Immunohistochemical distribution of COX-1 and COX-2 in the renal tissue of pubere rats treated with capsaicin

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SUMMARY

The objective of this study was to investigate the effects of capsaicin on the renal distribution of cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) in pubere rats. The expression of the 2 enzymes was analyzed using immunohistochemistry in 50 days old rats daily treated with capsaicin (1 mg/kg, subcutaneously) diluted in 10% ethanol, 1% Tween 20 and 80% distilled water for one week, as well as in sham rats (only injected with the vehicle) and in not injected control rats (n = 10 in each group). Weight growth was significantly deleted in the treated rats but no kidney damage was evidenced by conventional histology. The COX-1 and COX-2 localisations were similar in all rats but the COX-2 expression intensity was markedly depressed in the proximal and distal tubules, macula densa and loops of Henle from the capsaicin treated rats whereas the COX-1 expression appeared increased in proximal tubules compared to the sham and control rodents. These results demonstrate that capsaicin which is already known for affect gastrointestinal, cardiovascular and respiratory systems, also interfere with the expression of cyclooxygenases in kidney in rats.

Keywords: rats, capsaicin, kidney, cyclooxygenases, immunohistochemistry.

Introduction

Capsicum annuum L. is an important plant frequently used by humans, in the pharmaceutical industry, chemistry, medicine, and veterinary medicine. Hot green and red peppers are commonly used spices in human food. Capsaicin is the major active ingredient of hot green and red peppers of the plant genus Capsicum [8].

Some available studies state that capsaicin (CAP) decreases the triglyceride concentrations in liver and serum and the amount of fatty tissues by increasing lipid peroxidation and also that capsaicin has an inhibitor effect on the glycogen metabolism of skeletal muscles in an in vitro environment [10, 11]. Capsaicin also decreases the risk of developing arteriosclerosis by decreasing cholesterolemia, and serum triglyceride concentrations [10, 19].

Other studies aim to clarify the direct relation between capsaicin and pain sensation [2, 3, 23]. Capsaicin is included in the group of analgesic substances and is efficient on primary sensory neurons [2, 3, 27]. It is also specified that it is also used in post hepatic neuropathy, diabetic and other neuropathic pains by eliminating the sensitivity of such neurons [2, 3, 27].

A German chemist, Felix Hoffman, had produced acetylsalicylic acid (aspirin), which is the first non-steroid anti-inflammatory drug (NSAID) [25], for the first time in 1893. Although the NSAIDs have been considerably used in the last century, they are the drugs for which the mechanisms of action were not completely known until the structure of COX enzyme was identified in 1971. Until 1990, only one type of cyclooxygenase was considered but because of the considerable extend of molecular biology techniques, 2 types of cyclooxygenases, constitutive (also called COX-1) and inducible (also called COX-2) types, were identified.
Although COX-1 and COX-2 catalyze the same reactions, they have different structures and functions [5]. Besides, a third form called as COX-3, was recently found [26]. COX-1 has a protective effect mostly in the tissues and is efficient in regulating physiological protective functions such as the platelet aggregation and homeostasis in gastrointestinal system and kidneys and the COX-2 enzyme has the exact opposite effect [7]. While COX-1 is expressed in the kidney especially in the veins, smooth muscles and collecting drain cells, COX-2 is expressed in macula densa, cortical thick ascending limb cells of the loop of Henle and the medullary interstitial cells [1, 14].

The purposes of this study were to determine the immunohistochemical localisation of Cyclooxygenase-1 (COX-1) and Cyclooxygenase-2 (COX-2) in rat renal tissues by immunohistochemistry and to identify possible difference in COX-1 and COX-2 expression in pubere rats as example for a development period.

**Material and Methods**

**ANIMALS AND EXPERIMENTAL DESIGN**

This study, approved by the Animal Testing Local Ethics Committee of Kafkas University, was conducted on 30 male pubere (50 days old) Sprague-Dawley rats. Animals were kept in standard cages in an environment at 22 ± 2°C, and were exposed to 12-hour sunlight, and 12-hour darkness and were weighed every day for a week before they were treated.

The rats were randomly divided into three groups, experimental, sham and control groups. Capsaicin (CAP) (Sigma M 2028) was prepared daily by dissolution in 10% ethanol, 1% Tween 20 (Merck M8170772100) and 80% distilled water and was subcutaneously injected in rats from the experimental group at the same time every day for a week utilizing an insulin injector at 1 mg/kg of body weight according to previous studies of MORAN et al. [15] and TÜTÜNCÜ et al. [24]. Rats from the sham group received only the diluent (10% ethanol, 1% Tween 20 and 80% distilled water) according to the same modalities than in the experimental group whereas controls had no application. At the end of the week treatment, all rats were weighed, and then killed using deep ether anaesthesia before removing kidneys. Weight gains (expressed in %) were calculated using the formula: 100 \( \times \) (\( W_f - W_i \)/\( W_i \)), where \( W_f \) was the weight determined at the 7th or at the 14th days and \( W_i \), the initial weight.

