

Antiarrhythmic effects of newly developed propafenone derivatives

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Abstract

It is well known that the presence of different chemical groups in drug molecules influences their pharmacological properties. The aim of our study is to investigate whether newly synthesized derivatives of propafenone, with changes in benzyl moiety, have a different effect upon arrhythmia, compared to propafenone. 5OCl-PF and 5OF-PF are derivatives of propafenone with -Cl or -F substituent on the *ortho* position of the benzyl moiety. For verification of their antiarrhythmic effect, we used an *in vivo* rat model of aconitine-induced arrhythmia. 5OCl-PF speeded the appearance of supraventricular premature beats (SVPB) and death more than aconitine. All animals treated with 5OCl-PF developed ventricular premature beats in salvos (VPBS), bigeminies (VPBB) and paroxysmal ventricular tachycardia (PVT). 5OF-PF had a negative chronotropic effect and potentiated atrial excitability (more SVPB). It had a positive effect on the occurrence and onset time of supraventricular tachycardia, VPBS, and PVT. Based on the obtained results, it can be concluded that newly synthesized propafenone derivatives have no better antiarrhythmic effect than the parent compound. In the future, our research will be focused on the synthesis of different derivatives and examining their antiarrhythmic effects.

Key words: propafenone derivatives, experimental arrhythmia, aconitine, rats

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Introduction

Propafenone is an old anti-arrhythmic drug introduced in clinical medicine more than 30 years ago (1). It is an Na⁺ channel blocker with a relatively slow time constant for recovery from block (2). In addition, propafenone also blocks K⁺ channels and β receptors and has anticholinergic properties (3). Its major electrophysiological effect is to slow conduction in fast-response tissues. According to the new recommendations for the management of cardiac arrhythmias, propafenone is indicated for maintenance of normal sinus rhythm in patients with symptomatic atrial fibrillation (4, 5). Propafenone can be applied for the treatment of life-threatening ventricular arrhythmias as well. However, like other 1st class anti-arrhythmic drugs, propafenone had a limited use in patients with ventricular arrhythmias to those with life-threatening arrhythmias (6, 7). Its use in patients with less severe ventricular arrhythmias, even symptomatic, is not recommended because of propafenone arrhythmogenic potential (8). Propafenone has proarrhythmic effects, such as 1:1 conducted atrial flutter and re-entrant ventricular tachycardia. It was reported that the overall incidence of arrhythmias with propafenone is 5-10% (9). Rapid recognition of these complications allows discontinuing the medication and resolution of arrhythmia (10). Adverse effects during propafenone therapy also include exacerbation of heart failure and sinus bradycardia due to β -receptor blockade (7). Based on these facts, there is certainly a need to synthesize a new derivative of propafenone, which would be equally effective in the treatment of arrhythmias, but would have fewer side effects and better bioavailability. Namely, the bioavailability of propafenone is very low, which also limits its therapeutic application.

Chemical structure of propafenone is 1-(2-[2-hydroxy-3-(propylamino)propoxy]phenyl)-3-phenylpropan-1-one hydrochloride. It is well known that the presence of different chemical groups in drug molecules influences their potency and selectivity. Good examples of this are the beta-blockers which are structurally related to propafenone (Figure 1A). The change of the benzene ring with different hydrophilic groups improved propranolol cardioselectivity, which resulted in the reduction of its side effects (11). Previous structure properties studies and structure-activity relationship studies (*SPS*, *SAR*), as well as docking studies, have shown that protonated nitrogen, ether, carbonyl, aromatic moiety (phenyl or benzyl groups), as well as hydrophobic groups, are crucial for the interaction of propafenone and propafenone analogues with ion channels (12-14). These structural parts contribute to the lipophilicity and degree of ionization of molecules and they have a significant impact on the absorption, distribution, metabolism, and excretion of compounds (15). Recent research in the field of ion channel blockers has been focused on hydrophobic interactions between the inside pore of the ion channels and the molecules (16, 17). In this regard, our synthetic route was focused on the modification of the benzyl moiety in the molecule of propafenone.

