PhD Thesis – SUMMARY

Bisphenol A – effects on hemodynamic parameters, atrial electrical remodeling and atrial arrhythmogenicity

PhD student **Vasile-Bogdan Halațiu**

PhD coordinator **Prof. Dr. Dan Dobreanu**

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INTRODUCTION

Bisphenol A (BPA) is nowadays one of the most used synthetic chemical agents in the production of plastics and epoxy resins. This compound is present in a wide range of consumer products such as: plastic food containers, plastic bottles, baby bottles, cans, media products, plastic pipes, medical products and devices, etc. The presence of BPA in all these consumer products makes human exposure to this compound significant and continuous. In the United States, the presence of BPA has been detected in the urine of more than 90% of the general population. Exposure to this compound has been implicated in the occurrence of various pathologies, with epidemiological studies suggesting a possible link between exposure to BPA and a number of cardiovascular pathologies such as coronary heart disease and hypertension. The effect of BPA on the cardiovascular system is attributed to its effect on β-type estrogen receptors, BPA being an agonist for these receptors. In experimental studies, *in vitro*, acute exposure and often at supraphysiological doses, BPA has been involved in modulating Ca^{2+} ion dynamics. In addition, direct interactions with multiple ion channels and an important profibrotic effect were observed. Mechanistically, all these interactions may explain the ventricular proarrhythmic effect observed in previous studies.

Considering that these mechanisms are not only the basis of ventricular arrhythmias, but also of atrial fibrillation (AF), it is expected that BPA has important supraventricular proarrhythmic effects. However, the effect of BPA on atrial arrhythmogenicity has not been evaluated to date. Thus, the present research aims to evaluate for the first time, *in vitro* and *in vivo*, the effects of chronic exposure to concentrations relevant to human exposure, but also supraphysiological, of BPA on hemodynamic parameters, atrial electrical remodeling and atrial arrhythmogenicity in an experimental model of AF in the rat.

METHODOLOGY

The effects of BPA were evaluated in an experimental study carried out in the Laboratory of Physiology and Cardiovascular Pathophysiology within the Physiology Department of the "George Emil Palade" University of Medicine, Pharmacy, Sciences and Technology in Târgu Mureș. Due to the gender specificity of the response to BPA, the study was conducted on female rats, respecting the international norms in force (International Council for Laboratory Animal Science Guidelines - 2010/63/EU), with the prior approval of the University's Research Ethics Committee (no. 28/01.02.2018).

All animals were individually housed in a controlled environment with a 12/12 h light/dark cycle with free access to water and food throughout the study. The study included 22 adult female Wistar rats, randomized into 3 groups as follows: Control group (n = 7), BPA-exposed group at the clinically relevant dose for human exposure (n = 7) and hBPA-exposed group at toxic doses of BPA (n = 8). Bisphenol A was administered daily to rats in the BPA and hBPA groups in drinking water for 9 weeks prior to the start of the experimental protocols, while animals in the Control group received water without added BPA.

After 7 weeks of BPA administration the animals were implanted with the radiotelemetry ECG device. After one week of postoperative recovery, systolic blood pressure (SBP) was measured and a continuous/24-h ECG recording was performed in conscious, unrestrained rats, and heart rate and basal spontaneous atrial load were determined. Subsequently, all animals underwent a unique transesophageal electrical stimulation protocol described previously to assess the effect of BPA exposure on AF inducibility. At the end of the pacing protocol, a new continuous/24-h ECG recording was performed in all animals to determine atrial arrhythmic load post-scheduled electrical stimulation. Subsequently, the animals were euthanized and the left atrium was sampled from each animal for the electrophysiological study.

RESULTS

Study no. 1: Effects of bisphenol A on body mass and atrial fibrillation inducibility

The aim of this study was to evaluate the effects of chronic exposure to clinically relevant and toxic doses of BPA on body mass and AF inducibility in an animal model of AF by programmed transesophageal electrical stimulation in adult female rats.

The mean body mass of the animals at the end of the study was 276.00 ± 18.79 g. After 9 weeks of exposure to BPA, animals in the Control group (292 g [288-300 g]) had a significantly higher body mass than those in the BPA (275 g [264–282 g], p = 0.04) and hBPA (264 g [260–283 g], p = 0.02) groups. Analyzing the difference in body mass during the study, a significantly greater weight gain was observed after 9 weeks of the study in animals in the Control group (22 g [17-27 g]) compared to animals in the BPA groups (12 g [5- 21 g], p = 0.02) and hBPA (10 g [7-12 g], p = 0.001). No significant difference was found in the number of animals in which at least one episode of AF was induced following the application of the

programmed transesophageal electrical stimulation protocol between groups (all p values >0.05), the percentage of animals in which induced AF ranged from 60.00% of the total number of animals in the Control group to 83.88% of the total number of animals in the hBPA group. The number of episodes of induced AF per animal ranged from 0 to 12. Animals in the Control group had an average of 3.40 ± 1.99 episodes of AF during the programmed transesophageal electrical stimulation protocol, those in the BPA group 5.50 ± 1.77 episodes, respectively those in the hBPA group 1.57 ± 0.57 AF episodes. No statistical difference was observed between the mean number of induced AF episodes between the three groups (all p values >0.05.). Analyzing the total number of stimulation cycles applied, in the animals of the Control group 31.90% ± 13.59% of the stimulation cycles were followed by episodes of AF, in the animals of the BPA group 57.62% ± 17.56%, and in the hBPA group 10.48% ± 3.81%. No significant differences were observed between the Control group and BPA or hBPA (both p values >0.05), but in the BPA group it was observed that a significantly higher number of pacing cycles were followed by episodes of AF compared with the hBPA group (p = 0.04). No animal in the hBPA group experienced sustained AF, defined as an episode of AF lasting more than 10 min, during the programmed transesophageal electrical stimulation protocol. In the Control group two animals, and in the BPA group three animals presented episodes of AF lasting more than 10 minutes, for these animals, the stimulation protocol was stopped after recording the sustained arrhythmic episode (p = 0.28).

