Partial protection of verapamil against gentamicin nephrotoxicity in rats

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الخلاصة:

تم استخدام عدد (40) من ذكور الجرذان لاختبار الحماية الكافية لمادة عقارالفيراباميل لمثبطات موانع دخول الكالسيوم ضد التأثير السمي الكلوي لعقار الجينتمايسين وتم تقسيم الفئران إلى أربعة مجاميع: المجموعة الأولى مجموعة السيطرة والتي تم إعطائها الماء فقط، المجموعة الثانية تم إعطائها عقار الجنتمايسين عن طريق الغلاف البريتوني بجرعة (80 مل) بالكيلوغرام، المجموعة الثالثة تم إعطائها الفيراباميل عن طريق الفم بجرعة (10مل) بالكيلوغرام ، المجموعة الرابعة تم إعطائها عقارالجنتمايسين مع عقار الفيراباميل بنفس الوقت وكانت مدة الدراسة عشرة أيام.

تم إحداث التسمم الكلوي باستخدام عقار الجنتمايسين لمدة سبعة ايام وتم التأكد من التسمم الكلوي بواسطة ارتفاع اليوريا في الدم وارتفاع الكرياتنين في الدم عند المقارنة مع مجموعة السيطرة، كانت زيادة اليوريا في الدم من 20± 1.4 الى 205±7 وزيادة الكرياتنين من .75 0 ± 0.08 الى 4.2 ± 0.3 مل/100 مل. وجد إن إضافة عقار الفيراباميل مع عقار الجنتمايسين قلل ارتفاع اليوريا والكرياتنين الى 90±13 و1.5 ± 0.9 مل/100 مل بالتعاقب.

نتائج هذا البحث تبين إن إعطاء عقار الفيراباميل يقلل من شدة الفشل الكلوي الحاد في الفئران التجريبية هذا يدعم الدراسات السابقة التي تبين أن مثبطات دخول الكالسيوم لها القدرة على حماية الكلية والميكانيكية المسؤولة عن هذه الحماية يحتاج إلى دراسات أكثر.

Abstract:

A number of male rats (40) were used to test the protective efficacy of verapamil a calcium channel blocker (CCB) against gentamicin nephrotoxicity. They were divided into four groups: group-1 control, giving only water, group-2, they were administered gentamicin IP 80 mg/Kg, group-3, administered verapamil orally 10 mg/Kg and group-4 administered gentamicin and verapamil at the same time. The period of study was 10 days.

Nephrotoxicity was induced by using gentamicin for 7 days; this was evidenced by marked elevation in blood urea nitrogen (BUN) andplasma

creatinine (Pcr) when compared with control. BUN increased from 20±1.4 to 205±7 and Pcr from 0.75±0.08 to 4.2±0.3 mg/100 ml). Coadministration of verapamil with gentamicin decreased the rise in BUN and Pcr, their values reached to 90±13 and 1.5±0.9 mg/100 ml respectively.

Our data suggest that supplementation of verapamil decreased the severity of acute renal failure (ARF) in rats, this supports previous studies in which CCBs offer renoprotection in ARF, the exact mechanism of this protection need further study.

Key words: Acute renal failure, Plasma creatinine, blood urea nitrogen, calcium channel blockers, Endothelium derived relaxing factor

Introduction:

The aminoglycosides including gentamicin are used primarily to treat infections caused by aerobic gram negative bacteria. Although they are widely used, serious toxicity is a major limitation to their usefulness. Nephrotoxicity is the major side effects of aminoglycosides, accounting for 10-15% of all cases of acute renal failure ARF [1]. The toxicity results from accumulation and retention of aminoglycoside in the proximal tubular cells [2].

Recently the cellular and metabolic pathogenetic factors of ARF received consideration. These factors are of particular importance and may account for both hemodynamic and nephronal factors in the pathogenesis of ARF. Aminoglycosides inhibit various phospholipases, sphingomyelinases, and ATPases, and they alter the function of mitochondria and ribosomes. Because of the ability of cationic aminoglycosides to interact with anionic phospholipids, these drugs may impair the generation of membrane derived autacoids and intracellular second messangers such as prostaglandins, inositol phosphates, and diaceylglycerol [3].

Over the past several years, a large number of data has established that Ca plays a critical modifying role in the pathogenesis of both ischemic and toxic cell injury [4]. In this regard, drugs that block Ca influx across plasma membranes were used to test their protective efficacy in toxic and ischemic ARF. Verapamil and nifidipine which are chemically dissimilar CCBs have been shown to exert substantial functional, cellular and morphological protection against experimental ischemic ARF [5]. This study confirmed the findings of Malis in which verapamil was proposed to be protective in ischemic ARF [6]. Verapamil was also shown to prevent the first changes in renal functions during the cisplatine treatment in testicular patients [7].