ACEBUTOLOL – β blockers

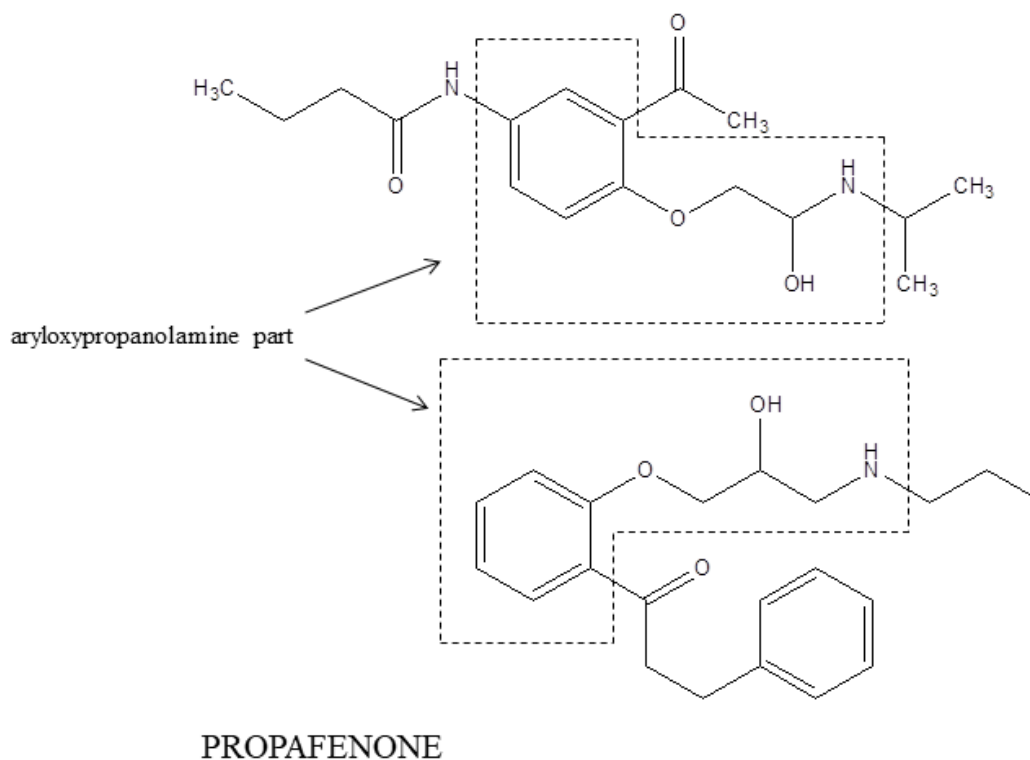


Figure 1A. Chemical structure of acebutolol, propafenone and novel propafenone derivatives; aryloxypropanolamine part – the common part of the structures of propafenone and beta blockers

Slika 1A. Hemijska struktura acebutolola, propafenona i novih derivata propafenona; ariloksipropanolaminski deo – zajednički deo strukture propafenona i beta blokatora

Thus, the aim of our study is to investigate whether newly synthesized derivatives of propafenone, with changes in benzyl moiety, have different effects upon arrhythmia, compared to propafenone. We decided to verify experimentally the expected antiarrhythmic effects of newly synthesized derivatives of propafenone on an *in vivo* animal model of aconitine-induced arrhythmia. Laboratory rats were chosen as experimental animals for better comparison with the existing data. No pharmaceutical company took part in the development of propafenone derivatives.

Experimental

The study reported in this work was carried out following the European regulations on the protection of animals, the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the United States National Institutes of Health.

Chemicals for synthesis and in vivo experiments

For chemical synthesis and analysis of propafenone derivatives, the following compounds were used: 2-fluorobenzaldehyde, (Merck Darmstadt, Germany), 2-hydroxyacetophenone 2-chlorobenzaldehyde, epichlorohydrin, propylamine (Sigma-Aldrich Inc., St. Louis, MO, USA). For *in vivo* experiments the following chemicals were used: aconitine, propafenone, and urethane (Sigma-Aldrich Inc., St. Louis, MO, USA), and newly synthesized derivatives: 3-(2-fluoro-phenyl)-1-[2-(2-hydroxy-3-propylamino-propoxy)-phenyl]-propan-1-one hydrochloride (5OF-PF) and 3-(2-chloro-phenyl)-1-[2-(2-hydroxy-3-propylamino-propoxy)-phenyl]-propan-1-one hydrochloride (5OCl-PF).

Chemical analysis

The structure of synthesized derivatives has been verified by IR, ¹H-NMR, ¹³C-NMR, and MS-TOF spectroscopy. The IR spectra were recorded on a Nicolet 6700 FT spectrophotometer using the ATR technique in the range 4000-600 cm⁻¹. The NMR spectra were recorded on a Varian Gemini 200 (¹H NMR spectra at 200 MHz and ¹³C NMR spectra at 50 MHz) for samples dissolved in deuterated chloroform. ESI-TOF analyses of the compounds were carried out on an Agilent 6210 time-of-flight LC/MS system (G1969A, Agilent Technology, Santa Clara, CA, United States).

Procedure of synthesis of new substances

Synthesis of the novel propafenone derivatives was carried out according to the general method first reported by Ivkovic et al. (18). Preparation included five steps (Figure 1B): 1. condensation of acetophenone with *o*-mono substituted benzaldehyde, 2. reduction of α,β -unsaturated ketone with 5% Pd/H₂, 3. addition of phenolic nucleophiles to epichlorohydrin, 4. aminolysis with propylamine and 5. reaction with 1M HCl solution in dry diethyl ether to give hydrochloride aryloxypropanolamine derivatives. The tested compounds are derivatives of phenylpropiofenone (as well as propafenone) with fluorine (-F) or chlorine (-Cl) atom on the *ortho* position of the benzyl moiety.

