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REVIEW ARTICLE

Three Generations of β-blockers: History, Class Differences and Clinical Applicability

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Abstract: ***Background*:** Beta-adrenergic receptors are expressed in cardiomyocytes and activated by either noradrenaline released from sympathetic synapses or circulating catecholamines. Their corresponding receptors have three subtypes, namely, β_1, β_2 and β_3, which are members of the G protein-coupled receptors (GPCRs) family. Activation of β_1-adrenergic receptors causes various physiological reactions including cardiac contraction and renin secretion from juxtaglomerular cells of the kidney. Antagonists of β-adrenergic receptors, known as β-blockers, have been used effectively for over four decades and have beneficial effects in the treatment of cardiovascular diseases. There are three generations of β-blockers according to their pharmacological properties. First-generation β-blockers are non-selective, blocking both β_1- and β_2-receptors; second-generation β-blockers are more cardioselective in that they are more selective for β_1-receptors; and third-generation β-blockers are highly selective drugs for β_1-receptors. The latter also display vasodilator actions by blocking α_1-adrenoreceptors and activating β_3-adrenergic receptors. In addition, third-generation β-blockers exhibit angiogenic, antioxidant, anti-proliferative, anti-hypertrophic and anti-apoptotic activities among other effects that are still under investigation.

***Conclusion*:** The objective of this review is to describe the evolution observed during the development of the three distinctive generations, thereby highlighting the advantages of third-generation β-blockers over the other two drug classes.

Keywords: β-blockers, antagonists, β-adrenergic receptors, cardiovascular diseases, clinical applicability, G protein–coupled receptors (GPCRs).

1. INTRODUCTION

Adrenergic receptors comprise a class of G protein-coupled receptors targeted by catecholamines, in particular noradrenaline and adrenaline. In 1906, Dale [1] was the first to introduce the concept of a receptor in association with the sympathetic nervous system (SNS). In 1948, Alquist divided adrenergic receptors into α (excitatory) and β (inhibitory) according to their functional effects of vasoconstriction and vasodilatation, respectively [2]. Two decades later, Richardson *et al*. showed that activation of β-receptors in the heart muscle mediated positive chronotropic and inotropic effects [3]. In the same year, Lands *et al.* subdivided β-adrenergic receptors into β_1 (cardiac effects) and β_2 (bronchodilator and vasodilator effects) [4]. Later on, Yarden *et al.* demonstrated that β-adrenergic receptors consisted of seven transmembrane domains, and Dixon *et al*. described that those were G-protein-coupled receptors [5, 6]. In 1989, Emorine *et al*. observed the existence of a third isoform of β-adrenergic receptors (β_3), which also belongs to the family of G-protein-coupled receptors [7].

As antagonists to these receptors, β-blockers comprise an essential class of cardiovascular drugs designed to reduce morbidity and mortality in patients with cardiovascular diseases. These drugs decrease the number of deaths, strokes, and heart attacks associated with hypertension. β-blockers reduce sympathetic nervous system activity through blockade of β-adrenergic receptor subtypes. The specificity of β-blockers is directly related to the greater affinity the drug has for β_1- over β_2-receptors at usual therapeutic levels. There have been several β-blockers developed with distinct pharmacological and hemodynamic properties, which may be divided into three distinct generations according to differences in those pharmacological properties. First-generation β-blockers were non-selective, blocking both β_1 and β_2-receptors; second-generation β-blockers are more cardioselective in that they show higher affinity for β_1-receptors; and

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third-generation β-blockers present varied selectivity for β_1-receptors. The latter also display vasodilator actions by blocking α_1-adrenoreceptors and activating β_3-adrenergic receptors.

In this review, we discuss the development of the three distinct generations of β-blockers and highlight the advantages of the third-generation drugs over the previous two β-blocker classes. For the selection of articles, a MEDLINE-based search was conducted using the following keywords: "β-blockers", "β-adrenergic receptor", "β_1-adrenergic receptor antagonist", "first generation", "second generation", "third generation", "evolution", "extra β_1-effect", "cardiovascular diseases" and "selectivity". The list of articles was subsequently narrowed down to those containing abstracts and to articles published in English. Information analysis started with the title, followed by the abstract and then the complete report.

2. β-ADRENERGIC SIGNALING

β-adrenergic receptors are activated by the catecholamines noradrenaline and adrenaline, and they are members of the seven-transmembrane superfamily of receptors. There are three β-adrenergic receptors subtypes, namely β_1, β_2, and β_3. β-adrenergic receptors are implicated in several physiological functions, particularly in the cardiovascular and pulmonary systems. Table **1** summarizes the responses produced by the stimulation of β-adrenergic receptors from distinctive tissues. Their influence on the cardiovascular system is exerted both directly through an increase of the cardiac contractions and indirectly by means of renin secretion by the juxtaglomerular cells of the kidney.

Activation of β-adrenergic receptors occurs primarily by noradrenaline released by sympathetic nerve terminals, which form a network around the cardiomyocytes. A secondary mode of activation is through circulating catecholamines [8, 9]. Cardiomyocytes express all three isoforms of β-adrenergic receptors: β_1, β_2 and β_3. While β_1-adrenergic receptors are coupled to a stimulatory G protein (Gs), β_2-receptors are coupled to both a stimulatory (Gs) and an inhibitory G-protein (Gi), with predominant activation of the stimulatory one (Gs) [9, 10]. Finally, β_3-adrenergic receptors are Gi-protein-coupled and additional intracellular signaling includes activation of nitric oxide synthases (NOS), activation of guanylate cyclase (GC) and formation of cGMP [9, 11].

In cardiomyocytes, there is the prevalence of β_1-adrenergic receptors with a molar ratio of 4:1 in comparison to the β_2 form. Cardiac expression of β_3-adrenergic receptors is low under physiological conditions but it has been shown to increase in the cardiac muscle of some patients with heart disease [12, 13]. Activation of β_1-adrenergic receptors in cardiomyocytes leads to changes in its conformation, which in turn promotes activation of Gs proteins by exchanging guanosine triphosphate (GTP) for the diphosphate form (GDP) and dissociation of the G protein into an activated Gα subunit and the allosteric Gβγ complex [13, 14]. Activation of adenylyl cyclase (AC) leads to cAMP formation, followed by activation of protein kinase A (PKA), which in turn is responsible for the phosphorylation of L-type calcium channels [9, 15] and sarcoplasmic reticulum calcium-release channels [9, 16]. These responses increase the intracellular calcium concentration thereby promoting contraction. In addition, PKA can phosphorylate myofilaments, such as troponin I, reducing its sensitivity to calcium [9, 17]. These responses are directly linked to cardiac chronotropic and inotropic effects. Conversely, phosphorylation of phospholamban-type calcium channels is responsible for reuptake of cytosolic calcium by the sarcoplasmic reticulum, causing relaxation of the cardiac muscle [13, 18]. Under conditions of chronic activation of β-adrenergic receptors, three types of intracellular enzymes may be activated as a compensatory mechanism: *i*) receptor G protein-coupled kinases (GRK), which are responsible for the phosphorylation of β_1-adrenergic receptors with subsequent desensitization; *ii*) phosphodiesterases (PDEs), which are capable of hydrolyzing cAMP; and *iii*) general phosphatases [19].

It is important to mention that renal juxtaglomerular cells are in contact with sympathetic nerve varicosities expressing post-junctional β_1-adrenergic receptors. Their activation induces renin release, increasing the activity of the renin-angiotensin system (RAS), which is involved in the pathophysiology of cardiovascular diseases [20].

