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Effects of Indomethacin on the Expression gene of cyp27b1 Gene in Kidney Cortex and on Aldosterone Renin Ratio in Male Rats

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Abstract

Indomethacin is Non-steroidal anti-inflammatory medications which suppresses the activity of cyclooxygenase (1 and 2). The kidneys participate in the excretion of many pollutants and medication making them susceptible to releasing significant amounts of free radicals. These free radicals lead to elevated oxidative stress, which have role in the tubular necrosis and the renal damage. The renal side effects, such as changes in kidney function and the excretion of fluid and urine electrolytes, depend on the degree of selectivity between COX-2 and COX-1 and the dosage of these medications. Our study explains the effect of indomethacin on cyp27b1 gene expression in the cortex of the kidney and Aldosterone renin ratio with different dosage. Four groups of 40 rats, control group with 10 rats, G2, G3 and G4 each one with ten rats also placed in the animal house of Al-qadyisiah university. G1 drugged P.B.S , G2, G3 and G4 drugged 5, 7, 10 mg/kg Indomethacin solution. After 3 weeks, the animal sacrificed and small part of the kidney is extract and placed in −70 °C liquid nitrogen for molecular study and serum is collected for physiological study. mRNA extraction is done by special kit. Analysis of gene expression data in Kidney shows down-regulation of cyp27b1 in all treated compared with the control. ARR show significant increase in 10 mg/kg indomethacin compared with control group and other treated groups. In conclusion the expression of cyp27b1 gene in kidney decrease with increase doses concentration., and aldosterone renin ratio increased with increase indomethacin doses

Key word: Indomethacin, cyp27b1, kidney, renin, rat

تأثير الاندوميثاسين على التعبير الجيني لجين CYP27B1 في قشرة الكلية وعلى نسبة الالدوستيرون الى الرينين في ذكور الجرذان

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خلاصة: الاندوميثاسين هودواء مضاد التهاب غير استيرويدي يعمل على كبح فعالية انزيم سايكلواوكسيجينيز (1 و2) . الكليتين تشارك في افراز العديد من الملوثات والادوية مما يجعلها عرضه لأفراز كميات محسوسه من

الجذور الحرة. هذه الجذور الحرة تؤدي الى زيادة الجهد التأكسدي، والذي له دور في تنخر نبيبات الكلية وتحطيم الكلية ز التأثير الجانبي على الكلية ، مثل التغيرات في وظيفة الكلية وفي افراز الالكترولايت في السوائل والادرار يعتمد على درجة الانتقائية بين انزيم سايكلواوكسيجينيز (1 و2) وجرعة الدواء . دراستنا توضح تأثير الاندوميثاسين بجرع مختلفة على التعبير الجيني لـ cyp27b1 في قشرة الكلية وعلى نسبة اللالدوستيرون للرينين . اربعة مجاميع و40 ذكر جرذ ، مجموعة السيطرة بـ 10 جرذان والمجموعة الثانية والثالثة والرابعة كل منها ب10 جرذان ايضا وضعت في البيت الحيواني بجامعة القادسية . المجموعة الاولى جرعت المحلول الفوسفاتي المنتظم ، المجموعة الثانية والثالثة والرابعة جرعت 5 و7 و 10ملغم/كغم محلول الاندوميثاسين. بعد ثلاثة اسابيع ، قتلت الحيوانات ، وجزء صغير من قشرة الكلية استخرجت ووضعت في النتروجين السائل لغرض الدراسة الجزيئية وجمع السيروم لغرض الدراسة الفسلجية .استخلاص الـ mRNA تم بعدة خاصة . بيانات التحليل الجيني في الكلية اظهرت تقليل في التعبير الجيني لـ cyp27b1 ($p< 0.05$) في كل المجاميع المعاملة مقارنة بمجموعة السيطرة. نسبة الالدوستيرون الى الرينين تظهر زيادة معنوية ($p< 0.05$) في المجموعة المعاملة ب 10 ملغم/ كغم اندوميثاسين مقارنة مع مجموعة السيطرة والمجاميع الاخر. نستنتج ان التعبير الجيني لـ cyp27b1 في الكلية ينخفض بزيادة تركيز الجرعة ، و نسبة الالدوستيرون الى الرينين تزداد بزيادة جرعة الاندوميثاسين .

