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The nephroprotective effect of some vasodilators and vitamins in experimental model of acute renal failure

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Abstract

***Background*: Myoglobinuric ARF is an example of intrinsic type of renal failure induced by intramuscular injection of glycerol.**

***Materials and methods*: this study has been conducted using 36 local domestic rabbits. They were separated in to 6 groups, each one has been pre-treated with a test agent (vitamin C and E, amlodipine, carvedilol and verapamil) 2 hours prior to administration of glycerol. The changes in renal function have been followed up by monitoring the levels of BUN, serum creatinine, $K^+$and Na^+ after 3 and 7 days of the induction.*Results:* pretreatment with vitamin C, vitamin E, carvedilol and verapamil cause significant reduction in the levels of BUN, serum creatinine and K^+ and significant elevation of serum $Na^+$when compared with the control group. While pretreatment with amlodipine causes significant elevation of BUN, serum creatinine and K^+. *Conclusion*: Vitamin C, E, carvedilol and verapamil have a significant nephroprotective effect at the tested doses in this model of acute renal failure with a possible use in prevention of acute renal failure.**

Key words: nephroprotective, vasodilators, vitamins, acute renal failure

ملخص البحث:

تمهيد: العجز الكلوي الحاد هو متلازمة تتميز بالانخفاض الحاد في معدل الترشيح الكبيبي، تراكم نواتج الايض النيتروجينية مع اضطراب في حجم السائل خارج الخلوي و اختلال التوازن الأيوني وتوازن الحامض و القاعدة في الجسم.

في هذه الدراسة تم استخدام العجز الكلوي الحاد الناجم عن الميوغلوبين كنموذج تجريبي، وهو احد أنواع الجز الداخلي المنشأ. اذ ينتج عن تلف العضلات الهيكلية و تحرر محتويات الخلية العضلية خاصة الميوغلوبين إلى الدورة الدموية مسببا تدهورا سريعا في وظيفة الكلية.

الهدف: الهدف من الدراسة هو استكشاف التأثير الواقي للكلية المحتمل لبعض الأدوية الموسعة للأوعية الدموية (كارفيدايلول، املودبين و فيراباميل) و بعض الفيتامينات المضادة للتأكسد (فيتامين سي و أي).

الطرق: تم استخدام ست و ثلاثون أرنب محلي حيث قسمت إلى ست مجاميع و كانت إحداها مجموعة سيطرة. لقد حقنت الحيوانات فيها بمادة الغليسيرول بتركيز ٥٠% عضليا و بجرعة ٩ مليلتر لكل كيلوغرام من وزن الجسم لغرض إحداث العجز الكلوي، أما بقية المجاميع فقد تمت معالجة كل مجموعة باحدى المواد المراد فحصها أعلاه وقبل إعطاء الغليسيرول بساعتين. قيمت وظيفة الكلية من خلال قياس مستوى اليوريانيتروجين في الدم و مستوى كل من الكرياتنين و البوتاسيوم و الصوديوم في مصل الدم لمرتين، إحداهما بعد مرور ثلاثة أيام على إحداث العجز الكلوي و الأخرى بعد مرور سبعة أيام، ثم قورنت النتائج بالقيم السوية و بنتائج مجموعة السيطرة لتقييم تأثير المواد المختبرة الواقي للكلية.

النتائج: بينت الدراسة أن المعالجة اليومية بجرعة واحدة من فيتامين سي (٢٥٠ ملغم/كغم/اليوم) أو فيتامين إي (٢٠٠ ملغم/كغم/اليوم) تعطى فمويا للحيوانات مع تكرارها لثلاثة أيام متعاقبة بعد إحداث العجز الكلوي أدت إلى انخفاض معتد لمستويات كل من اليوريانيتروجين و الكرياتنين و البوتاسيوم وارتفاع لمستوى الصوديوم في المصل عند مقارنته مع مجموعة السيطرة مع بقاء مستوى الصوديوم قريبا من المستوى الطبيعي.

