

# Candesartan Cilexetil Decreases Genes Expression of p53 and TNF- $\alpha$ in Mice Serum

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## Abstract

**Background:** Candesartan is an angiotensin II receptor blocker available as Candesartan Cilexetil tablets used for hypertension treatment. Candesartan Cilexetil is converted to active Candesartan during gastrointestinal absorption. Tumor necrosis factor - $\alpha$  (TNF- $\alpha$ ) is a cytokine associated with pro-inflammation, which has emerged as a critical modulator of blood pressure. Tumor suppressor protein (p53) is a cytokine responsible for cell apoptosis. Hypertension can induce apoptosis, especially in cardiac muscle. Recently, research referred to increased levels of TNF- $\alpha$  and P53 in serum with increasing angiotensin II releasing. This study is designed to investigate the impact of Candesartan Cilexetil on reducing these cytokines in serum.

**Methods:** Forty Albino Swiss male mice were incubated under ideal conditions. Mice were divided into two groups; one was given many doses of Candesartan Cilexetil (0.1, 0.3, and 0.5 mg kg<sup>-1</sup>day<sup>-1</sup>) and a control group. After treatment for 21 days the concentration of p53 and TNF- $\alpha$  in serum was determined using Elisa sandwich method.

**Results:** Candesartan Cilexetil reduced P53 and TNF- $\alpha$  concentration significantly at ( $p \leq 0.05$ ). But a highly significant decrease for the two cytokines was recorded in mice that were treated with 0.3 mg kg<sup>-1</sup> of Candesartan cilexetil.

**Conclusion:** Candesartan Cilexetil can reduce TNF- $\alpha$  and P53 concentration in mice serum by blocking the angiotensin II receptor because angiotensin II enhances releasing of these cytokines.

**Keywords:** Candesartan Cilexetil, TNF- $\alpha$ , P53 and Angiotensin II.

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## INTRODUCTION

Candesartan Cilexetil (CC) is a brand-new angiotensin II (Ang II) receptor antagonist medication. It is quickly absorbed by the digestive system after oral treatment. It functions pharmacologically by combining noncompetitively with type I of AngII receptors, which have strong, long-lasting effects and excellent selectivity. It works by blocking the effects of the hormone AngII in the body, thus decreasing blood pressure [1]. Candesartan Cilexetil prevents retina disorder and repairs damaged bodily organs [2].

Angiotensin II and its receptor and Ang II type I receptor (AT1R), are known as factors linked to the pathogenesis of cardiometabolic and cardiovascular diseases, such as obesity, atherosclerosis, and diabetes [3]. Angiotensin II also has relationships with the activation of TNF- $\alpha$ , which is a pro-inflammatory cytokine, causing endothelial cell apoptosis, while AngII-induced inflammation has been proven to participate in cardiac fibrosis and remodeling [4, 5].

TNF- $\alpha$  is well known as an inflammation central mediator in many diseases. AngII induces TNF- $\alpha$  expression, which

initiates endothelial cells pathological phenotype and can contribute to increasing plaque vulnerability [6]. Ang II induces expression of Interleukins 8 and 12 and TNF- $\alpha$  [7, 8].

TNF- $\alpha$  is a serious modulator of neural plasticity and blood pressure. Nevertheless, the contribution of TNF- $\alpha$  signaling to the development of hypertension is unclear [9]. Tumor suppressor protein p53 located in many cell organs, including the nucleus and mitochondria, maintains mitochondrial activity [10–11]. P53 plays a central role in organizing mitochondrial remodeling by rearrangement of their content, intracellular signaling molecules that are related to the pathways of mitochondrial autophagy and apoptosis and fusion/fission processes. [12]

Angiotensin II plays crucial roles in elevating blood pressure by influencing the vascular smooth muscle cells, endothelial cells, and immune cells to advance the development through simple inflammation. Macrophages create renin, Ang II, and angiotensin-converting enzyme accumulating in cardiac tissues and other organs. [13]. ongoing stress generated by Ang II produces damage and apoptosis in vascular endothelial cells, initiating blood vessel injury [14, 15]. Therefore, it is very important to

prevent continuous Ang II stress in order to protect blood vessels [16].

According to the above, this study was designed to evaluate Candesartan Cilixetil ability to reduce P53 and TNF- $\alpha$  concentration in mice serum.

