mg/kg)</th>
<th>PB ED(_{50}) (mg/kg)</th>
<th>PHT ED(_{50}) (mg/kg)</th>
<th>VPA ED(_{50}) (mg/kg)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imperatorin + AEDs</td>
<td>6.0</td>
<td>10.3</td>
<td>19.6</td>
<td>8.5</td>
<td>12.8</td>
</tr>
<tr>
<td>Osthole + AEDs</td>
<td>6.89</td>
<td>8.87</td>
<td>12.35</td>
<td>18.17</td>
<td>7.48</td>
</tr>
<tr>
<td>Umbelliferone + AEDs</td>
<td>11.76</td>
<td>13.97</td>
<td>21.78</td>
<td>35.39</td>
<td>10.84</td>
</tr>
<tr>
<td>Xanthotoxin + AEDs</td>
<td>5.01</td>
<td>13.97</td>
<td>27.87</td>
<td>35.39</td>
<td>12.21</td>
</tr>
</tbody>
</table>

Results are presented as median effective doses (ED\(_{50}\) in mg/kg; with 95% confidence limits in parentheses) required to protect 50% of animals tested against maximal electroshock-induced seizures. Bolded ED\(_{50}\) values correspond to the significantly reduced effective dose of combination in comparison to the AED itself. AEDs – antiepileptic drugs, CBZ – carbamazepine, PB – phenobarbital, PHT – phenytoin, VPA – valproate.

---

### Table II. Effect of imperatorin and xanthotoxin on the protective activity of various novel antiepileptic drugs against maximal electroshock-induced seizures in mice.

<table>
<thead>
<tr>
<th>Coumarins</th>
<th>LCM ED(_{50}) (mg/kg)</th>
<th>LTG ED(_{50}) (mg/kg)</th>
<th>OXC ED(_{50}) (mg/kg)</th>
<th>PGB ED(_{50}) (mg/kg)</th>
<th>TPM ED(_{50}) (mg/kg)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imperatorin + AEDs</td>
<td>-</td>
<td>-</td>
<td>6.11</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xanthotoxin + AEDs</td>
<td>3.96</td>
<td>8.70</td>
<td>5.01</td>
<td>5.96</td>
<td>7.54</td>
<td>12.49</td>
</tr>
</tbody>
</table>

Results are presented as median effective doses (ED\(_{50}\) in mg/kg; with 95% confidence limits in parentheses) required to protect 50% of animals tested against maximal electroshock-induced seizures. Bolded ED\(_{50}\) values corresponds to the significantly reduced effective dose of combination in comparison to the AED itself. AEDs – antiepileptic drugs, LCM – lacosamide, LTG – lamotrigine, OXC – oxcarbazepine, PGB – pregabalin, TPM – topiramate.
Coumarins in the treatment of epilepsy


Xanthotoxin

Xanthotoxin, known as methoxsalen (8-methoxypsoralen), is furanocoumarin commonly occurring in plants. A high content was found in the Apiaceae family plant Ammi majus (L.), possessing numerous pharmacologically active properties, including photoreactivity used to treat skin autoimmune diseases (Selim et al., 2012).

It has been shown that xanthotoxin effectively penetrates the blood-brain barrier (BBB) (Tian et al., 2015). Skalicka-Wozniak and coworkers (2018) indicated that xanthotoxin improved the ability to remember in mice with scopolamine-induced memory deficits. Additionally, another study by Skalicka-Wozniak et al. (2014) showed an anticonvulsant effect for xanthotoxin in the mouse MES model. The compound had the most effective protective effect (ED_{50}=219.1 ± 4.7 mg/kg) when administered 60 minutes before the test.

Zagaja et al. (2015a) examined the antiepileptic activity of umbelliferone alone and in combination therapy with classical AEDs in the MES model in mice. Umbelliferone was administered (i.p) at doses of 50, 100 and 150 mg/kg 30 minutes before electroshock. Umbelliferone alone at a dose of 150 mg/kg significantly increased the threshold for the maximal electroshock-induced seizure test CS_{50} in mice (about 37%). Interestingly, the same dose of umbelliferone enhanced the anticonvulsant effect of VPA and PB, reducing the ED_{50} from 281.4 to 215.5 mg/kg for VPA and 35.39 to 21.78 mg/kg for PB. Results from the total VPA and PB brain concentrations showed that the interaction between umbelliferone and AEDs was pharmacodynamic in nature. Moreover, no side effects such as impaired motor coordination, muscle strength or long-term memory were observed in the mice.