الكلمات المفتاحية : اندوميثاسين ، الجين cyp27b1 ، الكلية ، الجرذ ، الرنين

Introduction:

The drug indomethacin decreased prostaglandin synthesis is the cause of indomethacin's analgesic, antipyretic, and anti-inflammatory effects (1), also produces free radicals, which add to the body's sense of oxidative stress, according to (2). However, oxidative stress is a condition that is brought on by oxygen acting as a free radical (3). At 2016 research by (4) and associates Recent research has shown that indomethacin may be to blame for pre-oxidant activity and the activation of lipid peroxidation via the ROS generation.

Indomethacin binds to a site on the mitochondrial electron transport chain that is adjacent to complex I and ubiquinone, according to study by (5), as a consequence, proteins and lipids were oxidized and damaged DNA.

According to (6), the use of indomethacin may result in a significant reduction in renal function due to indomethacin can decrease the filtration rate and increase the urine volume. CYP27B1 has a role in the metabolic process of vitamin D (7). The conversion of vitamin D3 to its active form takes place in the kidney by the cytochrome P450 enzyme CYP27B1.

Because they prevent prostaglandin synthesis, NSAIDs have been linked to renal problems. These issues result from decreased renin and aldosterone production and increased ADH activity (8)

Because of the significant medical value of indomethacin as an anti-inflammatory medicine, and in order to determine the subsequent biological effect of his drug when it is used often and in large dosages, the purpose of the current

research is to emphasize the impact of indomethacin on the gene expression of (CYP27B1 in the kidney of a male Wister rat owing to the toxic effects of indomethacin.

Material and method

A total of forty adult male Wistar rats with a weight (200-220) grams and an age (2-3) months were plased in animals house of University of Al-Qadisiyah-Faculty of Science. There are four groups, with each group (ten male Rats).

Experimental Groups

The drug Indomethacin powder from MACKLIN company / china dissolves in P.B.S solution, with continuous shaking, administrated orally and daily for 21 day as followed:

• Control received PBS (3) ml.

• G2 received dose of 5 mg/kg (9)

• G3 received dose of 7 mg/kg

• G4 received dose of 10 mg/kg (10)

Molecular Assay

RNA Extraction

Small part of Kidney and tissues frozen in nitrogen at −70 °C. Protein or RNA may be obtained from tissue samples by using an extraction kit. The purity and concentration of RNA were determined using a NanoDrop. The RNA amount was determined by measuring the absorbance at 260 nm and calculating the ratio of A260/A280, which fell between 2.0 and 2.23. The process of increasing the amount of CYP27B1 was carried out by using the primers indicated in Table 1.

Table (1): the used primers for cyp27b1 gene

Primer	Sequence	gene
CYP27B1-F	TTCTCAGACACGATCTATGGCTGT	CYP27B1
CYP27B1-R	CTACTGTCTCTGCAGAAAGCGTA	
GAPDH-F	GTGGACCTCATGGCCTACAT	Hk gene
GAPDH-R	GGATGGAATTGTGAGGGAGA	

Quantitative Reverse Transcriptase PCR (RT-qPCR) Preparation

RT-qPCR:

The amplification was done by AddScript RT-qPCR Syber master (AddBio company, Korea). The reply included.

QPCR- PCR Master Mix for gene (CYP27b1)

Add 6 µl of H2O, 10 µl of Script RT-qPCR, 1 µl of F-primer (0.05 pmol), 1 µl of R-primer (0.05 pmol), and 2 µl of cDNA. The sum is 20 µ in total. The cDNA used in this study was derived from samples of the kidney cortex and targeted the CYP27B1 gene.The aforementioned components were added to a qPCR tube, which was then centrifuged at a speed of 3,000 rpm for a duration of 2 minutes. Subsequently, the tube was transferred to a qPCR machine.

The thermal condition

Is denaturation 95 C ,anneling 60 C, extension 72 C and melting point 95 C.

RT-qPCR data:

The delta-delta Ct was applied to normalise transcript amounts to the amount of 12SrRNA mRNA, as outlined by reference (11). This approach used the following formula: The formula for 2 -ΔΔCT is derived by subtracting the difference between the interested gene and the control in sample B from the variance between the interest gene and the control in sample A. Sample A denotes a particular cohort. Sample B pertains to a discrete and particular cohort.

Physiological study

Detection of Serum Parameter

Serum should be collected since it does not undergo hemolysis. The serum and cells were separated two hours after the sample was taken.

Detection of Kidney Hormone

Hormone was messured by Immunodignosticsystems IDS Direct Renin

Determination of Renin Hormone

Principle:

This test makes use of the chemiluminescence method, which was created by (12)

Determination of Aldosterone Hormone

Principle:

The IDS Aldosterone test uses a chemiluminescence technology that was developed by (12)

Statistical analysis

The F-test was used to compare the control group with the treatment group after the results were analyzed using (Mean Standard Error). Using a one-way analysis of variance (ANOVA) and the test (Tukey's multiple comparisons test) at a probability threshold of 0.05, the current study looked at whether there were any significant differences in the averages of the several groups that were included in it.

