Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology and Medicine
Volume 14 Issue 6 Version 1.0 Year 2014
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

The Cardioprotective Effects of Irbesartan and Candesartan in Isoproterenol Induced Cardiomyopathy in Rats

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Abstract- The presence of a wide selection of angiotensin receptor blockers and the conflicting evidence regarding their cardioprotective effect, led to the attempt to evaluate the impact of irbesartan and candesartan on cardiac hypertrophy and remodeling. Female Albino rats were divided into 3 groups. The first group served as the control group and was given 1 ml distilled water via oral gavage and 0.5 ml distilled water subcutaneously. The second group was the isoproterenol (ISO) group and was given a daily S.C. injection of ISO at a dose of 5 mg/kg. The third group served as the treatment group and it was subdivided into 2 groups, both received ISO as stated previously along with a treatment drug which was administered via oral gavage and they included: ISO-Irb(irbesartan 50 mg/kg/day), and ISO-Cand(candesartan 2.6 mg/kg/day). All groups were treated for a period of 14 days. The assayed parameters included; mean serum Matrix metalloproteinase 9 (MMP-9), Cardiac troponin I (cTn-I), and Heart weight to Body weight (Hw/Bw) ratio.

Keywords: angiotensin, isoproterenol, cardiomyopathy, ARBs, MMP-9, cTn-i, candesartan, irbesartan.

GJMR-B Classification : NLMC Code: QV 37.5

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The Cardioprotective Effects of Irbesartan and Candesartan in Isoproterenol Induced Cardiomyopathy in Rats

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Abstarct- The presence of a wide selection of angiotensin receptor blockers and the conflicting evidence regarding their cardioprotective effect, led to the attempt to evaluate the impact of irbesartan and candesartan on cardiac hypertrophy and remodeling. Female Albino rats were divided into 3 groups. The first group served as the control group and was given 1 ml distilled water via oral gavage and 0.5 ml distilled water subcutaneously. The second group was the isoproterenol (ISO) group and was given a daily S.C. injection of ISO at a dose of 5 mg/kg. The third group served as the treatment group and it was subdivided into 2 groups, both received ISO as stated previously along with a treatment drug which was administered via oral gavage and they included: ISO-Irb(irbesartan 50 mg/kg/day), and ISO-Cand(candesartan 2.6 mg/kg/day). All groups were treated for a period of 14 days. The assayed parameters included; mean serum Matrix metalloproteinase 9 (MMP-9), Cardiac troponin I (cTn-I), and Heart weight to Body weight (Hw/Bw) ratio.Irbesartan co-administered with ISO significantly reduced mean serum MMP-9 concentration, while candesartan significantly reduced MMP-9, and cTn-I concentrationscompared to the ISO group respectively. The Hw/Bw ratio was significantly reduced by both drugs. In conclusion both treatment drugs possessed some degree of cardioprotection; candesartan being the most beneficial in ameliorating isoproterenol induced cardiac injury.

keywords: angiotensin, isoproterenol, cardiomyopathy, ARBs, MMP-9, cTn-i, candesartan, irbesartan.

I. Introduction

The human heart is an exceptional organ, that's designed to function continuously for an average 70 year life span of a normal individual, thus a human heart beating at a rate of 70 beats per minute will exceed 2.5 billion beats throughout the life span of a human being (McCartan et al., 2012), this exceptional muscular pump displays extraordinary capacity to adapt to a broad range of genetic and extrinsic factors to sustain its contractile functions, failure to do so results in cardiac dysfunction and cardiomyopathy (Harvey and Leinwand, 2011). Cardiomyopathies are defined as "a heterogeneous group of diseases involving the myocardium which are associated with mechanical and/or electrical dysfunction that usually exhibits inappropriate ventricular hypertrophy or dilation and are due to a variety of causes that frequently are genetic" (Maron et al., 2006). They can be classified either into primary, or secondary; or according to the type of cardiomyopathy into dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy (RCM), and arrhythmogenic right ventricular cardiomyopathy (ARVC) (Wexler et al., 2009)

DCM is a progressive, irreversible condition with an estimated prevalence of 1:2500, and is considered one of the leading causes of heart failure (Burke, 2011). HCM is regarded as a leading cause of death among athletes, and has an incidence of 1:500 (Maron, et al., 2006), while RCM and ARVC are considered rare types of cardiomyopathy (Wexler et al., 2009). Many biomarkers have been associated with cardiac remodeling and cardiomyopathy (Gopal and Sam, 2013), among these is the cTn-I and MMP-9, their elevation is involved in cardiac injury and cardiomyopathy (Herman et al., 1999; Fairweather et al., 2011), in addition the renin angiotensin system (RAS) can induce left ventricular hypertrophy and fibrosis (Ocaranza et al., 2002), due to the direct effect of Ang II on myocardial cell hypertrophy through its action on the AT_1 receptor (Mehta and Griendling, 2007).

