UNIVERSITY OF MEDICINE AND PHARMACY OF TÂRGU MUREŞ

DOCTORAL SCHOOL

Summary of the thesis

BETA ADRENERGIC BLOCKERS FREE RADICALS TRAPS

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BETA ADRENERGIC BLOCKERS FREE RADICALS TRAPS

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Abstract

Beta blockers, or beta adrenergic blocking agents, are useful in the treatment of hypertension, especially high blood pressure associated with hearth failure, for patients with cardiac arrhythmias, angina and also in glaucoma therapy. Many of these pathologies were explained by imbalance between excess production of free radicals and the process of neutralizing them by natural or synthetic antioxidants. This brings into question the oxidative stress and lipid peroxidation particularly involved in the development of cardiovascular diseases. Nowadays, the extremely wide chemical and biological role of free radicals is very intensively studied. It is known that several compounds, oppose oxidative challenges by virtue of their trap very rapidly oxygen or carbon centered radicals and generating other radical species which are stable and biochemically less harmful than the original radicals. If some beta blockers molecules would capture free radicals, this would be an additional benefit for chronic patients taking these substances frequently. Several bibliographic data confirm the existence of some interactions between certain molecules from beta blockers class and oxygen and nitrogen- centered radicals.

The general part includes aspects concerning the current state of knowledge regarding beta blockers, free radicals chemistry and different methods of analysis, including molecular modeling techniques. The experimental part contains personal studies, to explain the mechanism of capture free radicals and identify structural elements involved in the reaction with various molecules.

To understand the relationship between chemical structure and physico-chemical, biological and pharmacological behavior of this class of drugs we used ***molecular modeling***. Based on soft and hard notions is important to stress that main difference between the two analogue classes of compounds is the metabolic pathway, hard compounds are metabolized oxidatively and the soft analogues turns hydrolytic. We turn our attention to the conditions and oxidative mechanism factors and during the analysis is clear that the transformation of hard to soft structure is frequently accompanied by increased steric energy, so emphasized a lower steric energy stress the hard character, increased reactivity of the compound with oxidants, which means a possible efficient free radical trap. We compared the total steric energy and other thermodynamic related terms for 16 beta blockers analogues.

The method of choice for reaction that involved free radicals is ***electron spin resonance (ESR).*** We carried out a qualitative and quantitative characterization of free radical generation and from interpretation of spectra we have analyzed the interaction with beta blockers molecules. Oxygen-centered radicals we have generated using the Fenton reaction, especially $OH^{\bullet}$ free radicals, extremely reactive and with a very short life time.

Comparatively we used a specific spin trap molecule, phenyl –N-tert-butyl nitrone, PBN, because the spin adduct formed has a characteristic ESR spectrum that represents a standard for the oxygen free radicals spin trapping process. First the reaction conditions was established, using ethanol solutions only, and a peroxide for the reaction with iron solution ($FeSO_4$ 100mM) TBH tert-butyl hydroperoxide, the control sample contains 170μl phosphate buffer, 2μl $FeSO_4$ 100 mM, 10 μl PBN 1M, 20μl t-BuOOH 77.7 M, this is the optimal composition for a good ESR spectrum. We added further to the control sample different beta blockers solutions such metoprolol , carvedilol in ethanol 1M and 100mM in different proportions, and the obtained ESR spectra were analyzed and interpreted. The magnetic field intensity was changed frequently in the first place in order to indentify the optimal frequency and the measurements were carried out in the flat cell and capillary, also. After adding the beta blockers solution we observed a certain modification of the ESR spectra, by calculating the double integrate parameters and demonstrated that the concentration of oxygen free radicals in the system is lower in the presence of beta blockers, of course in different proportions. We studied the interaction of beta blockers with a nitrogen-centered radical, diphenylpicrylhydrazyl (DPPH) because this radical is also a standard of spin trapping process.

The electron spin resonance studies were completed by ***electrochemical*** analysis, as well. Based on the analogy of unpaired electron- containing free radical and cathodic attack we can better understand the free radicals trap mechanism. For example the decrease of catalytic wave height can be explained by interaction between the $OH^{\bullet}$ free radicals and the trap, in case of a sufficiently high concentration of spin trap when the catalytic current density approaches to a calculable limit value. The half-wave potentials characterize the electron transfer (electron capture) energy, so the ESR data and the polarographic parameters are completing each other reciprocally.

Due to their DPPH free radical solution characterized by a purple color with a strong absorbtion maximum at 517nm, we can applied for our study a ***spectrophotometric*** assay too. The color turns from purple to yellow when the odd electron of DPPH radical becomes paired with a hydrogen from a free radical scavenging antioxidant. In this case we have tasted the scavenger ability of some beta blockers: metoprolol, atenolol, carvedilol and pindolol. The most reactive beta blocker with the DPPH free radical was pindolol, followed by metoprolol, atenolol and slightly carvedilol.

We can conclude that accordingly to the literature data some beta blockers interact with oxygen and nitrogen-centered radicals. Our purpose was also to identify the chemical structures involved in the spin trapping process and to elucidate the basic possible mechanism of interaction.

Key words: beta blocker, free radical, electron spin resonance, chemical structure