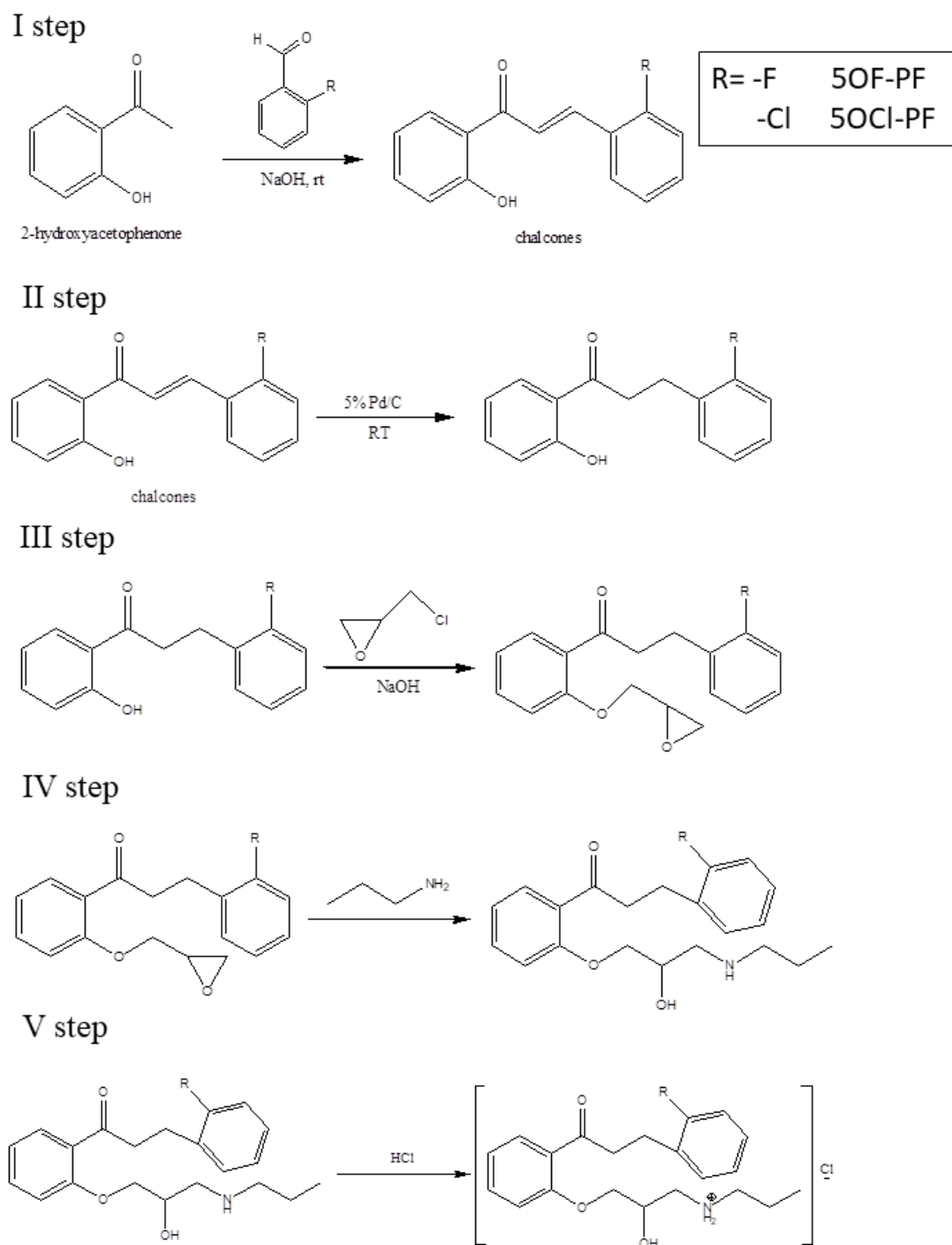


Figure 1B. Synthesis steps of propafenone derivatives
Slika 1B. Koraci sinteze derivata propafenona

Experimental animals

The experiments were performed *in vivo* on 45 male Wistar laboratory rats (average body weight of 310 ± 25 g). The animals came from a conventional breeding colony (Faculty of Medicine, Belgrade University, Belgrade, Serbia). The experimental protocol conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). All procedures were performed following protocols approved by the University Ethical Committee of the Faculty of Medicine, University of Belgrade, Republic of Serbia (license number 4211/2).

The animals were anesthetized with terminal anesthesia using urethane. An anesthetic agent was administered intraperitoneally at the dose of 1.0 g/kg of body weight (19).

Experimental design

The anti-arrhythmic effect of the novel propafenone derivatives was tested on the model of aconitine-induced arrhythmia (20). Aconitine was administered intravenously into the exposed *vena jugularis*, at a dose of 40 $\mu\text{g}/\text{kg}$ of the animal weight. The administration of aconitine took 10 seconds. This dose was determined based on the preliminary experiment ($n = 6$) whose object was to titrate the dose that would induce the occurrence of life-threatening arrhythmia.

We analyzed the effects of newly synthesized derivatives of propafenone, 5OCl-PF, and 5OF-PF on aconitine-induced arrhythmia, and compared their effects to the effect of propafenone. Those substances were analyzed as prophylactic agents (administered 5 min before the administration of aconitine). The tested substances were administered intravenously into the exposed *vena jugularis* at a dose of 2 mg/kg of animal body weight, the most effective dose as shown in preliminary experiments.

Four groups were designed (Figure 2): Group 1 ($n = 12$) - only aconitine was administered, Group 2 – propafenone was administered as a prophylactic agent ($n = 9$), Group 3 – 5OCl-PF was administered as a prophylactic agent ($n = 9$) and Group 4 - 5OF-PF was administered as a prophylactic agent ($n = 9$).

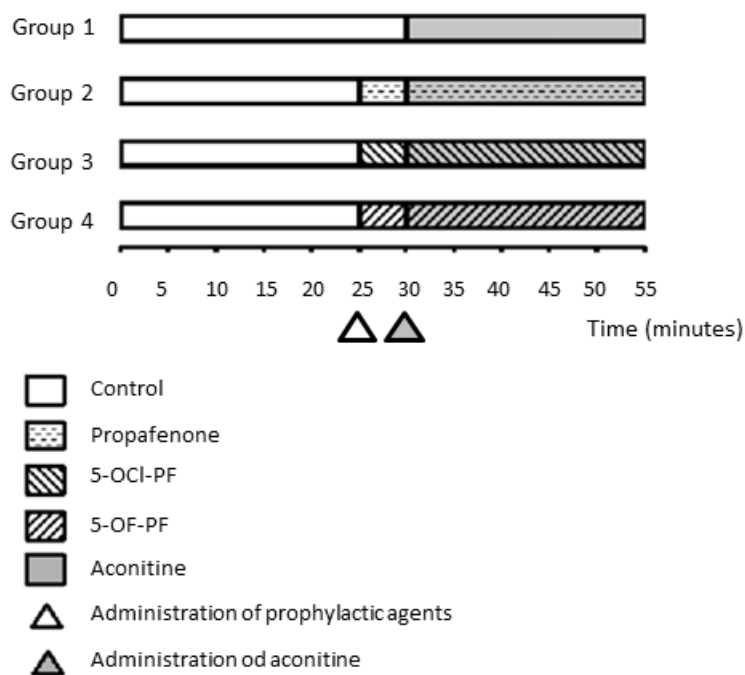


Figure 2. Schematic presentation of the experimental protocol. Each experimental group is represented by a rectangle. White parts of the rectangles represent drug-free periods (control). The moment of administration of prophylactic agents is marked by the white triangle (25 minutes of experiment). The parts of rectangles with lines represent periods with prophylactic agents. The moment of administration of aconitine is marked by the grey triangle (30 minutes of experiment) and grey parts of rectangles represent periods in which aconitine exerts its effect.