3. ANTAGONISTS OF β-ADRENERGIC RECEPTORS

β-adrenergic antagonists, also called β-blockers, are molecules that compete with catecholamines for the binding

Table 1. Responses stimulated by β-adrenergic receptors in distinctive tissues.

Tissue/Receptor	β_1 [21-23]	β_2 [24-26]	β_3 [27]
G Protein Activation	G_s	G_i and G_s	G_i
Adipocytes	-	-	Lipolysis and thermogenesis
Heart	Positive inotropism and chronotropism	Positive inotropism and chronotropism	Negative inotropism
Ileo and colon	-	-	Relaxation
Lung	-	Relaxation	Relaxation
Peripheral sympathetic nerves	Release of norepinephrine	Release of norepinephrine	-
Kidney justaglomerullar cells	Secretion of renin	-	-
Vascular	Relaxation	Relaxation	Relaxation

Table 2. First published article about each β-blocker.

β-Blocker	First Published Article
Propranolol	Black, *et al*. A new adrenergic betareceptor antagonist. Lancet. 1964.
Practolol	Dunlop D & Shanks RG. Selective blockade of adrenoceptive beta receptors in the heart. Br J Pharmacol Chemother. 1968.
Atenolol	Barrett AM, *et al*. A new type of cardioselective adrenoceptive blocking drug. Br J Pharmacol. 1973.
Metoprolol	Ablad B, Carlsson E, Ek L. Pharmacological studies of two new cardioselective adrenergic beta-receptor antagonists. Life Sci I. 1973.
Labetalol	Kennedy I & Levy GP. Combined alpha- and beta-adrenoceptor blocking drug AH 5158: further studies on alpha adrenoceptor blockade in anaesthtized animals. Br J Pharmacol. 1975.
Carvedilol	Bartsch W, *et al*. Pharmakologie und klinische Pharmakologie des neuen vasodilatierenden B-Rezeptoren-Blockers BM 14.190. Therapiewoche. 1982.
Nebivolol	Van de Water A, *et al*. Pharmacological and hemodynamic profile of nebivolol, a chemically novel, potent, and selective beta 1-adrenergic antagonist. J Cardiovasc Pharmacol. 1988.

site on β-adrenergic receptors. James Black was the pioneer in the development of this class of drugs. There are currently more than twenty antagonists commercially available for clinical use. The action of β-blockers in the cardiovascular system includes negative inotropic and bradycardic effects, which translate into a lower cardiac output. In addition, antagonism of β_1-receptors from the juxtaglomerular cells can reduce the activity of the renin-angiotensin system, resulting in decreased blood pressure [21-28]. Despite the common mechanism to all members of this class of drugs, there are several differences in their specific activities.

The most important pharmacodynamic difference among β-blockers is their selectivity for adrenergic receptors and their subtypes. There are also three generations of β-blockers. Representatives of the first generation are non-selective antagonists of receptors of type β_1 and β_2. Representatives of the second generation have selectivity for β_1 receptors compared to β_2, also called cardioselectivity, but this feature is dose-dependent. Representatives of the third generation are known as vasodilators as a direct result of their effects on the cardiovascular system in addition to blocking β_1 receptors. For example, third-generation β-blockers both block α_1-adrenoreceptors and activate β_3-receptors with further increase of NOS activity and NO generation. An understanding of the differences among the generations of β-blockers is critical to the correct utilization of these drugs [28].

4. FIRST GENERATION OF β-BLOCKERS

Based on isoprenaline structure, in 1958 Powel and Slater introduced the first β-receptor antagonist named dichrloroisoprenaline, but successive researchers have demonstrated that this compound effectively antagonized myocardial rate and tension, but also presented sympathomimetic activity [29]. At this time, Black and Stephenson (1962) tried several compounds, with small structural changes (Fig. **1**), that could antagonize β effects, without agonistics actions. Then, pronethalol was published as the first completely antagonist of β-adrenergic receptors without any sympathomimetic activity on the cardiovascular system [29]. On the other hand, pronethalol demonstrated a variety of side effects as lightheadedness and slight incoordination followed by nausea and vomiting that could be associated with non-specific action of pronethalol on the central nervous system. Black *et al*., started to look for a large number of compounds with better therapeutic effects and no aggressive toxicity until found the propranolol [30]. This compound presents one chiral center, constituting a racemic mixture of R- and S-enantiomers, which the R-stereoisomer (R- configuration at the hydroxyl) has no pharmacological effect while its S- isoform contains all the pharmacological properties of propranolol [31].

In this sense, propranolol, the first β-blocker used in the clinic, was developed by James Black in 1964, who demonstrated the antagonistic effect of this drug in reducing isoprenaline-induced increases in heart strength and heart rate [30]. Propranolol has a high lipophilicity and can cross the blood-brain barrier. When administered orally, it shows good absorption but suffers first-pass metabolism, with only 25% of the drug reaching systemic circulation. It has a large distribution volume (about 4L/kg) and 90% binding to plasma proteins. Propranolol clearance varies according to hepatic blood flow and it is consequently dependent on hepatic physiology such as the presence of pathologies of the liver and/or concomitant administration of other drugs that also affect the hepatic biotransformation of this drug. In addition, propranolol also shows a comparatively short half-life (3-6 hours) [28]. The main cardiovascular effect of propranolol is reduction of the systolic and diastolic blood pressures associated with decreased cardiac output and reduced activity of the renin-angiotensin system [28].

Based on its clinical applications, Hansson and Zweifer observed that administrations of either four or two daily doses of propranolol (160-320 mg) were able to reduce blood pressure in hypertensive patients to normal levels [32]. They also observed a decrease in diastolic blood pressure with decreased plasma renin activity after four weeks of treatment. However, the antihypertensive response was not observed after administration of a single dose of propranolol. MacLeod *et al.* developed a study involving 63 hypertensive patients with mean systolic blood pressure of 173 ± 5 mmHg and diastolic blood pressure of 110 ± 3 mmHg at the start of the treatment. Patients were initially subjected to a regimen of four daily administrations of propranolol (40-320 mg) for twelve weeks and were then switched to a twelve-week period wherein they were given the same total daily amount but through only two administrations. Both twelve-week treat-