## MATERIALS AND METHODS

### A- Animals

Forty Albino Swiss male mice were obtained from the Al-Kut Technical Institute, Department of Pathological Analysis. Mice were incubated under given conditions (12h dark/light cycles at ambient temperature of  $24 \pm 1^\circ$  Celsius) with free water and food and subdivided into groups of 8 mice each.

### B- Groups of the study:

Serum was collected from mice after 21 days.

1- Control group: Mice were kept in cages for 21 days with no treatments.

### 2-Treatments groups:

Group I: Mice were given water that contained 0.1% alcohol and left alone for 21 days. [17]. Using 0.1% ethanol facilitates the dissolution of the drug.

Group II: Candesartan Cilixetil (CC) treatment: Mice were treated using tablets of 8mg, manufactured by AstraZeneca, UK. Tablets were dissolved in 0.1% ethanol water as stated by Farghaly Aly *et al* [18]. Mice drinking water was replaced three times per week and weight of mice was calculated and the water volume consumed were recorded to calculate the average drug doses, which were found to be equal to (0.1, 0.3, and 0.5 mg kg<sup>-1</sup>day<sup>-1</sup>) [19].

### C- Blood Sampling and testing

At the end of the treatments, mice were anesthetized with chloroform. Blood samples were obtained from cardiac puncture by a 3ml syringe and collected in glass tubes and left for 30 minutes to clot at ambient temperature. Tubes were centrifuged at 5000 rpm for 10 minutes. Serum was collected and stored frozen until required [20]. Levels of p53 and TNF- $\alpha$  were determined by Elisa sandwich method, as noted with each kit. The kits used to check mouse p53/tumor protein levels (Catalog No: YLA0823MO) and mouse TNF- $\alpha$  (Catalog No: YLA0097MO) were manufactured by the Shanghai yl Biotech Company.

## STATISTICAL ANALYSIS

Utilizing the Student's t-test and F-test, experimental data were evaluated. Statistical significance was defined at a p value  $\leq 0.05$ . Data were analyzed using the Statistical Package for Social Sciences version 24 (SPSS 24), and one-way ANOVA was performed to see whether there were any significant differences between the scores and the variables.

## RESULT AND DISCUSSION

When mice were treated with 0.1% alcohol no significant difference ( $p=0.075$ ) in values of p53 and TNF were detected compared with mice in the control group, as shown in Table 1. This indicates that 0.1% alcohol has no effect on p53 and TNF levels in serum. Therefore all next treatments tests will be compared with the control group.

Table 1: Effects of 0.1% alcohol on concentration of p53 and TNF- $\alpha$  in mice serum.

Groups	M $\pm$ S.E. of P53 pg ml <sup>-1</sup>	M $\pm$ S.E. of TNF- $\alpha$ pg ml <sup>-1</sup>	*P value
Control	39.35 $\pm$ 3.9	98.54 $\pm$ 8.9	0.075
0.1% alcohol	37.65 $\pm$ 4.1	99.74 $\pm$ 4.4	

\*P  $\leq 0.05$

Values of p53 concentrations in the blood of treated mice were 33.29 $\pm$ 3.41, 29.41 $\pm$ 5.3 and 27.37 $\pm$ 6.6 pg ml<sup>-1</sup> at 0.1, 0.3, and 0.5 mg kg<sup>-1</sup> of Candesartan Cilixetil, respectively. These values are significantly less than those seen in the controls especially at a dose of 0.3 mg kg<sup>-1</sup>, as shown in Table 2.

Table 2: The concentrations of p53 in mice serum after treated with different doses of Candesartan Cilixetil

Experimental groups	M $\pm$ S.E. of p53 pg ml <sup>-1</sup>	*P value
Control	39.35 $\pm$ 3.9	0.0001
0.1 mg kg <sup>-1</sup> of CC	33.29 $\pm$ 3.41	
0.3 mg kg <sup>-1</sup> of CC	29.41 $\pm$ 5.3	
0.5 mg kg <sup>-1</sup> of CC	27.37 $\pm$ 6.6	

\*P  $\leq 0.05$

The results given in Table 3 show the ability of Candesartan Cilixetil at 0.1, 0.3, and 0.5 mg kg<sup>-1</sup> to reduce the concentration levels of TNF - $\alpha$  to 79.19 $\pm$ 2.8, 67.54 $\pm$ 5.4, and 73.55 $\pm$ 3.1pg ml<sup>-1</sup>, respectively. Generally, all Candesartan Cilixetil doses significantly reduced the levels of TNF - $\alpha$ , particularly at 0.3 mg kg<sup>-1</sup>.