Slika 2. Šematski prikaz eksperimentalnog protokola. Svaka eksperimentalna grupa je predstavljena pravougaonikom. Beli delovi pravougaonika predstavljaju periode bez leka (kontrole). Trenutak primene profilaktičkog agensa je obeležen belim trouglom (25. minut eksperimenta). Delovi pravougaonika sa linijama predstavljaju periode sa profilaktičkim agansom. Trenutak primene akonitina je obeležen sivim trouglom (30. minut eksperimenta), a sivi delovi pravougaonika predstavljaju periode u kojima akonitin ispoljava svoj efekat.

Monitored parameters

The heart rate and ECG were recorded continuously on a Hellige-Simpliscriptor ECG machine. Group 1 was monitored 30 min before and 25 min after the administration of aconitine. In experimental groups, 2–4 anti-arrhythmic drugs were

applied as prophylactic agents 25 min after the beginning of the experiment. Five minutes later, aconitine was administered. All experimental groups were monitored for 25 min after aconitine application (Figure 2).

Changes induced by aconitine administration were evaluated for their potential influence on heart rate and rhythm disturbances. Attention was paid to the onset time for the given types of arrhythmias and time of death. Moreover, the percentage occurrence of the given disorder in each group was assessed discretely. Animals that survived more than 25 min were sacrificed by *i.v.* administration of urethane according to a procedure defined by the University Ethical committee of the Faculty of Medicine, University of Belgrade, Republic of Serbia.

The following arrhythmia types (Figure 3) were monitored:

- Atrial arrhythmias: supraventricular premature beats (SVPB) and supraventricular tachycardia (SVT),
- Ventricular arrhythmias: discrete ventricular premature beats or ventricular premature beats in salvos (VPBS), ventricular premature beats with a firm bond to the basic rhythm, *i.e.* bigeminies (VPBB) and paroxysmal ventricular tachycardia (PVT).

Continued measurement of blood pressure was performed as well. Only animals with normal blood pressure levels (100 ± 30 mmHg) during whole experiments were subjected to the statistical analysis.

Statistical Analysis

Statistical calculations of the changes in the heart rate and of the onset time of the arrhythmia types between tested groups were carried out with the Student t-test and Rank Sum test. Statistical significance of the frequency of occurrence of the appropriate arrhythmia types was assessed with the Fisher test. All statistical tests were carried out in SPSS 15.0 for Windows Evaluation Version (IBM, USA). Software GraphPad Prism/Instant 1.1 (*GraphPadSoftware, California, USA*) and Microsoft Excel were used for the graphic presentation of results.

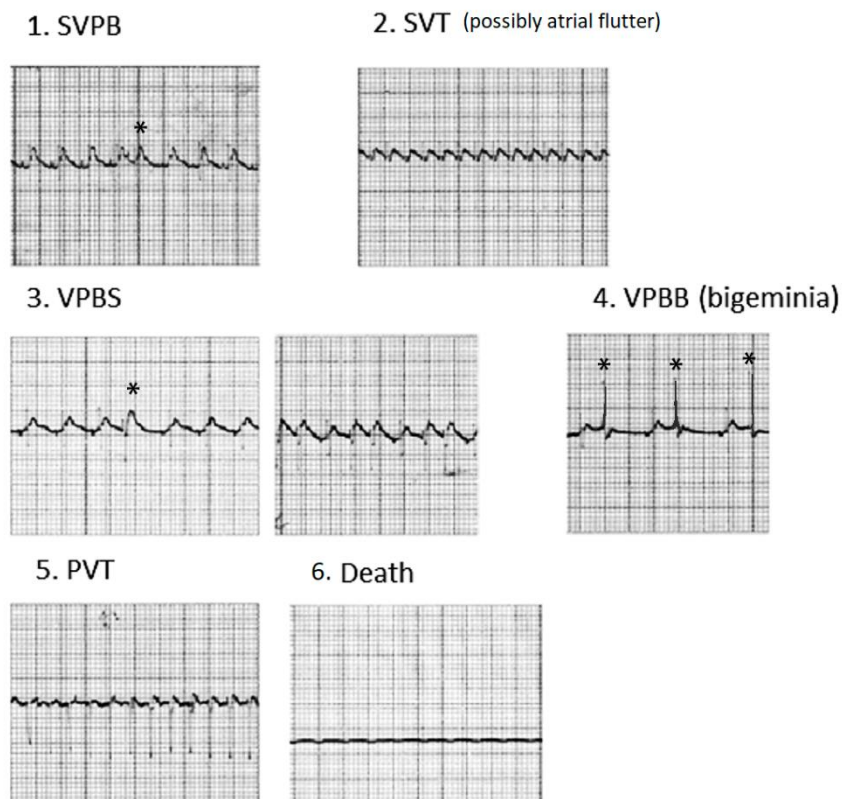


Figure 3. Original records of experimental arrhythmias induced by aconitine. The following types of arrhythmia are shown: 1. SVPB = supraventricular premature beats, 2. SVT = supraventricular tachycardia (it is possible that this differential ECG record is atrial flutter), 3. VPBS = discrete ventricular premature beats or ventricular premature beats in salvos, 4. VPBB = ventricular premature beats with a firm bond to the basic rhythm, i.e. bigeminia 5. PVT = paroxysmal ventricular tachycardia.