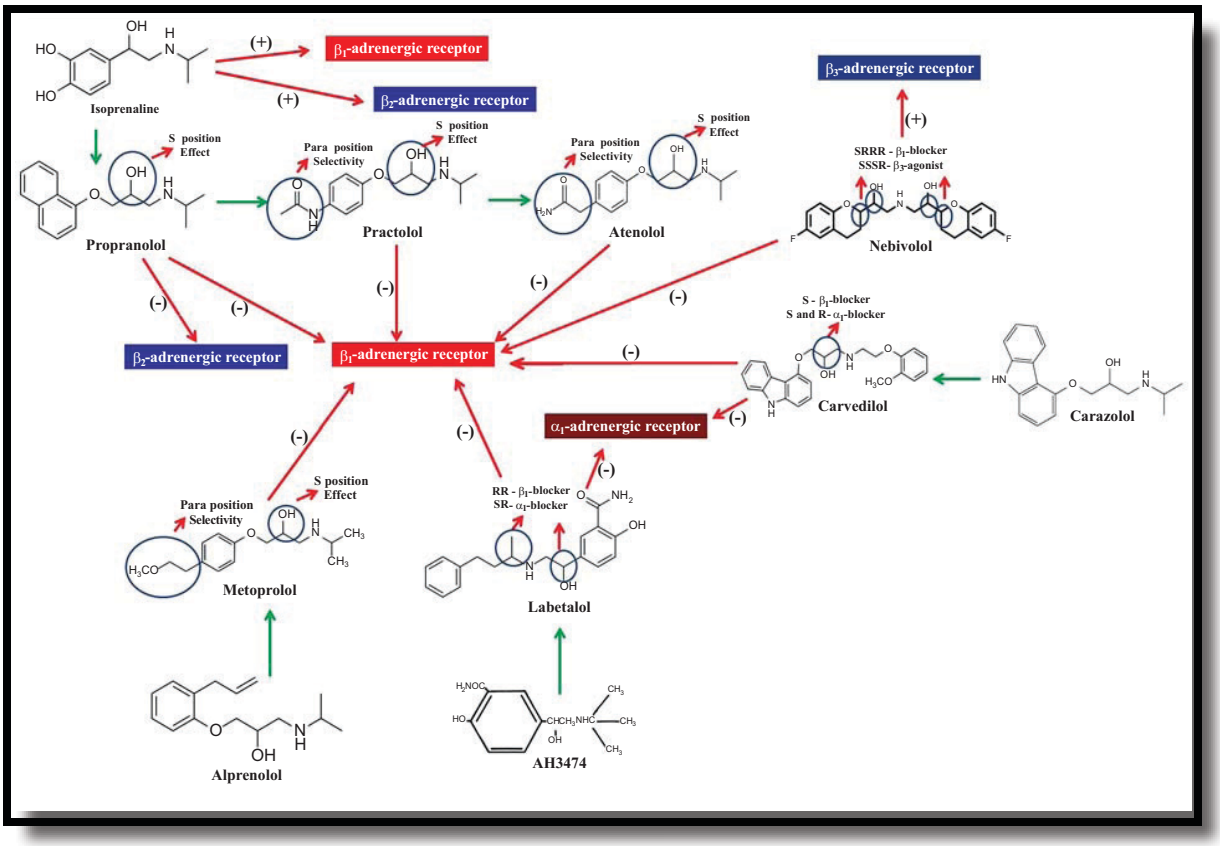

Fig. (1). Schematic summary of the chemical development of β-blockers. The development of each β-blocker was based in a primary compound (green arrow). According to the evolution of each antagonist some fundamental characteristics can be observed as the radical groups in para- position and R or S conformation of hydroxyl from chiral carbon (blue circle). Based on each chemical structures the β-antagonists presented its respective pharmacological actions (red arrow). (*The color version of the figure is available in the electronic copy of the article*).

ments with propranolol led to reduced diastolic and systolic blood pressures, therefore indicating the antihypertensive effect of this drug [33].

Propranolol is also effective in infarcted patients, whether with congestive heart failure or not. Chadda *et al.* accompanied patients who had been treated with propranolol (180-240 mg) or a placebo for 25 months and observed that treatment with propranolol was able to reduce the mortality rates of those patients with or without congestive heart failure compared to patients treated with placebo. The reduction in mortality was associated with decreasing cardiovascular events, such as reduction of sudden death, myocardial infarction recurrence and decrease in the number of coronary events. The beneficial effect of propranolol in infarcted patients, with or without congestive heart failure, is associated with its ability to decrease both contractile strength and heart rate, resulting in an anti-ischemic effect [34].

The administration of propranolol in individuals with angina may also have beneficial effects. Pine *et al.* observed that increasing doses of β-blockers (40-320 mg) improved patient performance during physical evaluations as measured by increased oxygen volume (VO_2) emitted during the test. Furthermore, decreased heart rates and systolic blood pressures were also observed during physical evaluation when compared to patients who had not been treated with the drug. Nevertheless, patients subjected to propranolol treatment showed an increase in peripheral vascular resistance, which may be associated with the antagonistic activity on β_2 receptors in the peripheral vasculature [35].

In addition to increased peripheral vascular resistance, the non-selective β_1-adrenergic antagonism of propranolol can cause serious adverse effects, which are associated with β_2-receptor antagonism, such as bronchospasm in patients with asthma or chronic obstructive pulmonary disease. Moreover, propranolol may increase peripheral vascular resistance due to β_2 antagonism on peripheral vasculature [28].

To address this adverse effect, Boskabady and Snashall evaluated the respiratory function in symptomatic and asymptomatic patients with asthma and healthy volunteers after the administration of isolated isoprenaline (0.65–22 nmol) and isoprenaline preceded by propranolol (4–20 μg/kg intravenously). They observed that healthy volunteers, without pulmonary pathologies, showed no significant changes in respiratory function after treatment with propranolol followed by isoprenaline. In contrast, patients with symptomatic or asymptomatic asthma showed a significant reduction of respiratory function after administration of propranolol. This difference in responses between symptomatic and healthy volunteers may be associated with the fact that asthmatics have a bronchodilator protective effect related to circulating adrenaline, which is lost following low doses of β_2-receptors antagonists [36].

Another important aspect to consider is that adrenaline exerts an essential role in hypoglycemia recovery, leading to symptoms such as tremor and tachycardia, which may be masked by β-blockers [37]. Finger tremor, for example, is associated with peripheral β-receptors activation [38], and propranolol was described to block such response during hypoglycemia [39]. The increase in tremor during treatment with β_1-selective antagonist was similar to that for the placebo administration, which suggests that tremor is only partly mediated by β_2-receptors [39]. Thus, by masking the effects of hypoglycemia, propranolol may represent a risk for diabetics under insulin treatment and its use may be limited for this class of patients.

In summary, first-generation β-blockers can lower blood pressure through decreasing contractile strength of the heart and its rate, and consequently, it can reduce cardiac output. These mechanisms of action allow for their use in patients suffering from hypertension, angina and post-myocardial infarction. However, the use of propranolol is not indicated for diabetics or patients with specific lung pathologies, such as asthma or chronic obstructive pulmonary disease.

5. SECOND GENERATION OF β-BLOCKERS

Based on the non-selective β-blockers structures, Dunlop and Shanks studied several compounds until practolol (4-(2-hydroxy-3 isopropylaminopropoxy) acetanilide) has been found in 1968 [40]. At this moment, the authors observed that some compounds as N-isopropylmethoxamine and dimethyl isopropylmethoxamine blocked β-receptor in canine peripheral blood vessels, suggesting that this response could be associated to compounds which presented a metal attached to the alpha carbon. Different from these molecules, practolol presents a benzylacetamide in para- position, characterizing the first β-blocker that displayed selectivity for β_1-adrenergic receptors (Fig. **1**) [40]. As propranolol, practolol presents one chiral center, constituting a racemic mixture of R- and S-enantiomers, which the R-stereoisomer (R- configuration at the hydroxyl) has no pharmacological effect while its S- isoform contains all β_1-adrenergic receptor antagonist effects [31].