Other studies have demonstrated an increase in renal cortical lipid peroxidation in gentamicin treated rats [8], so many different chemical agents were used to protect against nephrotoxicity on both animal models and human subjects because of their antioxidant properties, such as probucol [9], hydroxyl radicals[10]

against gentamicin nephrotoxicity, melatonin [11] against cyclosporine nephrotoxicity.

Based on these observations, we tested the effect of verapamil on the severity of gentamicin induced nephrotoxicity in rats.

Aim of study:

Our research aimed to test the effect of verapamil, a calcium channel blocker, on severity of ARF induced by gentamicin in rats.

Materials and Methods:

Experimental protocol:

All experiments were performed on male albino rats weighing 200-300 gram. The animals were divided into four groups; in each group we used 10 rats. The period of experiment is 10 days.

Group-1: (Control): They were administered 1 ml of water for 10 days.

Group-2: They were administered verapamil (10 mg/kg) orally three times daily for 10 days.

Group-3: They were administered gentamicin as daily injection IP in a dose of (80 mg/Kg) for 7 days to induce ARF.

Group-4: They were administered verapamil (1 0 mg/kg) orally three times daily 3 days prior to induction and cocurrently with gentamicine (80mg/Kg) for 7 days.

* Verapamil was supplied as tablets of 40 mg (Evans), at the time of administration; it was dissolved in water to get a solution of 2.5 mg/ml.

* Gentamicin was supplied as vials of 2ml.

Sample collection:

Rats were given heparin Sc in a dose of 2000 unit/Kg. Blood was collected in small glass tubes by cutting the tip of the tail immediately after injection. It was centrifuged and 1 ml of plasma was removed for creatinin determination and 0.01 ml for urea determination.

Biochemical assays:

Blood urea nitrogen BUN was determined manually by using Barthelot reaction. Plasma creatinin Pcr was determined by using Jaffe reaction.

Statistical methods:

For comparison between different groups, all results were expressed as mean +SE. The significance of difference between means was evaluated by using student's t-test. The difference was considered significant when ($p < 0.05$) or less.

Results:

The effect of gentamicine, verapamil and their coadministration to rats on BUN and Pcr are summarized in table-1 and plotted in figures (1and2).

Verapamil alone (Group-2) had no effect on the renal parameters; since BUN and Pcr were normal and non-significantly different when compared to control rats (Group-1).

A marked increase in BUN and Pcr was noted in gentamicin treated group (Group-3) compared to control (Table-1). This means that, ARF was developed after 7 days of gentamicin dosing. BUN and Pcr increased to (205±7 and 4.2±0.3) respectively which are significantly different when compared to control with ($P< 0.001$).

Coadminstration of verapamil with gentamicin (Group-4) decreased the rise in BUN and Pcr, (i.e. less severe ARF was developed in rats) because BUN and Pcr were high but significantly less than Group-3. There values were (90±13 and 11.5±0.9 respectively) with $p< 0.01$ compared to Group-3.

Renal parameters	Group-1 N=10	Group-2 N=10	Group-3 N=10	Group-4 N=10
BUN	20 ± 1.4	20± 0.5 Ns	205 ± 7*	90 ± 13.3**
Pcr	0.75 ±0.08	0.75±0.1Ns	4.2 ±0.3*	1.5 ±0.9**

Table-1: **Summary of BUN and Pcr values (mg/100 ml) during the period of the study (10 days).**

Group-1=Control
Group-2=Verapamil
Group-3=Gentamicin
Group-4=Verapamil+gentamicin.
N=number of rats
Ns=Non significant Vs control.
* = $P < 0.001$ Vs control
** = $p < 0.01$ Vs gentamicin