Result

Amplification CYP27B1 gene in kidney are manifestaed in figure (1).

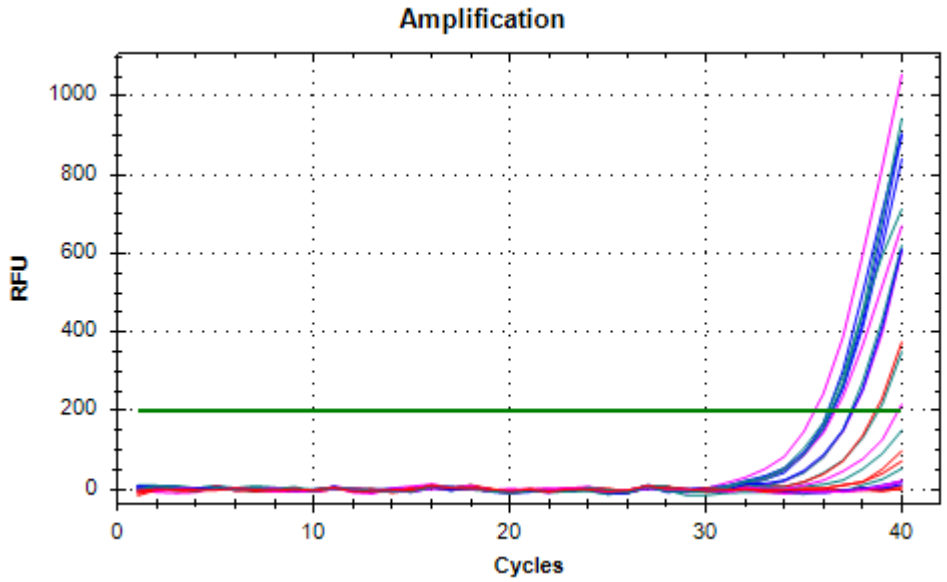

Figure ge CYP27B1 hesis.

Pink curves = the group treated with 5 mg of indomethacin.
Green curves = the group treated with 7 mg of indomethacin
Blue curves = control group.
Red curves = the group treated with 10 mg of indomethacin.

Analysis of expression of CYP72B1 gene in kidney:

This research examined the expression gene of cyp27b1. The results shown in Figure 2 demonstrate a marked reduce at ($P < 0.05$) in the expression of genes in the indomethacin treatment groups at doses of 5 mg, 7 mg, and 10 mg/kg throughout the 21-day trial period. There were no marked effects in gene

expression at (P< 0.05) among the groups treated with several doses (5, 7, and 10) mg, as shown in figure (2).

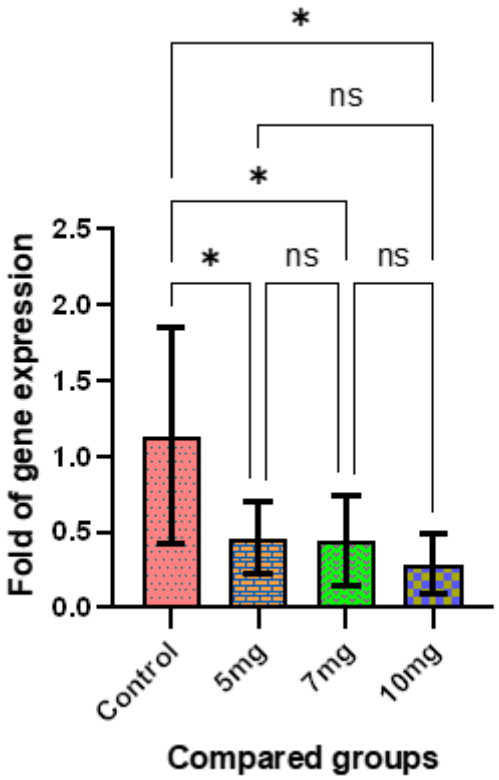

Figure (2): Fold change comparison between the groups expressed CYP27B1 gene. This shows significant down-regulation of the treatment groups (5, 7 and 10 mg of indomethacin) compared with control group.