II. Materials and Methods

Thirty six female albino rats, 8-12 weeks old, weighing 140-200 grams, were used. The animals were housed in groups of four per cage, on sawdust in the animal house facility, under conditions of controlled ambient temperature of 22-25 oC with a 12 hour light/ dark cycles. The animals were supplied with rodent chow and free access to tap water.

a) The Rats were allocated into 3 groups as follow

Group 1: (Control group) This group included 8 rats and served as the control group; they received 1ml distilled water orally via oral gavage and 0.5 ml distilled water subcutaneously for a period of 14 days. Group 2: (ISO group) included 8 rats and served as a model of isoproterenol induced cardiomyopathy. The animals were injected with isoproterenol hydrochloride in a dose of 5mg/kg/day (Tipnis et al., 2000; Heather et al., 2009; Chowdhury et al., 2013), S.C. for a period of 14 days to induce distinguishable cardiac hypertrophy and cardiomyopathy. Group 3: (Treatment group) included 20 rats, and served as the treatment group; they were

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further subdivided into 2 subgroups all of which received isoproterenol as stated previously for group 2, along with the treatment drug administered via oral gavage, and they include: Group 3.1 (ISO-Irb. group): This group included 10 rats that were given irbesartan 50mg/kg/day. Group 3.2(ISO-Cand. group): This group included 10 rats that were given candesartan 2.6 mg/kg/day. All groups were treated for a period of 14 consecutive days.

Isoproterenol hydrochloride solution was prepared by reconstitution of isoproterenol hydrochloride powder with distilled water daily under sterile conditions immediately before injection (Grimm et al., 1998). The rats were first weighed and then isoproterenol was injected S.C into each rat except control group which was injected with distilled water S.C. The subcutaneous route was used because of the higher levels of cTn-I associated with this route, and a greater degree of cardiac injury (Brady et al., 2010). Immediately after the injection, the rats received the corresponding treatment drug according to the stated dose for each group, (except for the control group and the ISO group). After 14 days, 24hr of the last dose, the rats were anesthetized by injecting thiopental sodium 100mg/kg/I.P (Grimm et al., 1998), then dissected to expose the beating heart, after which blood was withdrawn directly from the right ventricle. The withdrawn blood was placed in a graduated glass conical bottom centrifuge tubes and allowed to settle for 20 min after which it was centrifuged at 3000 RPM for 10 minutes. The obtained serum was placed in eppendorf tubes and stored at -20 oC for further analysis; the heart was extracted, dried with filter paper and weighed.

b) Serum Measurements

Rat Matrix Metalloproteinase 9 and Cardiac troponin I serum concentrations were measured bydouble-antibody sandwich enzyme-linked immune-osorbent assay (ELISA), purchased from Uscn life science/ Germany and QAYEE-BIO/ Germany respectively.The Hw/Bw ratio was calculated by dividing the heart weight (mg) over the body weight (gm.). (Suckowet al, 2005).

$$\boldsymbol{Hw\backslash Bw\ ratio} = \frac{\textbf{Heart weight in } \boldsymbol{mg}}{\textbf{Body weight in } \boldsymbol{gm}}$$

c) Statistical Analysis

All data are expressed as Mean ± standard deviation. Data was analyzed using the Statistical Package for Social Sciences (SPSS) version 16. Data analysis was made using one-way analysis of variance (ANOVA). Comparison between groups was done by using Post Hoc LSD test. P<0.05 was considered statistically significant.

III. Results

By the end of the study the following mortality was recorded: 2 of 10 rats in the ISO-Cand group. These animals were excluded from the study.

The table below shows the effect of co-administration of the treatment drugs with isoproterenol on the studied parameters. Irbesartan in its respective group, significantly reduced mean serum MMP-9 concentration to 8.10±2.32 ng/ml, while candesartan significantly reduced both serum concentrations of MMP-9 (8.25±1.96 ng/ml) andcTn-I (67.47±10.06 ng/ml,). The Hw/Bw ratio was significantly reduced by both treatment drugs.

Table 1: The effect of Irbesartan, and Candesartanco-administered with Isoproterenol on serum matrix metalloproteinase 9, cardiac troponin I, and heart weight to body weight ratio

		Biomarkers		
		MMP-9 ng/ml	cTn-I ng/ml	Hw/Bw ratio
Groups	Control	7.66±1.50	70.35±13.27	3.15±0.35
	ISO	11.38±3.41*	85.58±10.95*	4.53±0.31*
	ISO-Irb	8.10±2.32a	80.42±14.07	3.76±0.29a
	ISO-Cand	8.25±1.96a	67.47±10.06ab	3.65±0.20a
	P-Value	**0.015**	**0.019**	**<0.001**