**KIDNEY ANALYSES**

After removal, kidney tissues were immediately fixed in a 10% formaldehyde solution, were routinely embedded in paraffin blocks and 5 μm thick serial sections were performed for conventional histology. The 5 μm thick sections were stained with Crossman’s triple stain for histological investigations and other sections were immunostained for evidencing COX-1 and COX-2 in kidney [13].

For immunohistochemistry, the sections were incubated in 3% \( \text{H}_2\text{O}_2 \) for 15 minutes to inhibit endogenous peroxidase activity after undergoing deparaffinization and rehydration. Maximum heat was applied in a microwave for 10 minutes in citrate buffer solution to reveal antigens, after being washed with PBS (Phosphate Buffer Saline). Blocking solution A (Invitrogen Histostain plus Broad Spectrum (AEC) Ref. 85.9943) was dripped onto the sections to reduce nonspecific binding of antibodies. Sections were then incubated with primary antibodies (anti-COX-1 (5F6/F4, ab695, abcam) and anti-COX-2 (M-19, sc-1747, Santa Cruz)) diluted to 1:200 in PBS at room temperature for one hour. Only PBS was dripped on the tissues of the negative control group. Streptavidin-biotin peroxidase technique was used after primary antibody incubation [9]. For this purpose, a Broad Spectrum Antibody (Invitrogen Histostain plus Broad Spectrum (AEC) Ref. 85.9943) antibody, counter to the type produced by the primary antibody, was added onto sections, and left at room temperature for 15 minutes. Streptavidin-HRP (Horseradish Peroxidase) (Invitrogen Histostain plus Broad Spectrum (AEC) Ref. 85.9943) was dripped on the sections, and then incubated at room temperature for 15 minutes. After adding 3-Amino-9-Ethylcarbazole (AEC) Staining Kit solution, the sections were placed under a light microscope to check for immunoreactivity; the reaction was inactivated with distilled water in accordance with the immunoreactivity status. It was then dipped in haematoxylin for negative staining. A lamella was covered the kidney tissue after dripping water-based glue on them (Labvision, Large Volume Vision Mount, TA-060-UG). The slides were examined using light microscope, and their photographs were taken. Scoring was conducted using a semi-quantitative method on an area of the sections, based on criteria of the percentage of stained cells and the degree of staining. The degree of staining was evaluated as follows; 0 (no staining), 1+ (weak), 2+ (moderate) and 3+ (strong staining).

**STATISTICAL ANALYSIS**

Data were expressed as mean ± standard deviation and were compared using one-way ANOVA and Tukey HSD test. Differences were considered as significant when \( p \) value was less than 0.05.

**Results**

As shown in Table I, it was observed that the weekly weight gains were similar in the 3 groups (experimental, sham and control) before treatment. By contrast, at the end of the week treatment, weight gains have considerably differed according to the groups: weights measured on day 14 (157,78 ± 26,65 g) in rats treated with capsaicin (CAP) were slightly lower than those determined on day 7 (158,02 ± 24,65 g) and the overall weight gain was only 9.05% whereas it reach 17.46% and 25.60% in the sham and control groups, respectively (experimental group vs. sham and control groups: \( p < 0.05 \)).
Histologically, normal kidney structures were observed in all rats (experimental, sham and control groups). No difference was obtained between the 3 groups.

COX-1 and COX-2 immunoreactivities were observed by immunohistochemistry in kidneys from all rats (Table II). A weak (1+) COX-1 expression was seen in the medulla in all groups and in the proximal tubules of the sham and the control groups (figure 1B), whereas it was moderate (2+) in those of the capsaicin treated rats (figure 1A). No COX-1 immunoreactivity was detected in the glomeruli, vascular endothelium and distal tubules in the 3 groups. On the other hand, COX-2 immunolabelling was moderate to dense (proximal and distal tubules) and dense (macula densa and loops of Henle) in the renal structures from sham and control rats (figures 2C and 2D) and remained moderate in the experimental group except in some proximal convoluted tubules (figures 2A and 2B).

**Discussion**

In the present study, it was observed that capsaicin treatment for one week has significantly delayed weight growth in pubere rats. TUTUNCU et al. [24] also reported that the body weight of rats receiving capsaicin was lower in comparison to the control rats and numerous studies support the view that capsaicin reduces body weight gains [4, 11, 15, 17, 18, 21]. The effects on weight growth may be related to the increases in carbohydrate and lipid mobilisation and catabolism and in liver enzyme activities [24].