ان المعالجة بكل من كارفيدايلول (٦,٢٥ملغم/كغم) و فيراباميل (٢,٥ملغم/كغم) كجرعة واحدة تعطى فمويا قبل إحداث العجز الكلوي سبب نقصا معتدا لمستويات كل من اليوريانيتروجين و الكرياتنين و البوتاسيوم وارتفاع لمستوى الصوديوم في المصل عند مقارنته مع مجموعة السيطرة، في حين أن استخدام املودبين(١,٢٥ملغم/كغم) قبل إحداث العجز الكلوي لم يسبب تغيرا معتدا.

الاستنتاجات: من النتائج المتقدمة تبين أن هناك تأثيرا واقيا لكل من فيتامين سي و أي، كارفيدايلول و فيراباميل في الجرع المستخدمة في هذا النوع التجريبي من العجز الكلوي الحاد مما قد يعطي فرصا علاجية أو وقائية للمرضى المعرضين للإصابة بالعجز الكلوي الحاد.

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Introduction:

Acute renal failure (ARF) is a syndrome characterized by rapid decline in glomerular filtration rate (GFR), retention of nitrogenous waste products, and perturbation of extracellular fluid volume with electrolytes and acid-base homeostasis [1] Myoglobinuric acute renal failure is an example of intrinsic type of acute renal failure resulting from damage of skeletal muscles and release of muscle cell contents, notably myoglobin, into the circulation which can cause acute deterioration in renal function [2,3].This study was performed to explore the possible nephroprotective action of some vasodilators (carvedilol, amlodipine and verapamil), vitamins (C and E) in experimental model of Myoglobinuric acute renal failure.

Materials and Methods:

Thirty-six local domestic rabbits of both sexes weighing 750-1000 grams were used in this study.They were supplied by the animal house of the College of Medicine, Al-Nahrain University. They were fed standard oxoid pellets and were given water Ad libitum. Each rabbit was kept in a separate cage, which was provided with a wire mesh floor to avoid caprophagia. The animals were separated into six groups (each group contained six animals). The groups were treated by giving the tested agents at 9 a.m. and were injected with glycerol at 11 a.m for induction of myoglobinuric ARF. The effect of the tested agents was studied on the light of biochemical analysis of renal function. The treatment schedules were as follows:

Group One: the control group received 5 ml of distilled water orally 2 hours before induction of ARF by glycerol (SM chemical-Malaysia) in a dose of 9 ml/ Kg intra-muscularly.

Group Two: was given vitamin C (cetavit-Al-Shahba-Iraq) 250 mg/ Kg in a single daily dose orally started two hours prior to induction and continued for three days post-induction.

Group Three: given vitamin E (Himeco-Syria) 200 mg/ Kg in a single daily dose orally started two hours prior to induction and continued for three days post-induction.

Group Four: given amlodipine besylate (AMADY-5 Ajanta-India), 1.25 mg/ Kg as a single dose orally given two hours prior to induction

Group Five: given carvedilol (Dila-cardic-Domina pharm-Syria) 6.25 mg/ Kg as a single dose orally two hour before induction

Group Six: given verapamil hydrochloride (Danistole, MBC-

Syria) in a dose of 2.5 mg/ Kg orally as a single dose two hour before induction.

Blood samples were collected from the marginal ear vein for biochemical analysis of renal function at 3 occasions, before induction of ARF to determine the normal values of blood urea nitrogen (BUN), serum creatinine, Na^+, and K^+ by using spectro-photometric method [(4)], 3 days after induction and 7 days after induction. The obtained results were collected for analysis and assessment.

Results:

The results of this study revealed significant elevation in the levels of blood urea nitrogen(BUN), serum creatinine and K^+ with significant reduction of serum Na^+ levels in control group as compared to the levels of preinduction state (see table1, 2, 3, 4, 5).