In this sense, Dunlop and Shanks showed that when practolol was infused for 30 minutes (1-100 μg/kg/min) it was able to antagonize the effect of isoprenaline (0.2 μg/kg/min) and decrease heart rate, and at 0.5 mg/kg it counteracted the increase in contraction frequency and contraction strength of the cardiac muscle caused by the agonist. Practolol was also shown to antagonize the effects of propranolol (1-25 μg/kg/min) and pronethalol (4-100 μg/kg/min), both representatives of first-generation β-blockers. However, practolol did not antagonize isoprenaline-induced hypotension, suggesting a cardioselectivity for β_1-adrenergic receptors. To test this hypothesis, isoprenaline (0.1 mg/kg) was administered in conjunction with either propranolol (0.1-0.4 mg/kg) or practolol (1-4 mg/kg), followed by the administration of histamine, which causes bronchoconstriction. The animals that received treatment with propranolol followed by histamine died, demonstrating that propranolol antagonizes the bronchodilator effect of isoprenaline, thereby favoring the bronchoconstricting response of histamine. Conversely, animals that received treatment with practolol survived because there was no β_2-adrenergic receptor antagonism, that is, the bronchodilating effect of isoprenaline was counteracted by the bronchoconstricting effect of histamine. These experiments helped to establish the idea of a selectivity for β_1-adrenergic receptors by practolol [40].

Atenolol (4-(2'-hydroxy-3'-isopropylaminopropoxy) phenylacetamide), another β-blocker of second generation, was developed in 1973 by Barret *et al*. based on practolol characteristics. The drug has only one structural difference, the exchange of benzylacetamide for a phenylacetamide, but still in para- position (Fig. **1**) [41]. As practolol, atenolol presents one chiral center constituting a racemic mixture of R- and S-enantiomers, which the R-stereoisomer (R- configuration at the hydroxyl) has no pharmacological effect while its S- isoform contains all β_1-adrenergic receptor antagonist effects [31].

In this sense, the same selectivity for β_1-adrenoreceptors over β_2 analogs was observed for atenolol, which demonstrated similar antagonistic effect as practolol to antagonize isoprenaline-induced increase in heart rate *in vivo*. In addition, when its ability to antagonize the vasodilator effect of isoprenaline was measured, atenolol was shown to be less potent than propranolol. Studies *in vitro* showed that propranolol and atenolol both displayed comparable potencies as antagonists of the chronotropic effects induced by isoprenaline, while atenolol was shown to be less potent than propranolol in antagonizing isoprenaline-induced tracheal relaxation. These findings demonstrate a cardioselectivity for members of the second generation of β-blockers in comparison to those from the first generation [41].

Regarding its pharmacokinetic properties, atenolol is a hydrophilic drug with an absorption rate around 50%. It has a half-life in the range of 5–8 hours and it is eliminated primarily by the kidneys without any biotransformation where one finds it in urine in its original form [28]. Nobre *et al*. evaluated its antihypertensive effect at 90 mg/kg using the two-kidney-one-clip (2K1C) hypertension model, and they compared the effects of this β-blocker with other antihypertensives, such as hydrochlorothiazide (at 20 mg/kg) and losartan (at 10 mg/kg). After fifteen days of treatment, a reduction in blood pressure was observed for all these drugs, but atenolol also showed decreased heart rate [42]. A treatment of atenolol (25-100 mg) was also administered to patients submitted to three weekly dialysis sessions, which produces an increase in blood pressure by increased sympathetic activation and activity of the renin-angiotensin system. In this scenario, atenolol was administered after each dialysis session for a period of twelve months with evaluations conducted every three months, and the drug was shown to lower systolic and diastolic blood pressures. This indicated that the antihypertensive effect of atenolol is related to its ability to reduce cardiac output and its activity on the renin angiotensin system [43].

In addition, atenolol also showed beneficial effects in individuals with angina. Tardif *et al.* evaluated the effect of this second-generation β-blocker at a dose of 50 mg for one month followed by treatment with the same dose of 100 mg for three months. Both doses of atenolol were able to reduce heart rate during physical evaluation and repose. Addition-

ally, treatment with atenolol at both doses reduced the number of weekly angina attacks and it was shown to increase the patient's physical resistance during exercise assessment as well, based on the increasing length of the exercise and time it took for the patient to develop an angina attack. The beneficial effects of atenolol are due to its ability to reduce both heart rate and contractile force, and consequently decrease the oxygen demands by the heart muscle [44].

Metoprolol, a third representative of second-generation β-blockers was presented in 1973 by Ablad *et al.* [45]. This β_1-adrenergic receptor antagonist was developed based on alprenolol structure (1-(2-allylphenoxy)-3-isopropylamino-propan-2-ol), a non-selective β-blocker not approached in this review. A series of meta- and para- analogues of alprenolol were studied and para- compounds demonstrated a higher affinity to β_1-adrenergic receptor than β_2 isoform. This finding led to the synthesis of a variety of para-substituted phenoxy-isopropylaminopropanols with featured for metoprolol therapeutic effects (Fig. **1**) [45]. Metoprolol is also composed by on chiral center constituting a racemic mixture of R- and S-enantiomers, which the R-stereoisomer (R- configuration at the hydroxyl) has no pharmacological effect while its S- isoform contains all β_1-adrenergic receptor antagonist effects [31].

In this sense, metoprolol also showed similar potency to propranolol in antagonizing increased frequency and cardiac contractile force induced by isoprenaline (0.1 μg/kg). Metoprolol also showed a comparatively lower potency than propranolol in antagonizing the vasodilating and bronchodilating effects of isoprenaline. The cardioselectivity of metoprolol for β_1-receptors was established after these experiments [45]. The main pharmacokinetic properties of metoprolol include lipophilicity, high absorption rate, extensive first-pass metabolism and an elimination half-life of 3 to 4 hours [28].

Ljung *et al*. studied the antihypertensive effects of metoprolol in spontaneously hypertensive rats (SHR). The animals showed a blood pressure reduction after oral treatment with metoprolol (0.7 mmol/kg) for five months. The same effect was observed after administration of metoprolol intravenously (15 μmol/kg) for four days and orally (0.7 mmol/kg) for thirteen days [46]. Sumbria *et al*. also observed an antihypertensive effect in hypertensive patients treated with metoprolol (25-200 mg). After six months of treatment, metoprolol decreased both systolic and diastolic blood pressures to normal levels with no change in left ventricular mass. These results showed an antihypertensive effect without anti-hypertrophic effect [47].

Cocco and Chu showed that metoprolol (50-200 mg) also has a beneficial effect on patients with angina. Metoprolol treatment for twelve months reduced the number of attacks per week when compared to the placebo group. Moreover, reduction of the heart rate and both systolic and diastolic blood pressures at rest and during physical evaluation were also observed, and so was an increase in total exercise length and in exercise duration prior to an angina attack [48].

Merit also studied the use of metoprolol in patients with congestive heart failure. His findings showed a lower mortality rate in patients treated with metoprolol when compared to those in the placebo group. Additionally, other parameters were observed including a lower risk of death associated with cardiovascular events, a decreased risk of sudden death and a lower risk of death associated with worsening congestive heart failure. These responses are directly linked to the ability of metropolol in reducing the energy demands of the heart muscle, which are accompanied by a decrease in ventricular remodeling and reduction of ventricular dysfunction aggravations [49].

These findings show that second-generation β-adrenergic antagonists have β_1-receptor selectivity. As such, they are involved in reducing cardiac contractile strength and rate, leading to a decrease in cardiac output, and they are involved in lowering the activation of the rennin-angiotensin system as well, which also cooperates to reduce blood pressure. Thus, representatives of second-generation β-blockers are a useful pharmacological choice in the treatment of hypertension, angina and congestive heart failure, with less risk of adverse effects associated with β_2-receptor antagonism.