Slika 3. Originalni zapisi eksperimentalnih aritmija indukovanih akonitinom. Sledeći tipovi aritmija su prikazani: 1. SVBP = supraventrikularni preuranjeni otkucaji, 2. SVT = supraventrikularna tahikardija (moguće je da je ovaj diferencijalno dijagnostički EKG zapis atrijalni flater), 3. VPBS = diskretni ventrikularni preuranjeni otkucaji ili ventrikularni preuranjeni otkucaji u salvama, 4. VPBB = ventrikularni preuranjeni otkucaji sa čvrstom vezom sa osnovnim ritmom, bigeminia, 5. PVT = paroksizmalna ventrikularna tahikardija.

Results

Evaluation of blood pressure and heart rate during aconitine free period

The mean blood pressure in propafenone, 5OCL-PF, and 5OF-PF groups did not change significantly during the drug-free period and the period between tested compound administration until aconitine administration (100.7 ± 7 mmHg vs. 122 ± 7 mmHg, 103.8 ± 6 mmHg vs. 117.2 ± 6 mmHg and 90.8 ± 7 mmHg vs. 97.6 ± 9 mmHg, respectively, $p > 0.05$, all). The data have not been shown in this paper.

The effect of propafenone and its derivatives on heart rate is presented in Figure 4. Propafenone and 5OF-PF led to the inhibition of heart rate (290.8 ± 15 beats/min vs. 245.1 ± 11 beats/min, $p < 0.05$; 318.3 ± 14 beats/min vs. 254.3 ± 14 beats/min, $p < 0.01$, respectively). 5OCL-PF did not change the heart rate after 5 min of administration significantly (299.8 ± 22 beats/min vs. 274.3 ± 19 beats/min, $p > 0.05$).

Aconitine-induced arrhythmia

The mean heart rate before the administration of aconitine was 316.12 ± 12 beats/min and it was significantly lower than the heart rate after aconitine administration ($p < 0.01$, all). The initial peak in heart rate was developed in the first 10 min (max 545 beats/min in the 8th min). After that, the heart rate started to drop and became more stable from the 15th to 25th min after aconitine administration (in that interval heart rate was around 450 beats/min, see Figure 4).

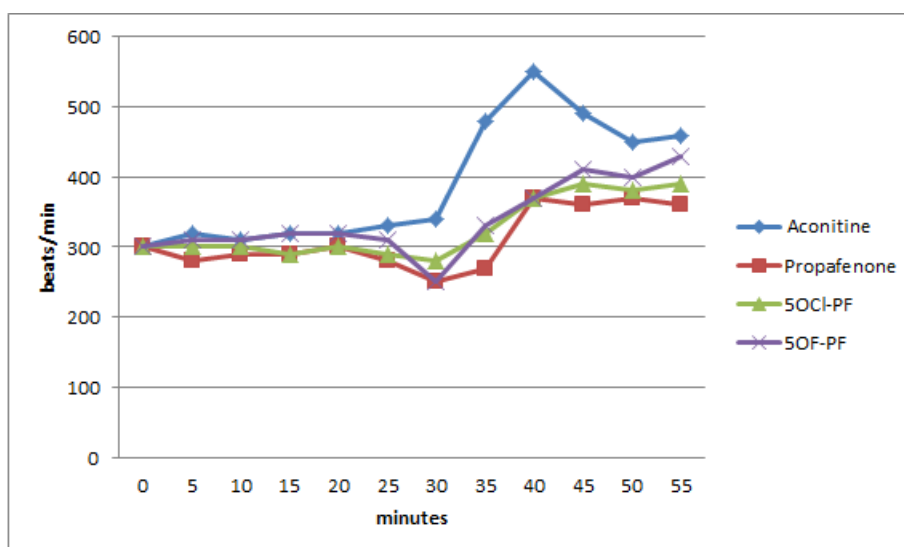


Figure 4. Analysis of the effect of propafenone and its derivatives at the heart rate after aconitine application. There are four curves which represent the heart rate during experiments (from start to the end of experiments) in four different experimental groups. Administration of prophylactic agents is marked by the white triangle in the 25th min, and administration of aconitine is marked by the grey triangle in the 30th min.

Slika 4. Analiza efekta propafenona i njegovih derivata na srčani ritam nakon primene akonitina. Prikazane su četiri krive koje prikazuju otkucaje srca tokom eksperimenta (od početka do kraja eksperimenta) u četiri različite eksperimentalne grupe. Primena profilaktičnih agenasa je obeležena belim trouglom u 25. minutu, a primena akonitina sivim trouglom u 30. minutu.

The effects of aconitine on cardiac rhythm are presented in Table I. After the administration of aconitine, there was an increase in the excitability of atria and ventriculans. An increase in atrial excitability was reflected in the appearance of SVPB and SVT in the majority of rats (83.3% both). As expected, experimental animals developed SVPB before SVT (0.7 min and 0.8 min).

Ventricular excitability was also increased and manifested as tachycardia and ventricular extrasystoles. VPBS were most commonly multiplex, discrete, or in salvos, and their occurrence was high (91.7%). VPBB was detected in 66.7% of rats with an average onset time of 1.1 min. PVT appeared in all animals with an average onset time of about 2 min.

In the control (aconitine) group, 41.7% (5/12) of animals died as a result of aconitine intoxication. The average survival time was 19.3 min. Interestingly, 7 animals survived even after 25 min, and they were sacrificed (see Methods).

Antiarrhythmic effects of propafenone

In experimental group 2, propafenone was administered 5 min before aconitine. During all 25 min after aconitine administration, propafenone managed to decrease the heart rate in comparison to the heart rate in the control group (Figure 4). The statistical difference between heart rates in these two experimental groups was high ($p < 0.01$) for all tested 5-min intervals after aconitine administration.