The results of both group 2 and 3 showed significant decrease in the levels of BUN (4.4±0.3, 4.5±0.4 versus 6.6±0.3 mmol/L), serum.creatinine(105±2.8, 110±3.4 versus 150±2 µmol/L) and serum. K^+ (3.5±0.2, 4±0.2 versus 5.4±0.3 mmol/L) ($p<0.05$) and significant increase in serum. Na^+ levels (150±2.3, 155±3.4 versus 144±2.4 mmol/L) ($p<0.05$) in comparison to the control group after 3 days and7days the latter were more evident.

The results of group 4 showed significant increase in the levels of BUN (8.3±0.7versus 6.6±0.3 mmol/L), serum-creatinine (180±3.4 versus 150±2 µmol/L) and serum. K^+ (6.2±0.1versus 5.4±0.3 mmol/L) ($p<0.05$) and significant decrease in serum.Na^+levels (145±3.4 versus 144±2.4 mmol/L) ($p<0.05$) in comparison to the control group after 3 days. These figures were more evident after 7 days.

The results of both group 5 and 6 showed significant lowering of BUN (5.2±0.6, 4.7±0.1 versus 6.6±0.3 mmol/L), serum-creatinine (107±4, 112±3.7 versus 150±2 µmol/L) and serum. K^+ (4±0.4, 4.2±0.3 versus 5.4±0.3 mmol/L) ($p<0.05$) and significant increase in serum-Na^+ levels (159±2.5, 155±3.7 versus 144±2.4 mmol/L) ($p<0.05$) in comparison to the control group after 3 days. These figures were more evident after 7 days. (See table 1, 2, 3, 4, 5).

Discussion:

Myoglobin nephrotoxicity was attributed to different mechanisms including oxidative stress in which oxygen free radicals and reduction in the antioxidant defense system were found to be the main events. It was observed that total antioxidant levels decrease within 24 hours of induction with spontaneous recuperation 72 hours after [5,6], in addition to renal vasoconstriction[7], these mechanisms justify treatment election. In the model of acute renal failure elevation of serum creatinine from 66±0.2 µmol/L before induction to 150±2 µmol/L 3 days after induction and then to 210±22.8 µmol/L after 7 days agreed with the results of [8] who reported that an increase in plasma creatinine concentration to greater than 200 µmol/L can be considered as biochemical confirmation to acute renal failure.

The antioxidant vitamins C and E (group 2 and 3) produced significant nephropro-tection at the tested doses. These results were similar to that reported by [9] who found that vitamin C is an effective chemoprotecive agent against cisplatin induced nephrotoxicity in rats and to the results reported by [10] who investigated the nephroprotective effect of vitamin E in cyclosporine A induced nephrotoxicity in rats.

Carvedilol (group 5) is beta and alpha adrenoceptor blocking agent with antioxidant effect produced significant nephroprotection at the tested dose, this result agreed with that of [11] who reported that carvedilol possess nephroprotective potential effect in cyclosporine induced nephrotoxicity.

verapamil (group 6) is calcium channel blocker with relatively short period of hypotensive effect, showed to have nephroprotective effect in this model of ARF that is similar to that of nefidipine which was successfully used in prevention of contrast media induced nephropathy [12].

Amlodipine(group 4), is another calcium channel blocker, was found to potentiate myoglobin nephrotoxicity. This effect may explained by prolonged hypotension caused by amlodipine(plasma half life 30-50 hours) which may result in renal hypoperfusion precipitating further renal ischemia.

In conclusion, vitamin C and E, carvedilol and verapamil have a significant nephroprotective effect at the tested doses in this model of acute renal failure with a possible use in prevention of acute renal failure.