Study no. 2: Effects of bisphenol A on hemodynamic parameters and on spontaneous and post-scheduled transesophageal electrical stimulation arrhythmic load in rats

The aim of this study was to evaluate the effects of chronic exposure to BPA on hemodynamic parameters (systolic blood pressure and heart rate) and on spontaneous and post-scheduled transesophageal electrical stimulation arrhythmic load in rats.

No statistically significant differences were observed between the three groups in terms of BP value after 7 weeks of BPA or drinking water administration (105 mmHg [97.5 – 117.5 mmHg]; 110 mmHg [100 – 127.5 mmHg]; 110 [110 – 120 mmHg]; p = 0.47). The mean/24-hour heart rate obtained from the 24-hour ECG recording, performed before the application of the programmed transesophageal electrical stimulation protocol, varied between 328 and 430 beats/min, with no statistically significant differences observed between the three groups (326.6 bpm [348.4 – 406.5 bpm]; 364.1 bpm [354.9 – 375.6 bpm]; 352.4 bpm [347.1 – 364.6 bpm /min]; p = 0.48). In the following, the results obtained from the ECG/24-hour recordings obtained immediately after the application of the esophageal stimulation protocol will be presented. The results also refer to supraventricular arrhythmias: the number of ESAs, respectively the number and duration of AF episodes. At ECG recording/24 hours after programmed transesophageal electrical stimulation, a statistically significant increase in the number of ESAs/24 hours was observed in the hBPA group compared to the BPA and Control groups (6.66 [3.78 – 15.38]; 7 .96 [3.29 – 12.54]; 20.02 [13.68 – 38.69]; p = 0.02). A statistically significant difference was also observed between the number of spontaneous AF episodes/24 hours after applying the electrical stimulation protocol in the 3 groups (1.11 [0.00 – 1.85]; 1.10 [0.54 – 2.02]; 4.97 [3.84 – 15.55]; p = 0.003). After programmed electrical stimulation, statistically significant differences were observed in the duration of spontaneous AF episodes assessed on ECG recordings/24 hours between the Control, BPA and hBPA groups (1.60 [0.84 – 2.96]; 5, 75 [2.37 – 14.04]; 27.53 [14.34 – 85.06]; p = 0.001). Analyzing the high-frequency components of HRV, a statistically significant difference was observed between the Control, BPA and hBPA groups (5.37 msec2 [4.68 – 5.71 msec2]; 8.78 msec2 [7.81 – 9.68 msec2]; 9.22 msec2 [7.60 – 11.68 msec2]; p = 0.003). Also, when analyzing the ratio between the low-frequency and high-frequency components, a statistically significant difference was observed between the Control, BPA and hBPA groups (0.36 [0.27 – 3.37]; 0.34 [0 .24 – 0.37]; 0.23 [0.17 – 0.29]; p = 0.01).

Study no. 3: Evaluation of the effect of chronic bisphenol A exposure on atrial electrical activity *in vitro*

The aim of this study was to evaluate the effects of chronic exposure to BPA on electrical activity in vitro, in the absence and after exposure to proarrhythmogenic stimuli: sympathetic stimulation, parasympathetic stimulation and Ca^{2+} overload.

No statistically significant differences were observed in depolarization velocity, DPA 50%, and DPA 90% among the three groups in the absence of proarrhythmogenic stimuli or upon Ca^{2+} overload (all p values > 0.05). In response to sympathetic stimulation, a statistically significant difference was observed between the three groups regarding DPA 50 (3.00 msec [-1.90 – 7.65 msec]; 0.95 msec [-2.75 – 3.00 msec];

8.42 msec [4.16 – 14.66 msec]; p = 0.02). Also, in response to sympathetic stimulation with adrenaline solution, a statistically significant difference was observed between the Control, BPA and hBPA groups in terms of DPA 90 (13.30 msec [1.15 – 20.7 msec]; -1.20 msec [-9.80 – 3.30 msec]; 19.96 msec [12.46 – 28.72 msec]; p = 0.004). Assessment of the response of cellular electrical activity in the presence of parasympathetic stimulation showed a statistically significant difference between the Control, BPA and hBPA groups with respect to the rate of depolarization (5.67 mV/sec [-11.40 – 8.00 mV/sec]; -6.26 mV/sec [-19.77 – -3.40 mV/sec]; 13.15 mV/sec [9.81 – 19.96 mV/sec]; p = 0.004). No statistically significant difference was observed between the three groups regarding the parameter DPA 50 in response to parasympathetic stimulation (-5.30 msec [-10.05 – -3.40 msec]; -5.00 msec [-8 .25 – -2.55 msec]; -9.90 msec [-14.72 – -6.82 msec]; p = 0.16). In response to parasympathetic stimulation with acetylcholine solution, no statistically significant difference was observed between the Control, BPA and hBPA groups in terms of the DPA 90 parameter (-16.60 msec [-21.95 – -12.35 msec]; -14.70 msec [-26.95 – -7.75 msec]; -29.74 msec [-35.74 – -13.12 msec]; p = 0.22).

CONCLUSIONS:

In conclusion, the present study is the first to investigate and demonstrate a supraventricular proarrhythmic effect of chronic exposure to BPA, an effect more pronounced in the case of supraphysiological doses. The exact mechanism by which BPA exerts these effects at the atrial level, i.e. what is the minimum dose of BPA at which proarrhythmic effects are likely to occur remains to be seen. The present research thus opens new directions of research towards the elucidation of the cardiotoxic effect of BPA.