Fold change in Control = 1.1.
Fold change in treatment with indomethacin (5 mg) = 0.45
Fold change in treatment with indomethacin (7 mg) =0.43
Fold change in treatment with indomethacin (10 mg) = 0.28

Effect of indomethacin in aldosterone renin ratio

Figure 3 demonstrated that indomethacin doses of 5 mg/kg and 7 mg/kg do not show significant changes in aldosterone renin ratio compared with control group and between themself (p< 0.05), while 10 mg/kg showed significant increase (p< 0.05) compared with 5 , 7 mg/kg and control groups.

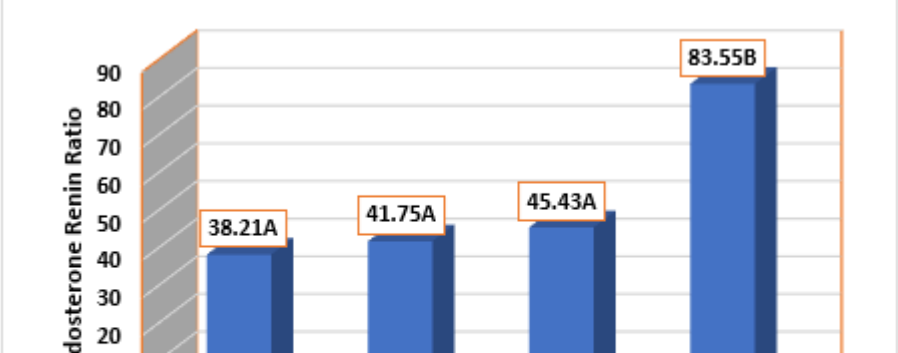

Figure 3: Effect of indomethacin at dose 5,7,10 mg/kg on Aldosterone Renin Ratio of male wister rat during 21 day.

(No. of animals=10 for each group, Values are mean ± SE, L.S.D. ($p<0.05$) =17.81, Different letters denote to the significant difference ($p<0.05$))

Disscusion

We found that indomethacin, which works by inhibiting the expression of the CYP27B1 gene, considerably reduce 25(OH)D and 1,25(OH)2D levels (13). Indomethacin is able to do this by inhibiting the formation of prostaglandins, which in turn disrupts the normally occurring in rabbits creation of 25(OH)D and 1,25(OH)D during the first stages of the pregnancy.

Indomethacin and prostaglandins normalized the production of vitamin D metabolites (13).According to (14), PGE and PGF2 stimulate 1,25(OH&D production in renal cells via cAMP. Without cAMP or PTH, PGE enhances the production of l,25(OH)2D in vitamin D-deficient rats (15). Other PG inhibitors change the vitamin D metabolites. In vitro, aspirin prevents 1,25(OH)2D and PG production (16).

Due to the fact that progesterone production was similarly lowered by indomethacin therapy and was then restored to normal levels by PG administration (17), PGs may mediate steroidogenesis.

Acute N-acytel-para- aminophenol (APAP) toxicity, a selective cox-2 inhibitor (18), may alter liver and kidney production and VD responses by inhibiting the synthesizing enzymes (cyp27b1) and vitamin D receptor VDR and upregulating the catalyzing enzyme and VDBP, (19) hypothesized in light of previous studies and our findings. The aberrant circulatory 25-OH VD levels and VD-regulatory molecule expression may be caused by APAP toxicity-induced cellular damage,

since liver and renal disorders change VD metabolism and systemic levels. The inflammatory and oxidative formting APAP-induced liver and kidney tissues damage, on the other hand, may be exacerbated or contributed to by aberrant expressions of the hepatorenal VD molecular network (20). This is supporting our result.

Recent studies show that NSAIDs block COX-1 and COX-2 activity, which are enzymes regulate the prostaglandins and thromboxanes production that are situated in the kidney. Inhibiting of the mentioned enzymes have an impact on several aspects of kidney function (21). Widespread necrosis, erosions, and renal glomerular, and tubular degeneration in the study of (22) this is another evidence for effect the kidney function.

The drug indomethacin mirrored hypoprostaglandinism syndrome symptoms, including sodium and potassium retention, hyporeninismb, hypoaldosteronism, decreased urine PGE clearance, hyperkalemia, and a small but significant increase in diastolic blood pressure, raising concerns about its use in renal diseases. [23,24]
Indomethacin significantly lowered aldosterone levels by 43% and plasma renin activity by 58% in hypertensive subjects with disturbed renin-aldosterone relationship.In our study, group treated with 10 mg/kg demonstrated significant increase and that disagree with previous study , this may be due to other reason such as diet, potassium concentration, animal type , doses given to animal .
In conclusions NSAID especially indomethacin inhibit both COX (1and 2) subsequently affect renal function and down regulate gene expression with increase dose concentration and increasing Aldosterone renin ratio may be because other reason.

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