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# Blocking of urotensin receptors as new target for treatment of carrageenan induced inflammation in rats

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ABSTRACT

This study investigated possible role of U-II and its receptor expression in inflammation by using UTR agonist and antagonist in carrageenan induced acute inflammation. Rats were divided into 5 groups as (1) Healthy control, (2) Carrageenan control, (3) Carrageenan +Indomethacin 20 mg/kg, orally, (4) Carrageenan +AC7954 (U-II receptor agonist, intraperitoneally) 30 mg/kg and (5) Carrageenan +SB657510 (UTR antagonist, intraperitoneally) 30 mg/kg. 1 h after drug administration, carrageenan was injected. At the 3rd hour after carrageenan injection, agonist produced no effect while antagonist 63% anti-inflammatory effect respectively. UTR and UT-II expression increased in carrageenan induced paw tissue. Antagonist administration prevented the decrease in an antioxidant system and also capable to decrease TNF-α and IL-6 mRNA expressions. This study showed the role of urotensin II receptors in the physiopathogenesis of acute inflammatory response that underlying many diseases accompanied by inflammation.



## 1. Introduction

Inflammation is an immune system response that activates many complex enzyme systems, mediates cytokine release form inflammatory cells, protects body from all kind of trauma and repairs damage [1,2]. However, long lasting and excessive inflammation induces tissue damage and contributes in pathogenesis of many diseases such as arthritis, cancer, stroke, neurodegenerative and cardiovascular diseases [3,4]. Non-steroidal anti-inflammatory drugs are commonly used for anti-inflammatory treatment; however, they have limitations for treating physiopathology of inflammation. Therefore, occurrence and development mechanism is still investigated and new anti-inflammatory drugs are being tried.

Vasoconstrictor potency of urotensin II (UT-II), is an order of magnitude greater than that of endothelin-1, suggests human U-II the most potent mammalian vasoconstrictor identified so far [5]. Effects of U-II on cardiovascular system alters dependent to type of mentioned vascular structure, receptor density and dose [6]. U-II have been suggested to have both endothelium-dependent vasorelaxation and endothelium-independent vasoconstriction effects and net effects occurs as a result of balance between these two effects [7]. U-II is considered to have many physiologic and pathophysiologic effects in addition to its vasoconstrictor effects. In another study U-II shown to contribute in the expression of pro-angiogenic cytokines in endothelial cells [8].

Latest studies demonstrated that U-II modulates its effects via U-II receptors also known as GPR14 that are G-protein coupled receptors on cell membrane [5]. UTR was discovered by 4 groups simultaneously [9–11]. Studies showed widespread expression of U-II receptors in cardiovascular, pulmonary, central nervous, renal and metabolic systems and especially in inflammatory regions [12–14]. Induction of macrophages and monocytes by U-II and especially increasing the expression of cytokines such as IL-6 sug-

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gests potential inflammatory function of U-II/UTR system [15]. Increased U-II receptor expression has been shown several diseases such as; hypertension, atherosclerosis, hearth diseases, pulmonary hypertension, diabetes mellitus, renal failures and metabolic syndrome [16]. Nowadays to inhibit mentioned effects of U-II, specific receptor blockers have been produced. Supporting our topic, U-II have been reported to increase level of specific cytokines such as IL-1 and IL-6, which antagonized these effects of urotensin-II by U-II receptor blockers [17]. Other studies showed U-II receptor antagonists decreased tumor necrosis factor alpha (TNF-α) levels during LPS/D-GAL induced hepatocyte apoptosis [18,19]. However, there is no study about expression and role of U-II receptors in rat paws or acute inflammatory reactions and this point remains as an open question in literature.

In the light of all these data, this study investigated possible role of U-II and its receptor expression in inflammation by using U-II receptor agonist and antagonist in carrageenan induced acute inflammation model in rats.

## 2. Materials and methods

### 2.1. Animals

A total of 40 male albino wistar rat were used in the experiments. Each rat weighed 200–250 g, and all were obtained from Ataturk University's Experimental Animal Laboratory at the Medicinal and Experimental Application and Research Centre. The animal experiments and procedures were performed in accordance with national guidelines for the use and care of laboratory animals and approved by Ataturk University's local animal care committee (31.05.2013-10/27). The rat were housed in standard plastic cages on sawdust bedding in an air-conditioned room at 22 ± 1 °C. Standard rat food and tap water were given ad libitum.

### 2.2. Chemicals

All of the chemicals used in our laboratory experiments and Urotensin receptor agonist (AC7954), Urotensin receptor antagonist (SB657510) which used intraperitoneally [20] and carrageenan were purchased from Sigma Chemical Co. (Munich, Germany). Indomethacin (Endol 25 mg; 25 cap.) was obtained from DEVA Holding A.S. (Istanbul, Turkey); and thiopental sodium was obtained from IE Ulagay A.S. (Istanbul, Turkey).