Imperatorin

Imperatorin (9-[(3-methyl-2-buten-1-yl)oxy]-7H-furo[3,2-g]chromen-7-one), a furanocoumarin, is produced as a secondary metabolite of plants from the Apiaceae and Rutaceae families. It possesses numerous pharmacological activities and, among other things, exerts a significant impact on the CNS (Kozioł et al., 2016).

Imperatorin affects the CNS through various mechanisms. It has been shown to act by inhibiting GABA transaminase and increasing the concentration of synaptic GABA in neurons—suggesting a similar activity to vigabatrin (Choi et al., 2005). In studies by Zaugg et al. (2011), imperatorin, like osthole, is modulator of the GABA_{A} receptor. Moreover, imperatorin inhibits volt-
age-gated Na\textsuperscript{+} channel (VGSC) activity and suppresses action potential amplitude (Wu et al., 2013). In addition, imperatorin was shown to desensitize transient reversal potential (TRP) V1 channels, and such action may account for the anti-nociceptive effects of imperatorin on formalin- and capsaicin-induced pain in rats (Chen et al., 2014). Furthermore, Wang et al. (2015) suggest that imperatorin inhibited both K\textsubscript{v} and K\textsubscript{ATP} channels.

It has also been shown that imperatorin inhibits acetylcholinesterase activity in thin-layer chromatography (TLC) bioautography (Urbain et al., 2005). Interestingly, results presented by Cao and colleagues (2007) were indicative of antidepressant properties for imperatorin. Long-term oral administration of imperatorin at 15 and 30 mg/kg/day for 28 days increased the 5-HT concentration in rat hippocampus.

Imperatorin anticonvulsant activity was demonstrated in preclinical studies by Luszczki et al. (2007a) in the mouse MES threshold model. In this study, imperatorin increased the convulsive threshold in a dose-dependent manner. Statistically significant results were obtained for doses of 50 and 100 mg/kg administered (i.p.) at 30, 60 and 120 minutes prior to the test.

The influence of imperatorin on the antiepileptic action of the four classic AEDs was demonstrated in a study by Luszczki et al. (2007b). The anticonvulsant activity of CBZ, PB, PHT and VPA was studied in combination with several doses of imperatorin (20, 30, 40 mg/kg) in the MES test. A significant reduction of ED\textsubscript{50} and an increase in the effect of the drugs were observed for PHT, PB and CBZ with 40 mg/kg of imperatorin (by 34, 38 and 42%, respectively). Statistically significant effects were also achieved for the combination of CBZ with imperatorin at a dose of 30 mg/kg (lowered ED\textsubscript{50} by 32%). Combinations of imperatorin with the drugs did not disturb the muscular strength of animals in the grip-strength test or long-term memory in the passive avoidance test. Analysis of the total brain concentration of the AEDs showed no effect of imperatorin (at a dose of 40 mg/kg) on the total concentration of PHT and PB, which proves that the observed interactions were pharmacodynamic in nature. In turn, the concentration of CBZ, which was significantly increased in comparison to control, indicated a pharmacokinetic nature of the imperatorin and CBZ interaction. Similarly, imperatorin at a dose of 50 mg/kg was shown to reduce the median effective dose of LTG from 6.11 to 2.47 mg/kg in mouse MES model (Luszczki et al., 2008). Moreover, imperatorin had no impact on the acute adverse effects of lamotrigine in the chimney test and results from total brain LTG concentration analysis did not indicate any pharmacokinetic interaction between drugs (Table II).

CONCLUSIONS

Looking for new anti-epileptic drugs is a long-term process requiring thorough research. Phytochemicals, commonly found in plants, are characterized by interesting bioactive properties, including pharmacological ones. Among these substances, coumarins and their derivatives demonstrate beneficial, significant anticonvulsant activity. Coumarins have similarly been shown to act as anticonvulsants in various in vitro and in vivo assays, yielding promising results at the preclinical stage of experimental models of epilepsy.

There are currently observable trends of supporting anti-epileptic treatment, especially drug-resistant epilepsy, with various “natural” diets. Therefore, patients treated with classic antiepileptic drugs may use “healthy” supplements prepared from medicinal plants and herbs, including coumarins, which may affect the anticonvulsant effect of antiepileptic drugs. In searching for novel medications, it is necessary to remember that the most important guideline is patient safety. Therefore, further evaluation of coumarins and approximations of the mechanism of their action is needed so that in the future they can support therapies for epileptic patients.

REFERENCES