Normal kidney histological results were obtained in rats treated or not with capsaicin in the current study, suggesting that capsaicin is probably not nephrotoxic.

It was stated that capsaicin or chili pepper administration to rats for a long time is effective in the treatment of the lesions produced by agents such as alcohol, aspirin or stress in the stomach, and plays a protective and analgesic role against damages [22]; it was determined that afferent neurons that are sensitive to the capsaicin are effective on this situation [22]. Cytokines, growth factors, tumour necrosis agents, pro-inflammatory stimulants such as bacterial endotoxin rapidly causes COX-2 expression in inflammatory cells [20]. Prostaglandins (PGs) produced by COX-2 play a major role in inflammatory reactions and are responsible for characteristic inflammatory symptoms such as rubor, fever, tubercle, pain and functional loss [20]. As capsaicin acts on primary sensory neurons, the analgesic effect of capsaicin may be mediated by removing sensitivity of these neurons [2, 3, 27].

In humans, mice and rats, COX-1 immunoreactivity is evidenced in proximal convoluted tubules both in cortex and medulla but the enzyme is expressed neither in glomerulus nor in epithelial cells of distal tubules [1, 14, 28]. In this

<table>
<thead>
<tr>
<th>Renal structures</th>
<th>COX-1</th>
<th>COX-2</th>
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<tr>
<td></td>
<td>Experimental group</td>
<td>Sham and Control groups</td>
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<tr>
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<tr>
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<td>Weak</td>
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<tr>
<td>Distal tubules</td>
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<td>No</td>
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<tr>
<td>Macula densa</td>
<td>No</td>
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<td>Loop of Henle</td>
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Table II: Semi-quantitative COX-1 and COX-2 expression detected by immunohistochemistry in kidneys from pubere rats subcutaneously treated daily at the 7th day for one week with capsaicin (1mg/kg in 10% ethanol, 1% Tween 20 and 80% distilled water, experimental group), with diluent (10% ethanol, 1% Tween 20 and 80% distilled water, sham group) or no treated (control group) (10 rats per group).
Figure 1: Kidney section from a rat treated with capsaicin (1 mg/kg/day SC for 7 days). Moderate COX-1 immunoreactivity is seen in the proximal convoluted tubules (A.); Kidney section from a control rat (no treatment). Weak COX-1 immunoreactivity is seen in the proximal convoluted tubules (B.). G: Glomerulus; PCT: Proximal convoluted tubule; TD: Distal tubule.

Figure 2: Kidney section from a rat treated with capsaicin (1 mg/kg/day SC for 7 days). The COX-2 expression was dense in some proximal convoluted tubules and moderate in distal tubules and macula densa (A.) and moderate in the loops of Henle (B.); Kidney section from a control rat (no treatment). The COX-2 immunoreactivity was moderate in distal tubules, moderate to dense in proximal tubules and dense in macula densa (C.) as well as in the loops of Henle (D.). G: Glomerulus; PCT: Proximal convoluted tubule; TD: Distal tubule; MD: Macula densa; LH: Loop of Henle. Immunohistochemistry, Bar:100 µm.
study, the presence of COX-1 was similar. The presence of low amount of COX-2 mRNA was determined in the renal tissues in 1993 for the first time [16]. In situ hybridisation and immunohistochemical methods demonstrated that the COX-2 was located in the macula densa cells, in distal tubules, medullary interstitial cells, and in the epithelial cells of the thick ascending limb of the loop of Henle in the cortex [6]. In humans, the COX-2 protein has been revealed in the endothelium and smooth muscle cells in arteries and veins and in podocytes in glomeruli, as well as in the macula densa cells [12].

The COX-2 expression in the present study was also found in all parts of the nephron except in glomerulus. However, in the present study, the COX-1 expression in rats treated with capsaicin for one week was strengthened in the proximal tubules and the COX-2 expression was depressed mainly in macula densa, distal tubules and loops of Henle compared to the rodents not receiving the analgesic substance (sham control groups), indicating that the inflammatory prostaglandin synthesis involving the COX-2 enzyme was markedly alleviated in kidneys.

As a conclusion, it is well known that Non-Steroid Anti-Inflammatory Drugs have very different activities in kidneys. Studies conducted with specific COX-2 inhibitors such as capsaicin, would open up the route to clarify with more accuracy the particular roles of COX-1 and COX-2 in renal physiology.

Notes

This study has already been presented at the XIth National Histology & Embryology Congress (With International Contribution), 16-19 May 2012, pp.:34 (Abstr.) Denizli Turkey.

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