6. THIRD GENERATION OF β-BLOCKERS

In 1972, Farmer *et al*., described for the first time the characteristics of labetalol (5- {1-hydroxy-2- [(1-methyl-3-phenylpropyl) amino] ethyl} salicylamide), a molecule that is chemically related to AH3474 (5-(2-t-butylamino-1-hydroxyethyl) salicylamide) which is a β-receptor antagonist with less potency than propranolol [50]. Labetalol has 2 chiral centers which results in four stereoisomers with RR- responsible for β_1-blocking effect, while SR- is responsible for α_1-blocking activity (Fig. **1**) [31].

In 1975, Kennedy and Levy demonstrated that labetalol, the first representative of the third-generation of β-blockers, antagonized isoprenaline-induced increase in contractile force and heart rate. Besides β-adrenergic antagonism, labetalol showed α_1-adrenergic antagonist effect. They also reported that labetalol (1mg/kg) shifted the diastolic pressure curve for phenylephrine and noradrenaline, which are potent α_1-adrenergic agonists. Furthermore, it was observed that labetalol (3 mg/kg) reduced the hypertensive effect of noradrenaline *in vivo* [51]. Regarding the pharmacokinetic characteristics, labetalol is a drug with high absorption, which undergoes an intensive first-pass metabolism and has an elimination half-life in the range of 3 to 8 hours [28].

Others third-generation β-blockers were developed a few years later. Carvedilol (Carbazolyl-(4)-oxy)-3-(2-methoxy-phenoxy ethyl)-amino)-propranol-(2)), a second representative of third generation β-blockers has structural similarity to carazolol, an antagonist with cardiotoxicity in higher doses. A 2-methoxy-phenyl-ethyl residue at the aliphatic nitrogen is responsible for carvedilol vasodilating properties. Carvedilol also presents one chiral center constituting a racemic mixture in which the S- stereoisomer presents β_1-adrenergic receptor antagonism, while R- and also S-stereoisomer blockade α_1 receptor (Fig. **1**) [31].

In this sense, in 1982, Bartsch *et al.* showed that carvedilol was a potent β-adrenergic antagonist that also blocked α_1-adrenergic receptors [52]. Carvedilol is a lipophilic drug

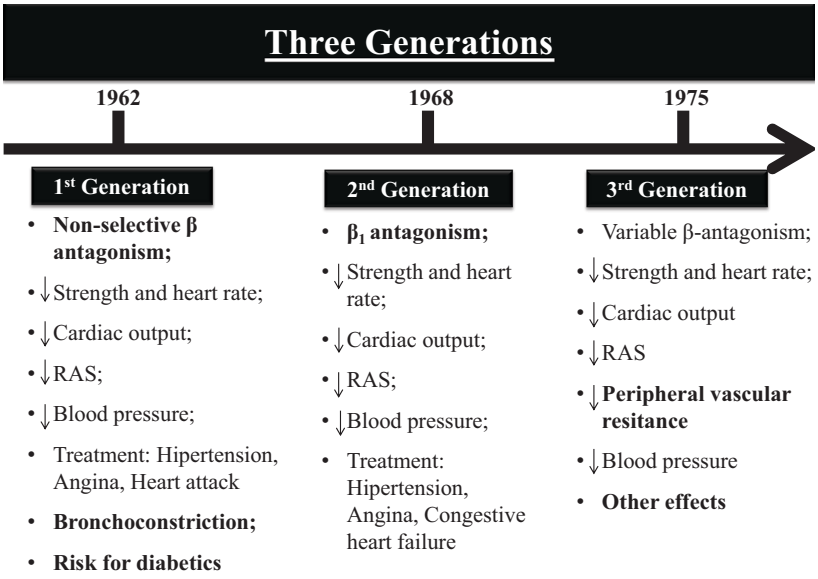

Fig. (2). Summary of the evolution of β-blockers and their basic differences. The therapeutic effects and disadvantages of first-generation β-blockers relative to the second and third generations are shown in bold. For second-generation β-blockers, therapeutic effects and advantages over representatives of the first generation are shown in bold. Finally, the therapeutic effects and activity on β_1-adrenergic receptor are shown in bold for the third-generation β-blockers as an advantage over the two previous generations.

with a high absorption rate which undergoes extensive first-pass biotransformation and has an elimination half-life in the range of 7-10 hours [28].

Eggertsen *et al.* treated hypertensive patients with third-generation antagonist at 25 mg and they observed a rapid reduction of both systolic and diastolic blood pressures in conjunction with a steep reduction in peripheral vascular resistance. The latter would turn out to be a peculiar characteristic of third-generation β-blockers, and it is associated with the representatives of this class to act as antagonists to α_1-adrenergic receptors [53].

Sabellek *et al.* treated hypertensive individuals with either two doses of carvedilol daily for twelve months or a single dose for six months and they noticed a reduction of both systolic and diastolic blood pressures to normal levels. More importantly, carvedilol reduced blood pressure within two hours of its administration. This response was maintained for 24 hours, a characteristic not observed for β-blockers of the first and second generations [54]. More recently, Chen *et al.* studied the effects of carvedilol in hypertensive rats. After eight weeks of treatment at 25 mg/kg, it was observed that carvedilol administration reduced blood pressure independently of NOS activation. These researchers also showed that carvedilol treatment produced anti-fibrotic and protective effects on the myocardial structure in these animals [55].

Kaski *et al.* reported the beneficial effects of carvedilol (25 mg) in patients with angina. Treatment with carvedilol for one week reduced the rate of angina attacks, accompanied by an increase in physical resistance during exercise evaluation. This beneficial effect of carvedilol is associated with its ability to decrease the cardiac contractile force and rate with a consequent reduction of the demands of the heart muscle for oxygen [56]. Kowalski *et al.* also showed that angina patients treated with carvedilol at 25 and 50 mg for four months had their antioxidant enzyme activities increased, such as superoxide dismutase, catalase and glutathione peroxidase [57]. This antioxidant effect showed by third-generation β-blockers is not observed in treatments with representatives of the second generation [58].

Zepeda *et al.* described that the antihypertensive effect of carvedilol (12.5mg for twelve weeks) was accompanied by an improvement in endothelial function, which was independent of increased plasma NO levels but rather associated with a reduction in oxidative stress, represented by decreased plasma levels of 8-isoprostane and erythrocyte malondialdehyde [59]. Le *et al.* also observed that carvedilol displayed antioxidant effects. In the latter study, the effect of carvedilol was compared to that of metoprolol in animals subjected to congestive heart failure. Carvedilol showed anti-hypertrophic, anti-fibrotic and pro-angiogenic effects. Both metoprolol and carvedilol reduced blood pressure, but metoprolol did not show the extra β -adrenergic effects displayed by third-generation β -blockers [60]. Jonsson *et al.* compared the effects of carvedilol and atenolol in patients with acute myocardial infarction. Although both drugs reduced systolic and diastolic blood pressures as well as heart rates to normal levels, carvedilol showed a stronger antioxidant action than atenolol, highlighting the clinical superiority of third-generation β-blockers [61].

Nebivolol is the latest third-generation β -blocker and it was introduced in 1988 by Van de Waters *et al*. [62]. This is the only antagonist which differs completely from the molecular structure of propranolol [31, 62]. Nebivolol (1-(6-fluorochroman-2-yl)-2-[2-(6-fluorochroman-2-yl)-2-hydroxyethylamino] ethanol), presents 4 chiral centers, while the others β -blockers contain only 1 or 2. Surprisingly, different from the majority of β-blockers, nebivolol presents β_1 antagonistic effect in SRRR-enantiomer, while SSSR- acts as a vasodilator (Fig. **1**) [31].