- *Values are expressed as mean ± standard deviation*
- *Difference between individual groups were detected using post hoc LSD test*
- *p<0.05 is considered significant*
- **indicates a significant difference from the control at P<0.01*
- *[a]indicates a significant difference from the ISO group at p<0.01*
- *[b]indicates a significant difference between ISO-Cand and the ISO-Irb group*
- *P value refers to the significance of the difference detected by ANOVA.*
- *MMP-9: Matrix metalloproteinase 9. cTn-I: Cardiac troponin I. Hw/Bw: Heart weight to body weight.*

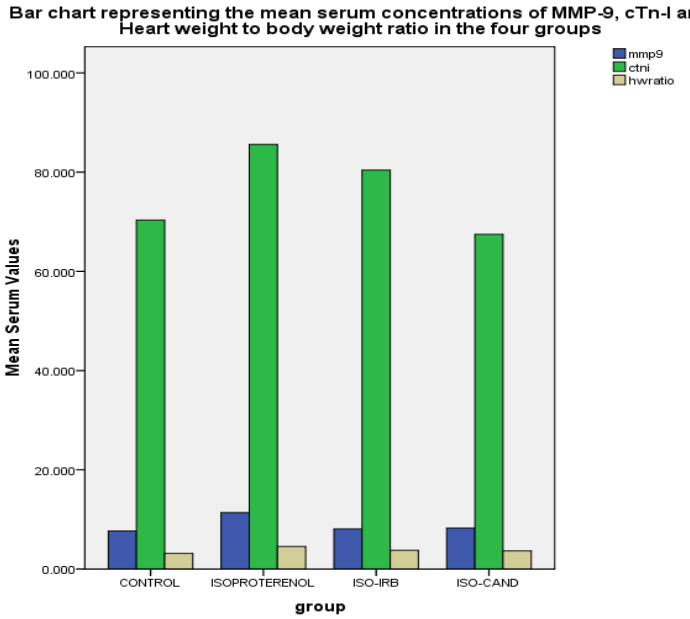

Figure 1 : Bar chart comparing the mean serum levels of MMP-9, cTn-I and cholesterol in the control group, ISO group, ISO-Irb group, and ISO-Cand group

IV. Discussion

Isoproterenol through its non-selective β-adrenoceptor activation causes severe cardiac injury and myocardial hypertrophy through inflammation, cytosolic Ca2+ overload and generation of reactive oxygen species (ROS) (Serra et al., 2008).

The mean serum MMP-9 concentration was significantly increased in the ISO group when compared to the control group, which is consistent with Li et al., (2008) and Cheng et al., (2009) as wasthe mean serum cTn-I concentration which is consistent with York et al., (2007). The elevated levels of cTn-I and MMP-9 are associated with cardiomyopathy and cardiac remodeling (Babuin and Jaffe, 2005; Roldán et al., 2008), and may reflect the myocardial injury produced by the administration of isoproterenol in the present study. Irbesartan in its respective groups, produced a significant reduction in MMP-9 serum concentrations which is in agreement with Montalescotet al., (2009), while candesartan in its respective group significantly reduced both mean serum MMP-9 and cTn-I concentrations, which is consistent with Palaniyappan et al., (2009), who found that candesartan is capable of normalizing MMP-9 (activity, protein, and mRNA) in rats after reperfused myocardial infarction.

The effects of ARBs on MMP-9 and cTn-I may be mediated through the inhibition of Ang II, Deschamps and Spinale, 2006 stated that Ang II stimulation of neonatal rat ventricular myocytes can trigger the mobilization of cytoplasmic Nuclear Factor-κB to the nucleus which in turn increases MMP-9 transcription.

Isoproterenol increased the mean Hw/Bw ratio significantly above control and this is consistent with Boluyte et al., (1995). This increase was significantly reduced in both treatment subgroups, and is consistent with the findings of Richer et al., (1999), Shirai et al., (2005).The effectiveness of ARBs in reducing heart weight to body weight ratio can be explained on the bases of their ability to block the action of Ang II, since accumulating evidence suggest that Ang II is involved in pathologic cardiac hypertrophy processes including myocyte hypertrophy, myocyte gene reprogramming, fibroblast proliferation, and extracellular matrix protein accumulation (Gray et al., 1998; Kim and Iwao 2000; Ichihara et al., 2001).

The observed differences among individual ARBs seen in this study may be attributed to the different binding affinity to the AT1 receptor (Kakuta et al., 2005).

The observed differences among individual ARBs seen in this study may be due to the different binding affinity to the AT1 receptor (Kakuta et al., 2005). Burnier (2001) stated that candesartan has the best Ang II antagonistic activity profile. Verdecchia et al., (2009) concluded that despite the shared mechanism of action, each ARB is characterized by specific pharmacological properties that could influence its clinical efficacy. . In conclusion both treatment drugs expressed cardioprotective abilities, candesartan being the most beneficial since it was capable of normalizing serum cTn-I levels as well as the MMP-9 and Hw/Bw ratio.

V. Acknowledgment

The professional assistance of Dr. Marwan Alnamir, and Dr. Nithal Abdulkader are gratefully acknowledged. This work was supported by Hawler Medical University/ College of Pharmacy.

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