Propafenone reduced the occurrence of SVPB (83.3% in group 1 vs. 44.4% in propafenone group, $p < 0.05$) and SVT (83.3% vs. 33.3%, $p < 0.05$). The onset time of SVT was significantly postponed (0.8 min vs. 2.6 min, $p < 0.05$) (Table I).

Propafenone delayed the onset of VPBS (1.1 min in group 1 vs. 1.9 min in the propafenone group, $p < 0.05$) and VPBB (1.1 min vs. 2.3 min, $p < 0.05$). All animals treated with propafenone developed VPBB, while in the control group the occurrence of VPBB was lower. Propafenone delayed the onset of PVT (2 min in group 1 vs. 4.5 min in the propafenone group, $p < 0.05$). All these data are summarized in Table I.

Table I Time onset (min) of the given types of arrhythmias and the percentage (%) occurrence of the given disorder in each group

Tabela I Vreme početka (min) zadanih vrsta aritmija i procenat (%) pojave datog poremećaja u svakoj grupi

Type of arrhythmia	Control group (n = 12)		Administration of propafenone (n = 9)		Administration of 50Cl-PF (n = 9)		Administration of 50F-PF (n = 9)	
	(min)	n (%)	(min)	n (%)	(min)	n (%)	(min)	n (%)
SVPB	0.73	10 (83.33)	0.86	4 (44.44) ^a	0.16 ^{a, b}	6 (66.67)	0.63	9 (100) ^b
SVT	0.79	10 (83.33)	2.63 ^a	3 (33.33) ^a	0.65 ^b	5 (55.55)	1.68 ^{ab}	4 (44.44)
VPBS	1.10	11 (91.67)	1.90 ^a	7 (77.78)	0.86 ^b	9 (100)	1.83 ^a	8 (88.89)
VPBB	1.06	8 (66.67)	2.28 ^a	9 (100)	0.96 ^b	9 (100)	1.19 ^b	7 (77.78)
PVT	1.97	12 (100)	4.54 ^a	7 (77.78)	2.26 ^b	9 (100)	3.92 ^a	9 (100)
Death	19.31	5 (41.67)	15.67	4 (44.44)	13.5 ^a	3 (33.33)	16.5	3 (33.33)

SVPB = supraventricular premature beats, SVT = supraventricular tachycardia, VPBS = discrete ventricular premature beats or ventricular premature beats in salvos, VPBB = ventricular premature beats with a firm bond to the basic rhythm, *i.e.* bigeminies PVT = paroxysmal ventricular tachycardia

a - Statistically significant difference vs. control group (p<0.01)

b - Statistically significant difference vs. propafenone group (p<0.01)

SVPB = supraventrikularni preuranjeni otkucaji, SVT = supraventrikularna tahikardija, VPBS = diskretni ventrikularni preuranjeni otkucaji ili ventrikularni preuranjeni otkucaji u salvama, VPBB = ventrikularni preuranjeni otkucaji sa čvrstom vezom sa osnovnim ritmom, bigeminia, PVT = paroksizmalna ventrikularna tahikardija

a – statistički značajna razlika u odnosu na kontrolnu grupu (p<0.01)

b - statistički značajna razlika u odnosu na grupu koja je primala propafenon (p<0.01)

Antiarrhythmic effects of 5OCl-PF

During all 25 minutes of experiment duration after aconitine administration, 5OCl-PF managed to decrease the heart rate in comparison to the heart rate in the control group (Figure 4). The statistical difference between the heart rate in these two experimental groups was high from the 1st min to the 15th min after aconitine administration and in the last 5 min of the experiments. The heart rate was lowest in the 1st min (288 beats/min) after aconitine administration and varied from 316 beats/min in the first 5 min to around 400 beats/min in the period from the 10th min after aconitine administration till the end of the experiment.

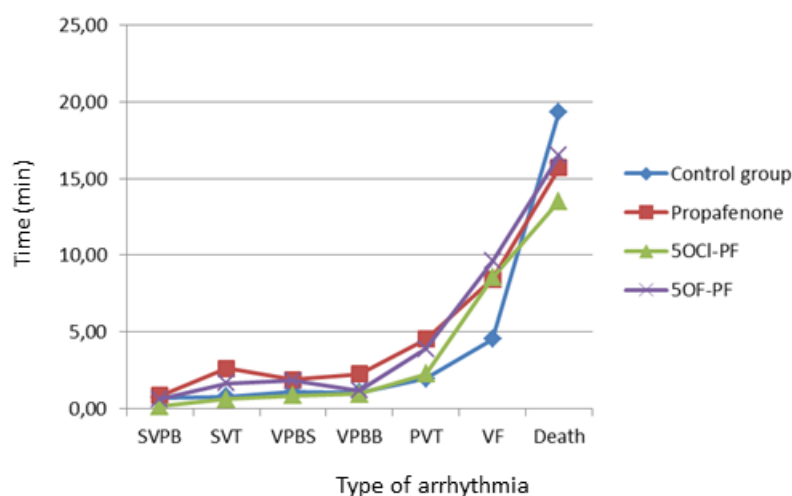


Figure 5. Graphical presentation of arrhythmia onset. (SVPB = supraventricular premature beats, SVT = supraventricular tachycardia, VPBS = discrete ventricular premature beats or ventricular premature beats in salvos, VPBB = ventricular premature beats with a firm bond to the basic rhythm, i.e. bigeminia, PVT = paroxysmal ventricular tachycardia)

Slika 5. Grafički prikaz početka aritmije. (SVBP = supraventrikularni preuranjeni otkucaji, SVT = supraventrikularna tahikardija, VPBS = diskretni ventrikularni preuranjeni otkucaji ili ventrikularni preuranjeni otkucaji u salvama, VPBB = ventrikularni preuranjeni otkucaji sa čvrstom vezom sa osnovnim ritmom, bigeminia, PVT = paroksizmalna ventrikularna tahikardija)

In comparison with group 2, there was no statistical difference in the heart rate from the 1st to the 20th min after the administration of aconitine ($p > 0.05$). However, in the last 5 min of experiments, propafenone decreased the heart rate more than 5OCl-PF ($p < 0.01$, Figure 4).