In low concentrations, nebivolol has been shown to antagonize the effects of increasing heart rate caused by isoprenaline although comparatively higher doses of nebivolol were needed to antagonize the relaxant effects of the tracheal smooth muscle, showing that nebivolol displayed a high selectivity for β_1-adrenergic receptors. In addition, nebivolol was able to decrease blood pressure in hypertensive rats at lower doses to those of propranolol and atenolol, representatives of the first and second generation of β-blockers, respectively. The antihypertensive response was associated with rapid lowering of peripheral vascular resistance, a peculiar characteristic of third-generation β-blockers. Regarding its pharmacokinetic properties, nebivolol is a well-absorbed drug and undergoes extensive first-pass biotransformation. Its elimination half-life is around twelve hours and occurs mainly through feces (44%) and urine (37%) [63]. Based on its pharmacological characteristics, several clinical trials have evaluated its antihypertensive effect with doses ranging from 5 to 40 mg per day. In these studies, reduction of both systolic and diastolic blood pressures was observed, consolidating its antihypertensive effect [64-66].

In 2014, Zang *et al.* showed beneficial effects of nebivolol in mice that were subjected to acute myocardial infarction. Treatment with nebivolol for four weeks reduced fibrous tissue, decreased the diameter of the left ventricle at end-systole and diastole, improved ejection fraction and cardiac shortening, and showed anti-apoptotic effect in cardiomyocytes. These protective effects of nebivolol were all associated with β_3-adrenergic receptors and they were accompanied by activation of NOS [67].

Ceron *et al.* compared the effects of nebivolol and metoprolol in hypertensive rats. Both β-adrenergic antagonists had antihypertensive effects but only nebivolol showed antihypertrophic effects in the aortic tissue, accompanied by systemic and vascular antioxidant effect. In addition, treatment with nebivolol reduced gelatinolytic activity and aortic levels of the matrix metalloproteinase-2 (MMP-2) as well as tissue hypertrophy. Neither of these effects was observed after treatment with metoprolol [68]. Rizzi *et al.* addressed the effects of nebivolol in the heart of hypertensive rats and showed, much like in the previous study, that nebivolol displayed antihypertensive, anti-hypertrophic and antioxidant effects, whilst reducing gelatinolytic activity and cardiac levels of MMP-2 [69].

Zepeda *et al.* demonstrated the beneficial effects of nebivolol in hypertensive patients subjected to administrations of 5 mg/day for twelve weeks in that it produced an improvement in endothelial function, represented by an increase in dilation of the brachial artery associated with increased plasma levels of NO [59]. This endothelial effect of nebivolol might be associated with increased tissue expression of endothelial NOS (eNOS), as observed by Zhou *et al.* [70]. However, it was subsequently shown that this effect in particular was due to the activation of β_3-adrenergic receptors [71].

The vasodilator effect of nebivolol, which is mediated by an increase in NO levels, is also associated with beneficial effects in patients with erectile dysfunction. Doumas *et al*. studied 29 hypertensive patients treated with metoprolol or atenolol for six months, after switched to nebivolol. Upon switch to the β-adrenergic antagonist, erectile function was observed to improve in twenty of these patients, with eleven individuals later reporting normalization of this function [72]. When treated with metoprolol for twelve weeks, Brixius *et al*. observed that hypertensive patients with a history of erectile dysfunction actually displayed decreased erectile function, based on the International Dysfunction Index Scale Function. Conversely, patients treated with nebivolol did not present alterations of erectile function [73].

Based on these findings, nebivolol is recommended for treatment of hypertension and heart attacks, with or without congestive heart failure. Furthermore, although other β-adrenergic antagonists are not usually considered first choice in the treatment of hypertension [74, 75], nebivolol has been shown to have a similar efficacy to calcium channels blockers, antagonists of AT_1 receptors and angiotensin converting enzyme (ACE) inhibitors in reducing both systolic and diastolic blood pressures in adults with mild to moderate hypertension [76-78].

In summary, third-generation β-blockers have beneficial effects in patients with cardiovascular diseases when compared to the representatives of previous two generations. Nebivolol and carvedilol are able to reduce peripheral vascular resistance expediently and thereby lower cardiac work, which is accompanied by a decrease in oxygen demands by the heart muscle. In addition, representatives of the third generation of β-blockers exhibit angiogenic, anti-hypertrophic, antioxidant, antifibrotic and anti-apoptotic effects, leading to lowering of the blood pressure, reduction of cardiac remodeling and decrease in endothelial and cardiac dysfunction.

CONCLUSION

Since the development of propranolol, the selectivity of β-blockers for β_1-adrenergic receptor has been increasing with concomitant improvement of their therapeutic safety, especially for diabetics and patients with pulmonary dysfunctions. More recently, with the development of third-generation β-blockers, antagonism of β_1-adrenergic receptors have been shown to be a part of the effects, which include reduction of peripheral vascular resistance due to α_1-adrenergic receptors, increase of eNOS activity, anti-hypertrophic and antioxidant properties. In this manner, the use of nebivolol and carvedilol, as examples of third-generation β-blockers, has improved the survival rate of patients suffering from hypertension, angina and congestive heart failure.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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REFERENCES

[1] Dale HH. On some physiological actions of ergot. J Physiol 1906; 34(3): 163-206.

[2] Alquist RP. A study of the adrenotropic receptors. Am J Physiol 1948; 153(3): 586-600.

[3] Richardson DW, Kontos HA, Raper AJ, Patterson JL. Modification by beta-adrenergic blockade of the circulatory responses to acute hypoxia in man. J Clin Invest 1967; 46(1): 77-87.

[4] Lands AM, Arnold A, McAuliff JP, Luduena FP, Brown TGJ. Differentiation of receptor systems activated by sympathomimetic amines. Nature 1967; 214(5088): 597-8.

[5] Yarden Y, Rodriguez H, Wong SK, *et al*. The avian beta-adrenergic receptor: primary structure and membrane topology. Proc Natl Acad Sci USA 1986; 83(18): 6795-9.

[6] Dixon RA, Kobika BK, Strader DJ, *et al*. Cloning of the gene and cDNA for mammalian beta-adrenergic receptor homology with rhodopsin. Nature 1986; 321(6065): 75-9.

[7] Emorine LJ, Marullo S, Briend-Sutren MM, *et al*. Molecular characterization of the human beta 3-adrenergic receptor. Science 1989; 245(4922): 1118-21.

[8] Goldstein DS, Eisenhofer G, Kopin IJ. Sources and significance of plasma levels of catechols and their metabolites in humans. J Pharmacol Exp Ther 2003; 305(3): 800-11.

[9] Galougahi KK, Liu CC, Bundgaard H, Rasmussen HH. Beta-adrenergic regulation of the cardiac Na^+-K^+ ATPase mediated by oxidative signaling. Trends Cardiovasc Med 2012; 22(4): 83-7.

[10] Audigane L, Kerfant BG, El Harchi A, *et al*. Rabbit, a relevant model for the study of cardiac beta 3-adrenoceptors. Exp Physiol 2009; 94: 400-11.

[11] Massion PB, Balligand JL. Modulation of cardiac contraction, relaxation and rate by the endothelial nitric oxide synthase (eNOS): Lessons from genetically modified mice. J Physiol 2003; 546: 63-75.