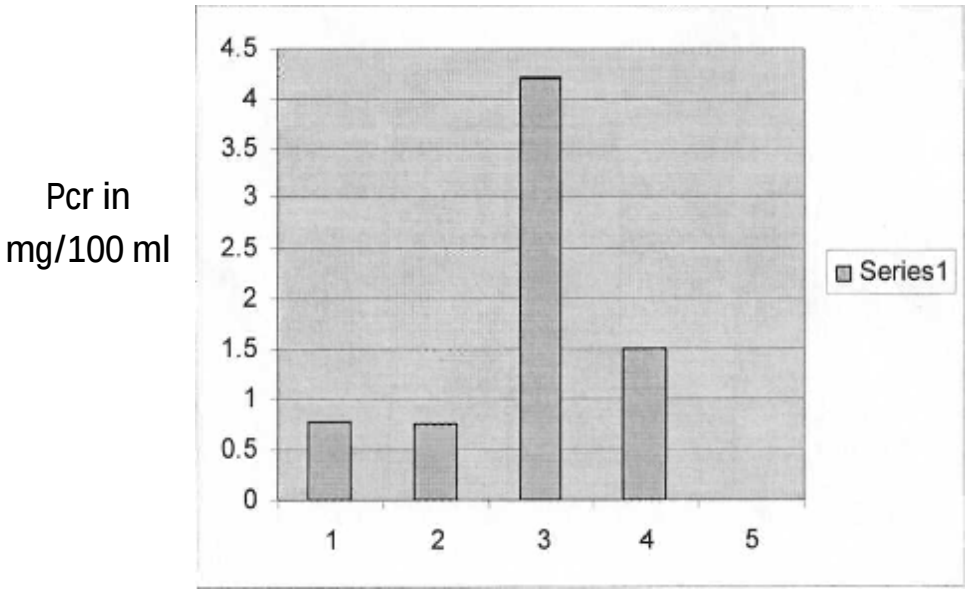

Figure-1: Effect of gentamicin and verapamil on Pcr in different groups of rats.

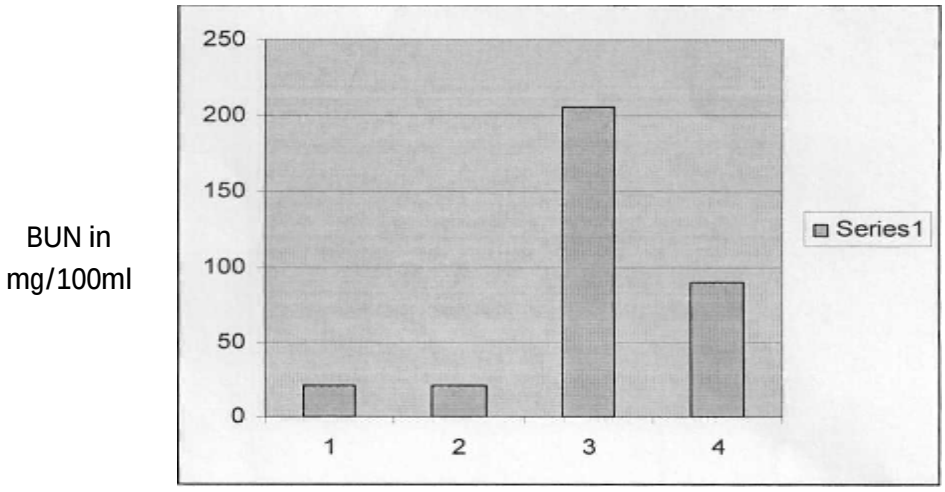

Figure-2: Effect of gentamicin & verapamil on BUN in different groups of rats.

Group-1=Control Group-2=Verapamil
Group-3=Gentamicin Group-4=Verapamil+gentamicin.

Discussion:

Gentamicin nephrotoxicity which was used in this study is one of the main methods have been used to induce ARF in rats and has been extensively investigated. The dose and route of administration of this antibiotic that used are variable.

In our work, we used 100 mg/Kg of gentamicin IP for 7 days. The results confirmed that gentamicin at this dose produces significant nephrotoxicity and this evidenced by increase in BUN and Pcr.

Calcium channel blockers CCBs have been shown to prevent or reduce the severity of postischemic ART [12]. Other study proved that nifdipine has beneficial effect in cyclosporine nephrotoxicity [13]. Dilitiazem, which is one of CCBS also can offer some benefit in cyclosporine induced nephrotoxicity [14].

Coadminstration of verapamil with atrial natriuretic peptide can prevent gentamicin induced ARF in the rat [15]. Verapamil when coadminstered with cimetidine also provide partial protection against cisplatine induced ARF in human [16].

Several mechanisms were proposed for the protection of ARF by CCBs. The mechanism of protection is different between the different chemical groups; nifidipine decreases the glomerular hypertrophy, while dilitiazem and verapamil reduce the intraglomerular pressure [17].

Other study reported that CCBs protected animals from cyclosporine nephrotoxicity because of their antioxidant properties [18]. Verapamil could prevent the rise in Pcr and due to contrast media in which it proved that there is a decrease in nitric oxide formation [19]. Another study reported that verapamil and nifidipine could block the abnormal sensitivity of the renal vessels to ischemic ARF which was contributed to a change in smooth muscle Ca and endothelial derived relaxing factor (EDRF) [20].

The present study provides evidence that coadministration of verapamil along with gentamicin decreased the severity of gentamicin induced nephrotoxicity in rats.

The mechanism of its renoprotective cannot explained exactly, but we expect that it was either due to its effect on Ca channels and dilation of renal vessels that preserve the renal function, or it may be due to its antioxidant properties (like other CCBs) that inhibit renal injury.

Conclusion;

In summary, the present study provides evidence that support previous studies in which the administration of verapamil prior and with gentamicin can offer partial protection against ARF in rat. The mechanism of this effect needs further study.

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