The onset of SVPB (0.16 min) was found earlier than in group 1 (0.7 min) and group 2 (0.9 min, $p < 0.05$ both). Similarly, 5OCl-PF accelerated the onset of SVT (0.65 min) in relation to group 2 (2.63 min, $p < 0.05$) (Table I).

All animals in group 3 developed VPBS, VPBB, and PVT, and there were no statistically significant differences in the occurrence of these types of arrhythmia in comparison with groups 1 and 2. These types of arrhythmias developed earlier than in group 2 ($p < 0.05$ all).

After the administration of 5OCl-PF, only 3 animals died, but deaths emerged earlier than with aconitine, and there was no statistically significant difference in the rate and onset time of death in comparison with group 2 ($p > 0.05$) (Table I, Figure 5).

Frequency of supraventricular arrhythmias and adverse haemodynamic effects have not been observed during these experiments. None of the animals suffered atrial fibrillation.

Antiarrhythmic effects of 5OF-PF

In the experimental group 4, 5OF-PF was administered 5 min before aconitine. The heart rate varied from 270 beats/min in the 1st min (average value in the first 5 min after aconitine administration was 326 beats/min) to around 400 beats/min in the 20th min. In the last 5 min of the experiments, the heart rate continued to increase and the mean value in this period was 430 beats/min. 5OF-PF decreased the heart rate in the first 15 min after aconitine administration in comparison with group 1. In the last 10 min, there was no statistical difference in the values of heart rate in these two experimental groups ($p > 0.05$). There was no statistically significant difference in the effect of propafenone and 5OF-PF on the heart rate after aconitine administration till the 10th min and from the 15th min to the 20th min ($p > 0.05$). Propafenone decreased the heart rate from the 11th to the 15th min and from the 21st to the 25th min after aconitine administration more than 5OF-PF.

5OF-PF had the opposite effect to propafenone on SVPB. It has shown the potential to develop SVPB in all animals, after only 0.6 min ($p < 0.01$). 5OF-PF postponed the onset time of SVT compared to the aconitine group (0.8 min vs. 1.7 min, $p < 0.05$), but the rats treated with this agent developed SVT significantly earlier than rats in the propafenone group (2.63 min vs. 1.68 min $p < 0.05$, Table I, Figure 5).

5OF-PF had a comparable effect on the development of VPBS as propafenone. It delayed the onset time (1.1 min in group 1 vs. 1.8 min in 5OF-PF group, $p < 0.05$) of VPBS (Table I). VPBB was developed similarly to group 1. Interestingly, animals were able to return to sinus rhythm after the development of VPBB, and later developed VPBS. All tested animals developed PVT after 5OF-PF administration. As well as propafenone, 5OF-PF was able to significantly delay the onset time of PVT (2 min in group 1 vs. 3.9 min in group 4, $p < 0.05$). Only 33.3% of animals (3) died in this group.

As stated in the previous chapter for 5OCl-PF, frequency of supraventricular arrhythmias and adverse haemodynamic effects were not observed during these experiments. None of the animals suffered atrial fibrillation.

Discussion

Aconitine has been widely used as a tool for the induction of cardiac arrhythmias in various animals (21, 22). It is a pentacyclic diterpenic alkaloid derived from the *Aconitum napellus* plant and has a great binding affinity to the so-called “neurotoxin receptor binding site 2” of the voltage-sensitive Na^+ channels in the cell membranes of various excitable tissues, including the myocardium (23-25). Modification of the Na^+ channel by an aconitine is generally considered to be irreversible. Aconitine causes a permanent activation of the Na^+ channels at the steady-state potential by blocking the inactivation of Na^+ channels (26). The result is a permanent depolarization of membranes and an increase in automaticity (27). In cardiac tissue, it can lead to an increase in intracellular concentration of Na^+ ions. The direct consequence of this is the subsequent secondary increase in the intracellular concentration of Ca^{2+} ions via the $\text{Na}^+/\text{Ca}^{2+}$ exchange mechanism. This Ca^{2+} overload may induce the triggered activity (23). In our study, after the administration of aconitine, there was an increase in excitability of atrials and ventriculums. The first type of arrhythmia which appeared was SVPB, then in order: VPBS, VPBB, SVT and PVT. Life-threatening cardiac arrhythmias were presented in all tested animals. Here, aconitine has a positive chronotropic effect, which is in line with observations of Bartosova et al. (2007) (28). According to Sampson and Kass (2011), there are three major underlying mechanisms of cardiac arrhythmia: enhanced automaticity (SVPB, SVT, VPBS), triggered automaticity (PVT) and re-entry. It seems that all of them were involved in aconitine-induced arrhythmias in our experimental model (29).

Anti-arrhythmic drugs of the 1st class (lidocaine, flecainide, procainamide and propafenone) can inhibit the effect of the aconitine. Propafenone is a Na^+ channel blocker with a relatively slow time constant for recovery from block. Some data also suggest that propafenone blocks K^+ channels. Moreover, it has β adrenergic receptor blocking properties. Propafenone blocks ryanodine receptor type 2 channels, like flecainide. Its major electrophysiological effect is to slow conduction in fast-response tissues.