[12] Cannavo A, Liccardo D, Koch WJ. Targeting cardiac beta-adrenergic signaling *via* GRK2 inhibition for heart failure therapy. Front Physiol 2013; 4: 264.

[13] Najafi A, Sequeira V, Kuster DWD, Van der Velden J. Beta-adrenergic receptor signalling and its functional consequences in the diseased heart. Eur J Clin Invest 2016; 46(4): 362-74.

[14] Post SR, Hammond HK, Insel PA. Beta-adrenergic receptors and receptor signaling in heart failure. Annu Rev Pharmacol Toxicol 1999; 39: 343-60.

[15] Kamp TJ, Hell JW. Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. Circ Res 2000; 87: 1095-102.

[16] Kushnir A, Marks AR. The ryanodine receptor in cardiac physiology and disease. Adv Pharmacol 2010; 59: 1-30.

[17] Walker JS, Walker LA, Margulies K, Buttrick P, de Tombe P. Protein kinase A changes calcium sensitivity but not crossbridge kinetics in human cardiac myofibrils. Am J Physiol Heart Circ Physiol 2011; 301(1): H138-46.

[18] Koss KL, Kranias EG. Phospholamban: A prominent regulator of myocardial contractility. Circ Res 1996; 79(6): 1059-63.

[19] Xiao RP, Cheng H, Zhou YY, Kuschel M, Lakatta EG. Recent advances in cardiac beta 2-adrenergic signal transduction. Circ Res 1999; 85: 1092-100.

[20] Montezano AC, Nguyen Dinh Cat A, Rios FJ, Touyz RM. Angiotensin II and vascular injury. Curr Hypertens Rep 2014; 16(6): 431.

[21] Johnson JA, Davis JO, Gotshall RW, *et al*. Evidence for an intrarenal beta receptor in control of renin release. Am J Physiol 1976; 230(2): 410-8.

[22] Kaumann AJ, Hall JA, Murray KJ, Wells FC, Brown MJ. A comparison of the effects of adrenaline and noradrenaline on human heart: the role of β_1- and β_2-adrenoceptors in the stimulation of adenylate cyclase and contractile force. Eur Heart J 1989; 10(Suppl B): 29-37.

[23] Berg T. $Beta_1$-blockers lower norepinephrine release by inhibiting presynaptic, facilitating β_1-adrenoceptors in normotensive and hypertensive rats. Front Neurol 2014; 5: 51.

[24] Liggett SB. Molecular and genetic basis of beta 2-adrenergic receptor function. J Allergy Clin Immunol 1999; 104(2 Pt 2): S42-6.

[25] Roberts SJ, Papaioannou M, Evans BA, Summers RJ. Characterization of beta-adrenoreceptor mediated smooth muscle relaxation and the detection of mRNA for beta 1-, beta 2- and beta 3-adrenoceptors in rat ileum. Br J Pharmacol 1999; 127(4): 949-61.

[26] Peterson L, Ismond KP, Chapman E, Flood P. Potential benefits of therapeutic use of β_2-adrenergic receptor agonists in neuroprotection and Parkinson's disease. J Immunol Res 2014; 2014: 1037-80.

[27] Skeberdis VA. Structure and function of beta 3-adrenergic receptors. Medicina (Kaunas) 2004; 40(5): 407-13.

[28] Reiter MJ. Cardiovascular drug class: Beta-blockers. Progr Cardio Dis 2004; 47(1): 11-33.

[29] Black JW, Stephenson JS. Pharmacology of a new adrenergic beta-receptor-blocking compound (Nethalide). Lancet 1962; 2(7251): 311-4.

[30] Black JW, Crowther AF, Shanks RG, Smith LH, Dornhorst AC. A new adrenergic betareceptor antagonist. Lancet 1964; 1(7342): 1080-1.

[31] Siebert CD, Hänsicke A, Nagel T. Stereochemical comparison of nebivolol with other beta-blockers. Chirality 2008; 20(2): 103-9.

[32] Hansson L, Zweifer AJ. The effect of propranolol on plasma renin activity and blood pressure in mild essential hypertension. Acta Med Scand 1974; 195: 397-401.

[33] MacLleod SM, Hamet P, Kapian H, *et al*. Antihypertensive efficacy of propranolol given twice daily. Can Med Assoc J 1979; 121(6): 737-40.

[34] Chadda K, Goldstein S, Byington R, Curb JD. Effect of propranolol after acute myocardial infarction in patients with congestive heart failure. Circulation 1986; 73(3): 503-10.

[35] Pine M, Favrot L, Smith S, McDonald K, Chidsey CA. Correlation of plasma propranolol concentration with therapeutic response in patients with angina pectoris. Circulation 1975; 52(5): 886-93.

[36] Boskabady MH, Snashall PD. Bronchial responsiveness to beta-adrenergic stimulation and enhanced beta-blockade in asthma. Respirology 2000; 5: 111-8.

[37] Lager I, Blohmé G, Smith U. Effect of cardioselective and non-selective beta-blockade on the hypoglycaemic response in insulin-dependent diabetics. Lancet 1979; 1(8114): 458-62.

[38] Marsden CD, Foley TH, Owen DA, McAllister RG. Peripheral beta-adrenergic receptors concerned with tremor. Clin Sci 1967; 33(1): 53-65.

[39] Kerr D, MacDonald IA, Heller SR, Tattersall RB. Beta-adrenoceptor blockade and hypoglycaemia. A randomized, double-blind, placebo controlled comparison of metoprolol CR, atenolol and propranolol LA in normal subjects. Br J Clin Pharmacol 1990; 29(6): 685-93.

[40] Dunlop D, Shanks RG. Selective blockade of adrenoceptive beta-receptors in the heart. Br J Pharmacol Chemother 1968; 32(1): 201-18.

[41] Barrett AM, Carter J, Fitzgerald JD, Hull R, Le Count D. A new type of cardioselective adrenoceptive blocking drug. Br J Pharmacol 1973; 48(2): 340P.

[42] Nobre F, da Silva CA, Coelho EB, Salgado HC, Fazan RJ. Antihypertensive agents have different ability to modulate arterial pressure and heart rate variability in 2K1C rats. Am J Hypertens 2006; 19(10): 1079-83.

[43] Agarwal R, Sinha AD, Pappas MK, Abraham TN, Tegegne GG. Hypertension in hemodialysis patients treated with atenolol or lisinopril: A randomized controlled trial. Nephrol Dial Transplant 2014; 29(3): 672-81.

[44] Tardif JC, Ford I, Tendera M, Bourassa MG, Fox K, INITIATIVE Investigators. Efficacy of ivabradine, a new selective I(f) inhibitor, compared with atenolol in patients with chronic stable angina. Eur Heart J 2005; 26(23): 2529-36.

[45] Ablad B, Carlsson E, Ek L. Pharmacological studies of two new cardioselective adrenergic beta-receptor antagonists. Life Sci I 1973; 12(3): 107-19.

[46] Ljung B, Ablad B, Drews L, Fellenius E, Kjellstedt A, Wallborg M. Anti-hypertensive effect of metoprolol in spontaneously hypertensive rats. Clin Sci Mol Med Suppl 1976; 3: 443S-5S.

[47] Sumbria M, Negi PC, Sahai AK, Kaundal PK. To compare the effect of Telmisartan with Metoprolol on arterial stiffness in hypertension: Prospective randomized parallel group trial. Indian Heart J 2014; 66(4): 415-21.