### 2.3. Carrageenan induced inflammatory paw oedema model of rat

#### 2.3.1. Experimental groups

1. Healthy control.
2. Carrageenan control.
3. Carrageenan +Indomethacin 20 mg/kg.
4. Carrageenan +AC7954 (U-II receptor agonist) 30 mg/kg.
5. Carrageenan +SB657510 (U-II receptor antagonist) 30 mg/kg.

Rats were divided in separate groups each consist of 8 animals. Before administration of drugs all rat's basal paw volume was measured with plethysmometer. 1 h after drug administration, carrageenan (0.1 ml, %1 w/v) was injected in right paws of the animals. At the third hour after carrageenan injection, paw volume measured with plethysmometer and anti-inflammatory effect was calculated as% inhibition. At the end of experiment rats were sacrificed under thiopenthal anaesthesia and paw tissues were obtained. Collected tissue were stored at −80° C for biochemical and molecular analyses and at 10% formalin for histopathological analyses.

### 2.4. Biochemical analyses

#### 2.4.1. Biochemical investigation of paw tissues

After conducting the macroscopic analyses, the rat paw tissues were kept at −80 °C. All tissue samples from each rat were grinded in liquid nitrogen using a TissueLyser II grinding jar set (Qiagen, Hilden, Germany). Approximately 100 mg of ground tissue were homogenized in 1 ml PBS homogenate buffer in an Eppendorf tube using the TissueLyser II, and then centrifuged. Superoxide dismutase (SOD) activity [21], glutathione (GSH) [22] and malondialdehyde (MDA) levels [23], from each sample supernatant and standards were measured at room temperature in duplicate, using a modified method, with an ELISA reader. The average absorbance of each sample and standard were calculated. A standard curve was plotted and the equation was obtained from the absorbance of standards. Linear SOD, GSH, and MDA concentrations were calculated according to this equation. The results of the SOD, GSH, MDA levels in the tissues were expressed as U/mg protein, nmol/mg protein, pg/mg protein, and ng/mg protein, respectively. All data are presented as mean ± standard deviation (SD) based on per mg protein.

#### 2.4.2. Protein determination

Protein concentrations were determined by the Lowry method using commercial protein standards (Total protein kit-TP0300-1KT; Sigma Chemical Co. (Munich, Germany)).

### 2.5. Molecular investigations

#### 2.5.1. Total RNA extraction and cDNA synthesis

Total RNA extraction and cDNA synthesis were performed according to our previous data [24]. Briefly, the tissues (20 mg) were stabilized in RNA stabilization reagent (RNAlater, Qiagen), and then disrupted using the TissueLyser II (2 × 5 min for paw tissues). Total RNA was purified using an RNeasy Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, in a QIAqube (Qiagen, Hilden, Germany). The RNA samples were reverse-transcribed into complementary DNA by a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA). Then, 10 μl total RNA were treated with 2 μl 10 X RT Buffer, 0.8 μl 25 X dNTPs mix, 2 μl 10X RT Random Primers, 1 μl MultiScribe Reverse Transcriptase, and 4.2 μl DEPC-$H_2O$. Reverse transcription was carried out at 25 °C for 10 min, followed by 37 °C for 120 min, and finally, 85 °C for 5 min, using a Veriti 96-well thermal cycler (Applied Biosystems). cDNA concentration and quality were assessed and quantified using an Epoch spectrophotometer system and Take3 plate (Biotek, Highland Park, USA).

#### 2.5.2. Relative quantification of gene expression

Relative tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and urotensin-2 receptors (UT2R) expression analyses were performed with StepOne Plus Real Time PCR System technology (Applied Biosystems) using cDNA synthesized from rat paw tissues RNA. qPCR was run using TaqMan Probe mix, TaqMan Probe-based technology (Applied Biosystems). Real-time PCR was performed using primers generated for rat TNF-α forward, 5′-GCT CCC TCT CAT CAG TTC CA-3′; reverse, 5′- CTC CTC TGC TTG GTG GTT TG-3′; and rat IL–6 F:5′ TCC ATC CAG TTG CCT TCT TG 3′ R:5′ GGT CTG TTG GGA GTG GTA TC 3′ and rat UT2R forward, 5′-GAC TGG CAC TTT GGA GAT GT-3′; reverse, 5′- CGT TCG CTG CTC ATT ATG GT-3′;, rat β-actin F: forward, 5′-TGG TGG GTA TGG GTC AGA AG-3′; reverse, 5′-GAC AAT GCC GTG TTC AAT GG-3′; Results are expressed as relative-fold compared to control animals. Expression data of β-actin in each tissue was used as endogenous controls. Primers and probes for β-actin were designed by Primer Design (Southampton, UK). For each tissue, triplicate determinations were performed in a 96-well optical plate for both targets using 9 μl of cDNA (100 ng), 1 μl of Primer