Table (1): the mean BUN, serum creatinine, K^+ and Na^+ levels of the tested animals measured before induction

Analyte	Mean level
BUN	4 ± 0.7 mmol/L
S. creatinine	65 ± 8.9 μmol/L
S. K^+	3.3 ± 0.8 mmol/L
S. Na^+	160 ± 4 mmol/L

Table (2): mean BUN levels of the studied groups measured after induction of ARF

Group	Agent	Dose	BUN (mmol/ L) after 3 days	BUN (mmol/ L) after 7 days
1	Control: Glycerol	9 ml/Kg	6.6 ± 0.3	8.9 ± 0.2
2	Vit. C	250 mg/Kg	4.4 ± 0.3	4.5 ± 0.4
3	Vit. E	200 mg/Kg	4.5 ± 0.4	5 ± 0.9
4	Amlodipine	1.25 mg/Kg	8.3 ± 0.7	14.6 ± 1
5	Carvedilol	6.25 mg/Kg	5.2 ± 0.6	6.8 ± 1.2
6	Verapamil	2.5 mg/Kg	4.7 ± 0.1	5.1 ± 0.2

Table (3): mean serum Creatinine levels of the studied groups measured after induction of ARF

Group	Agent	Dose	S. C r. (□mol/L) after 3 days	S. C r. (□mol/L) after 7 days
1	Control: Glycerol	9 ml/Kg	150 ± 2	210 ± 22.8
2	Vit. C	250 mg/Kg	105 ± 2.8	132 ± 2.3
3	Vit. E	200 mg/Kg	110 ± 3.4	130 ± 2.5
4	Amlodipine	1.25 mg/Kg	180 ± 3.4	280 ± 3.4
5	Carvedilol	6.25 mg/Kg	107 ± 4	120 ± 3.4
6	Verapamil	2.5 mg/Kg	112 ± 3.7	134 ± 2.2

Table (4): mean serum K+ levels of the studied groups measured after induction of ARF

Group	Agent	Dose	S. K+ (mmol/L) after 3 days	S. K (mmol/L) after 7 days
1	Control: Glycerol	9 ml/Kg	5.4 ± 0.3	6.3 ± 0.2
2	Vit. C	250 mg/Kg	3.5 ± 0.2	5 ± 0.4
3	Vit. E	200 mg/Kg	4 ± 0.2	5.7 ± 0.4
4	Amlodipine	1.25 mg/Kg	6.2 ± 0.1	7 ± 0.3
5	Carvedilol	6.25 mg/Kg	4 ± 0.4	4.5 ± 0.3
6	Verapamil	2.5 mg/Kg	4.2 ± 0.3	5.5 ± 0.2

Table (5): mean serum Na+ levels of the studied groups measured after induction of ARF

Group	Agent	Dose	S. Na (mmol/L) after 3 days	S. Na (mmol/L) after 7 days
1	Control: Glycerol	9 ml/Kg	144 ± 2.4	135 ± 4.5
2	Vit. C	250 mg/Kg	150 ± 2.3	153 ± 4.8
3	Vit. E	200 mg/Kg	155 ± 3.4	150 ± 2.8
4	Amlodipine	1.25 mg/Kg	145 ± 3.4	135 ± 3.4
5	Carvedilol	6.25 mg/Kg	159 ± 2.5	150 ± 3.2
6	Verapamil	2.5 mg/Kg	155 ± 3.7	153 ± 3.7