[48] Cocco G, Chu D. The anti-ischemic effect of metoprolol in patients with chronic angina pectoris is gender-specific. Cardiology 2006; 106(3): 147-53.

[49] [No authors listed]. Effect of metoprolol CR/XL in chronic heart failure: Metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF). Lancet 1999; 353(9169): 2001-7.

[50] Farmer JB, Kennedy I, Levy GP, Marshall RJ. Pharmacology of AH 5158; a drug which blocks both - and -adrenoceptors. Br J Pharmacol 1972; 45(4): 660-75.

[51] Kennedy I, Levy GP. Combined alpha- and beta-adrenoceptor blocking drug AH 5158: further studies on alpha adrenoceptor blockade in anaesthetized animals. Br J Pharmacol 1975; 53(4): 585-92.

[52] Bartsch W, Sponer G, Strein K, Muller-Beckmann B, Von Mollendorff, Abshagen U. Pharmakologie und klinische Pharmakologie des neuen vasodilatierenden B-Rezeptoren-Blockers BM 14.190. Therapiewoche 1982; 32: 46.

[53] Eggertsen R, Sivertsson R, Andren L, Hansson L. Hemodynamic effects of combined beta-adrenoceptor blockade and precapillary vasodilation in hypertension. Acta Med Scand Suppl 1985; 693: 115-20.

[54] Sabellek WM, Schulte KL, Streitberg B, Gotzen R. Two-year follow-up of 24-hour indirect blood pressure monitoring: An open study. Drugs 1988; 36: 66-112.

[55] Chen J, Huang C, Zhang B, Huang O, Chen J, Xu L. The effects of carvedilol on cardiac structural remodeling: The role of endogenous nitric oxide in the activity of carvedilol. Mol Med Rep 2013; 7(4): 1155-8.

[56] Kaksi JC, Rodriguez-Plaza L, Brown J, Maseri A. Efficacy of carvedilol (BM 14.190), a new beta-blocking drug with vasodilating properties, in exercise-induced ischemia. Am J Cardiol 1985; 56(1): 35-40.

[57] Kowalski J, Banach M, Barylski M, Irzmanski R, Pawlicki L. Carvedilol modifies antioxidant status of patients with stable angina. Cell Mol Biol Lett 2008; 13(2): 230-9.

[58] Van der Does R, Hauf-Zachariou U, Pfarr E, *et al.* Comparison of safety and efficacy of carvedilol and metoprolol in stable angina pectoris. Am J Cardiol 1999; 83(5): 643-9.

[59] Zepeda RJ, Castilo R, Rodrigo R, *et al*. Effect of carvedilol and nebivolol on oxidative stress-related parameters and endothelial function in patients with essential hypertension. Basic Clin Pharmacol Toxicol 2012; 111(5): 309-16.

[60] Le DE, Pascotto M, Leong-Poi H, Sari I, Micari A, Kaul S. Anti-inflammatory and pro-angiogenic effects of beta-blockers in a canine model of chronic ischemic cardiomyopathy: Comparison between carvedilol and metoprolol. Basic Res Cardiol 2013; 108(6): 384.

[61] Jonsson G, Abdelnoor M, Seljeflot I, *et al*. The antioxidative effects of long-term treatment are more pronounced for carvedilol than for atenolol in post-myocardial infarction patients. J Cardiovasc Pharmacol 2007; 49(1): 27-32.

[62] Van de Water A, Janssens W, Van Neuten J, *et al*. Pharmacological and hemodynamic profile of nebivolol, a chemically novel, potent, and selective beta 1-adrenergic antagonist. J Cardiovasc Pharmacol 1988; 11(5): 552-63.

[63] Fongemie J, Felix-Getzik E. A review of nebivolol pharmacology and clinical evidence. Drugs 2015; 75: 1349-71.

[64] Weiss R, Weber M, Carr A, Sullivan W. A randomized, double blind, placebo-controlled parallel-group study to assess the efficacy and safety of nebivolol, a novel beta-blocker, in patients with mild to moderate hypertension. J Clin Hypertens 2007; 9(9): 667-76.

[65] Saunders E, Smith W, DeSalvo K, Sullivan W. The efficacy and tolerability of nebivolol in hypertensive African-American patients. J Clin Hypertens 2007; 9(11): 866-75.

[66] Greathouse M. Nebivolol efficacy and safety in patients with stage I–II hypertension. Clin Cardiol 2010; 33(4): E20-7.

[67] Zhang Z, Ding L, Jin Z, *et al*. Nebivolol protects against myocardial infarction injury *via* stimulation of beta 3-adrenergic receptors and nitric oxide signaling. PLoS One 2014; 9(5): e98179.

[68] Ceron CS, Rizzi E, Guimarães DA, Martins-Oliveira A, Gerlach RF, Tanus-Santos JE. Nebivolol attenuates prooxidant and profibrotic mechanisms involving TGF-β and MMPs, and decreases vascular remodeling in renovascular hypertension. Free Radic Biol Med 2013; 65: 47-56.

[69] Rizzi E, Guimarães DA, Ceron CS, *et al*. Beta1-Adrenergic blockers exert antioxidant effects, reduce matrix metalloproteinase activity, and improve renovascular hypertension-induced cardiac hypertrophy. Free Rad Biol Med 2014; 73: 308-17.

[70] Zhou X, Ma L, Habibi J, *et al*. Nebivolol improves diastolic dysfunction and myocardial remodeling through reductions in oxidative stress in the zucker obese rat. Hypertension 2010; 55(4): 880-8.

[71] Vanhoutte PM, Gao Y. Beta blockers, nitric oxide, and cardiovascular disease. Curr Opin Pharmacol 2013; 13: 265-73.

[72] Doumas M, Tsakiris A, Douma S, *et al*. Beneficial effects of switching from betablockers to nebivolol on the erectile function of hypertensive patients. Asian J Androl 2006; 8(2): 177-82.

[73] Brixius K, Middeke M, Lichtenthal A, Jahn E, Schwinger RH. Nitric oxide, erectile dysfunction and beta-blocker treatment (MR NOED study): Benefit of nebivolol versus metoprolol in hypertensive men. Clin Exp Pharmacol Physiol 2007; 34(4): 327-31.

[74] Weber MA, Schiffrin EL, White WB, *et al*. Clinical practice guidelines for the management of hypertension in the community. A statement by the American Society of Hypertension and the International Society of Hypertension. J Hypertens (Greenwich) 2014; 16(1): 14-26.

[75] James PA, Oparil S, Carter BL, *et al*. 2014 evidence-based guideline for the management of high blood pressure in adults: Report from the panel members appointed to the Eighth Joint National Committee (JNC 8). JAMA 2014; 311(5): 507-20.

[76] Lacourcière Y, Lefebvre J, Poirier L, Archambault F, Arnott W. A double-blind crossover comparison of nebivolol and lisinopril in the treatment of ambulatory hypertension. Am J Ther 1994; 1(1): 74-80.

[77] Van Nueten L, Lacourcière Y, Vyssoulis G, *et al*. Nebivolol versus nifedipine in the treatment of essential hypertension: A double-blind, randomized, comparative trial. Am J Ther 1998; 5(4): 237-43.

[78] Van Bortel L, Bulpitt C, Fici F. Quality of life and antihypertensive effect with nebivolol and losartan. Am J Hypertens 2005; 18(8): 1060-6.