Previously, the electrophysiological effects of propafenone have been described in atrial and ventricular muscle fibers and Purkinje fibers (30-39). In this preparation, it was described that propafenone decreases the amplitude of the action potential. Also, propafenone exerted some adrenoceptor mediated sympatholytic actions, and in high concentrations, propafenone is a Ca-antagonist on the cat ventricular muscle fibers (32, 35, 40). These mechanisms suggest negative inotropic and chronotropic effects, especially if the dose exceeds that which was predicted. Propafenone, as a highly potent blocker of fast Na_v channels ($\text{Na}_{1.5}$) shows all the features of antiarrhythmics of the Ic group. In clinical practice, it is used in the prophylaxis and treatment of ventricular

tachycardias (VT), including persistent ventricular tachycardia (PVT), supraventricular tachycardia (SVT), including Wolff-Parkinson-White (WPW) syndrome, as well as in the conversion of atrial fibrillation, but only in the absence of structural damage.

In our study, heart rate decreased after the administration of propafenone. Contrary to this, Riou et al. did not demonstrate significant negative chronotropic effects in the myocardium of rats treated with propafenone (41). Furthermore, propafenone inhibited tachycardia induced by aconitine. It is very well known that a major effect of propafenone is to slow conduction in fast-response tissues (30).

The antiarrhythmic effect of propafenone in different animal models has been well described previously. Propafenone is highly effective in SVT, including atrial fibrillation and post-infarction ventricular arrhythmias in experimental models during acute myocardial infarction in conscious dogs, and it may be effective in lidocaine-resistant arrhythmia during acute ischemia (42, 43). In our study, we used propafenone only to compare the effects of the newly synthesized derivatives of propafenone to the antiarrhythmic effects of the initial compound. Here, propafenone had favorable effects on the excitability and automaticity of the atrium. It reduced the occurrence of SVPB and SVT and delayed the onset of SVT. It is well known that all members of class 1C of anti-arrhythmic drugs significantly slow conduction velocity of the atrial tissue. Moreover, propafenone inhibited ventricular excitability. It delayed the onset of VPBS, VPBB and PVT. A higher occurrence of VPBB in the propafenone group than in the aconitine group indicates its potential to produce ventricular arrhythmias.

5OCl-PF is a derivative of propafenone with -Cl substituent on the *ortho* position of the benzyl moiety. As shown in Results and Figure 4, heart rate decreased after the administration of 5OCl-PF, but its effect was lower than the propafenone effect. In contrast to propafenone, it is possible that 5OCl-PF did not interact with the β_1 receptor and/or L-type Ca^{2+} channels in the heart. However, 5OCl-PF inhibited tachycardia induced by aconitine in the same manner as propafenone, indicating its selectivity for fast-response tissues. This is typical for blockers of Na^+ channels (29).

The antiarrhythmic effect of 5OCl-PF was not satisfactory. It strongly speeded the appearance of SVPB. In contrast to propafenone, 5OCl-PF did not slow down the occurrence of any type of ventricular arrhythmias. All animals treated with 5OCl-PF developed VPBS, VPBB and PVT, indicating that 5OCl-PF potentiates excitability, automaticity and triggered automaticity of ventricles. It is obvious that 5OCl-PF produces arrhythmia. 5OCl-PF accelerated the occurrence of death more than aconitine.

5OF-PF is a derivative of propafenone with -F substituent on the *ortho* position of the benzyl moiety. 5OF-PF inhibited aconitine-induced tachycardia. It also decreased the heart rate, but its effect was shorter than the effect of propafenone (Figure 4). Maybe, 5OF-PF binds to the voltage-gated Na^+ channels in the activated state and dissociates faster from its receptor site than propafenone. However, this needs further investigation.

5OF-PF had the opposite effect to propafenone on SVPB and potentiated atrial excitability. In contrast, it had a favorable effect on the occurrence and onset time of SVT. The same as propafenone, 5OF-PF delayed onset time of VPBS and PVT. Accordingly, it seems that 5OF-PF decreased atrial and ventricular automaticity and triggered automaticity. This is in line with its inhibitory effect on heart rate.

In order to confirm the preventive effect of 5OF-PF derivative of propafenone on ventricular arrhythmia (considering possible side effects), additional experiments are planned to be reported in the future.

Conclusions

Based on the results presented in this paper, it can be concluded that newly synthesized propafenone derivatives (5OCl-PF and 5OF-PF) did not show better antiarrhythmic effects compared to propafenone. Based on the obtained results, new research would be directed towards the synthesis of propafenone derivatives with different changes in structure.

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Antiaritmički efekti novosintetisanih derivata propafenona

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Kratak sadržaj

Dobro je poznato da prisustvo različitih hemijskih grupa u molekulima leka utiče na njegova farmakološka svojstva. Cilj našeg istraživanja je ispitati da li novosintetisani derivati propafenona, s promenama u benzilnoj grupi, imaju drugačiji efekat na aritmiju u odnosu na propafenon. 5OCl-PF i 5OF-PF su derivati propafenona sa -Cl ili -F supstituentom na orto položaju benzilnog dela. Za proveru njihovog antiaritmičnog efekta koristili smo *in vivo* model na pacovima sa aritmijom izazvanom akonitinom. 5OCl-PF je ubrzao pojavu supraventrikularnih prevremenih otkucaja (SVPB) i smrt više nego akonitin. Sve životinje lečene sa 5OCl-PF razvile su ventrikularne prevremene otkucaje (VPBS i VPBB) i paroksizmalnu ventrikularnu tahikardiju (PVT). 5OF-PF je imao negativan hronotropni efekat i potencirao atrijalnu ekscitabilnost (više SVPB). Pozitivno je uticao na pojavu i vreme početka supraventrikularne tahikardije, VPBS i PVT. Na osnovu dobijenih rezultata se može zaključiti da novosintetisani derivati propafenona nemaju bolji antiaritmijski efekat od polaznog jedinjenja. U budućnosti, istraživanje će biti usmereno ka sintezi hemijski drugačijih derivata i ispitivanju njihovog antiaritmijskog efekta.

Ključne reči: derivati propafenona, eksperimentalna aritmija, pacovi, akonitin