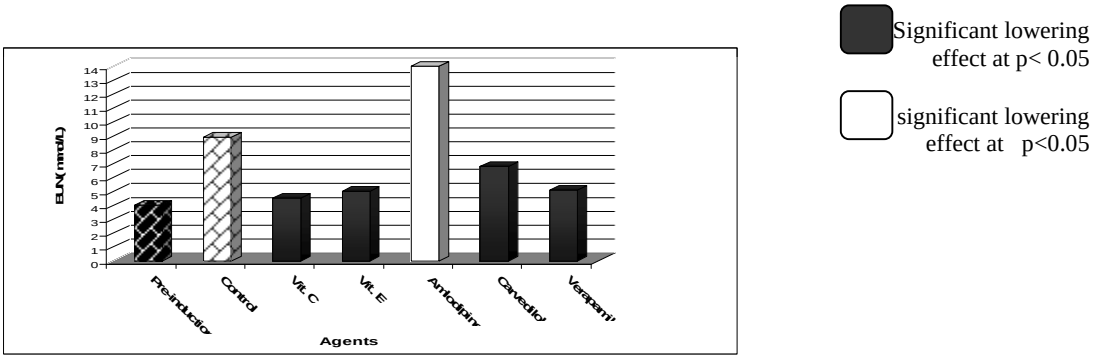

Figure (1): mean BUN measured 7 days after induction

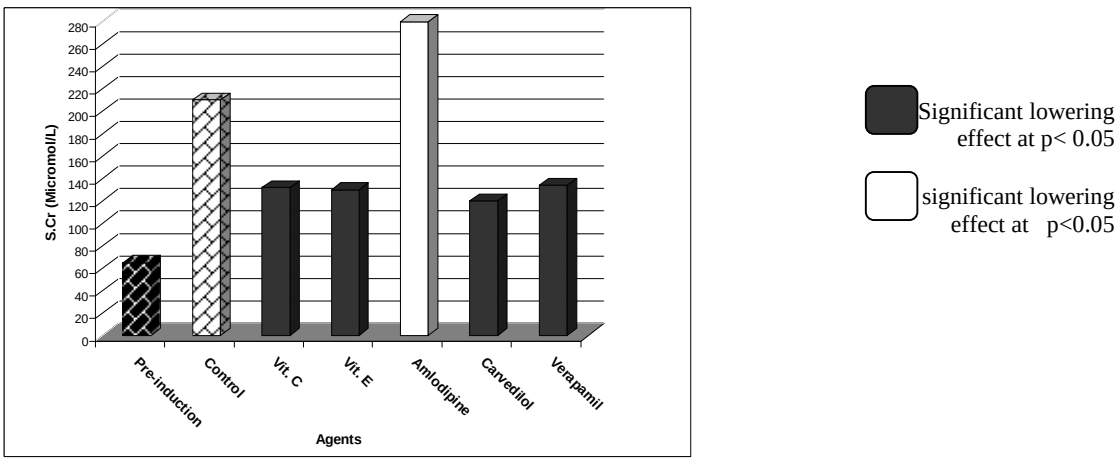

Figure (2): mean serum Creatinine measured 7 days after induction

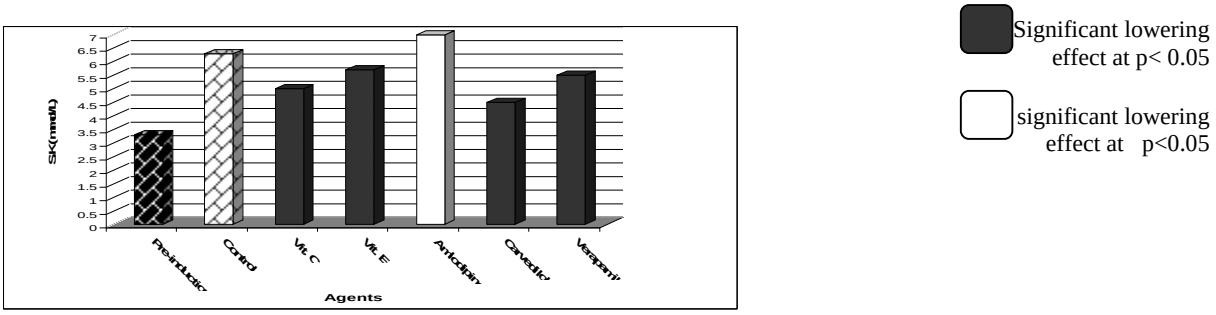

Figure (3): mean serum K^+ measured 7 days after induction

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