Table 3: The TNF- $\alpha$  concentration in mice serum decreased when treated with different doses of Candesartan Cilixetil

Experimental groups	M $\pm$ S.E. of TNF- $\alpha$ pg ml <sup>-1</sup>	*P value
Control	98.54 $\pm$ 8.9	0.0001
0.1 mg kg <sup>-1</sup> of CC	79.19 $\pm$ 2.8	
0.3 mg kg <sup>-1</sup> of CC	67.54 $\pm$ 5.4	
0.5 mg kg <sup>-1</sup> of CC	73.55 $\pm$ 3.1	

\*P  $\leq 0.05$

It is clear from the foregoing data that Candesartan has a

tendency for reducing levels of p53 and TNF- $\alpha$  in the blood serum, as shown in Tables 2 and 3. This drug has a great effect on stopping the production of angiotensin II and it appears that there is a relationship between the level of angiotensin II and the levels of these two factors [1]. Numerous studies have shown that there is a close relationship between inflammation and increased levels of AngII in the blood. Candesartan can reduce certain organ damage in human body [2]. This may be due to that Candesartan is able to reduce levels of p53 and TNF- $\alpha$ , while Liu T *et al* referred to a good relationship between the expressions of TNF- $\alpha$  and Angiotensin II levels in serum [4]. TNF- shows relevance with blood pressure and especially contributes to the development of hypertension [9]. Increasing release of AngII led to enhancement of apoptosis [14,15]. P53 is also an essential protein for apoptosis; therefore, AngII can activate production of P53. This action gives Candesartan the ability to reduce and curb production of P53 because Candesartan is considered as AngII blocker. AngII can activate NADPH oxidase complex and this enhances inflammation [21], which leads to production of superoxide radicals by AngII and significantly increased the levels of superoxide production and can induced cellular DNA damage and apoptosis in mice [22, 23]. However cellular stresses like increasing oxidation state can induce apoptosis leading to p53 rise [24]. Notably, a low dose of candesartan in mice can reduce inflammation and the effects of peroxisomes leading to reduce oxidation state [25]. Overexpression of AngII causes cellular damage and increases apoptosis [16]. Therefore candesartan can reduce free radical and cellular damage that lead to reduce P53 levels. This is clearly shown in Table 2 and 3. Additional research works have reported that AgnII inhibitors minimize TNF- $\alpha$  release *in vivo* and *in vitro* [26]. Price *et al.* noticed in a rat model that TNF- $\alpha$  production and syndromes of diseases were significantly decreased when using AgnII inhibitors [27]. These results were also supported by others that receptor type1 blockers (ARBs) are related to decreasing reactive oxygen species produced, inflammation, and disease activity [28–30]. Moreover, animal models of renal injury examining inhibitors indicates that impeding of AngII functions through angiotensin converting enzyme (ACE) inhibitors or AngII receptor blockers (ARBs) minimize pro-inflammatory cells and catabolic gene expression [31–32]. Intravenous injection of fairly higher doses of AngII can induce higher releasing of TNF $\alpha$ . This increase in the TNF $\alpha$  level performs a protecting role in stopping additional salt retention in medical conditions related to raise AngII levels in hypertension [33].

Many studies have referred to the contribution of TNF- $\alpha$  in the pathophysiology of AngII-dependent hypertension and the progress of organs injury [34–35]. Treatment by AngII initiates the release of TNF- $\alpha$  in the kidney [35]. There have been numerous renal dysfunction processes that have been linked to a functional interaction between AngII and TNF

[34]. TNF- $\alpha$  participates in the inflammatory process in AngII induced renal pathology and causes chronic dysfunction. It can regulate the releasing of many chemokines and cytokines, and is participate in the signaling of extended cellular responses such as cell division and differentiation of inflammatory cells causing renal injury leading to chronic diseases in the kidney [34–35]. AngII induced acute responses are increased renin-angiotensin system (RAS) and promotes the release of many pro-inflammatory cytokine, such as TNF- $\alpha$  [36] and an important rise in TNF- $\alpha$  mRNA and protein biosynthesis within 30–60 min [37– 38].

In the present study, we demonstrate the ability of Candesartan Cilexetil to decrease the TNF- $\alpha$  and P53 concentrations by blocking AngII pathway in mice.

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