STUDY ON THE CALCIUM ROLE IN PROPRANOLOL-VERAPAMIL INTERACTION ON THE KIDNEY

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INTRODUCTION

Propranolol, a drug which has both beta-1 and beta-2 adrenoceptor antagonistic action which plays an important role in the function of the heart (Ichihara et al., 1979; and Pieper et al., 1980), is widely used in the treatment of hypertension (Lewis and Haeusler, 1975; Privitera et al., 1979; and O'Connor and Richard, 1982). It was found also to reduce the renal blood flow and glomerular filtration rate (O'Connor and Richard, 1982) and suppress renin release (Buhler et al., 1972; Privitera et al., 1979; and Kida et al., 1985).

Verapamil, a calcium channel

blocking agent, originally introduced for the treatment of myocardial ischaemia (Sandler et al., 1968; and Sowton, 1969), has been shown to have antiarrhythmic properties (Schamroth et al., 1972) and antihypertensive effect which was the greatest in lower renin groups (Nicholson et al., 1987).

Because of the increasing use of propranolol and verapamil in combination, a study of their interaction was important. Kandil et al., 1981 and 1983 reported that simultaneous administration of propranolol and verapamil is toxic to the experimental animals. These drugs evidently induce their toxic interaction through the blockade of Ca-channels.

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Therefore this study aims to investigate the effect of the concomitant administration of propranolol and verapamil on the renal function, and morphology and the possible role of calcium supplementation.

MATERIALS AND METHODS

Male white Wister rats weighing about 190 g were used in this study. The animals were arranged in five different groups; the control (given saline solution), propranolol (P) treated animals (100 mg/kg b.w.), verapamil (V) treated rats (80 mg/kg b.w.), propranolol + verapamil (P+V) treated rats, and propranolol + verapamil + CaC12 (P+V+Ca) (300 mg/kg b.w.) treated animals. All drugs were dissolved in saline solution and were given daily by the gastric intubation for four weeks.

Blood, urine and tissue samples were collected 24 hours after last treatment. Serum and urine electrolytes (Na, K, Ca) were determined using flame photometer (Jenway, 8505. PFP7). Serum urea and creatinine were estimated by enzymatic colorimetric method using biomerieux kits.

Renal Na-K-ATPase activity was measured according to Bonting et al., 1961 technique. Nephrons were enumerated using the method of Damadian et al., 1965. After weighing the right and left kidneys, parts of them were fixed in 10% neutral formalin and processed as paraffin sections. These were stained with haematoxylin and eosin for histopathological examination. Another fresh parts were taken for preparing cryostat sections for histochemical demonstration of lipids (oil Red O stain).

RESULTS

Fig. (1) illustrates a reduction in the percentage change of the mean total body weight gain of the treated groups comparing to the control in the following order: P. + V > P. + V. + Ca > V. > P.

The absolute and relative weights of the right and left kidneys, and also the number of nephrons were decreased in all treated animals (Table 1). This decrease was more pronounced in P.+V. treated group. CaC12 administration improved the

changes which induced by the concomitant treatment of P.+V.

Table (2), shows significant decrease in serum Na and urinary K concentrations, while serum K and Ca; urinary Na and Ca; and urinary Na/K ratios were increased significantly. The changes were more obvious in P.+V. treated rats. Administration of CaC12 reduced some changes which induced by P.+V.

Serum concentrations of urea and creatinine were significantly increased specially in P.+V. treated group. This increase was diminished by CaC12 administration ($P>0.01$). Meanwhile Na-K-ATPase activity was decreased significantly in the renal tissues of all treated groups specially P.+V. treated group.

Histopathological examination of the kidneys of V. treated group revealed tubular cells degenerations in the form of cloudy swelling and vacuolations (Fig. 2). Oil red O stain was -ve for fat deposition in this group. In P. treated group, in addition to the

tubular cells degenerations, focal interstitial mononuclear cells infiltration was seen oil red O staining for fat was +ve with mild intensity(+) (Fig. 3).

Kidneys of P.+V. treated rats showed both glomerular and tubular changes. Some glomeruli appeared atrophic and small in size with associated periglomerular fibrosis. Others showed hypertrophy and increase in size (Fig. 4). The tubules revealed degenerative changes in the form of cloudy swelling, vacuolation (Fig. 5) and fatty changes(Oil red o stain was +ve with moderate (++) intensity)(Fig. 6). Focal tubules showed atrophy of their epithelial lining (Fig. 7). Interstitial nephritis as well as focal fibrosis were present.

In P.+V.+CaC12 treated group, the previous histopathological changes disappeared and mild cloudy swelling of the proximal convoluted tubules was the only detectable change.

DISCUSSION

The results of this study exhibited a marked reduction in the percentage

change of the mean body weight gain in all treated groups. Our results are in accordance with prior observations which recorded a significant reduction in the body weight gain in propranolol treated animals compared to control in both sexes (Donta et al., 1982). Conversely other reports failed to find any effect on the body weight in propranolol treated patients (O'Connor and Richard, 1982) or mice (Dulloo and Miller, 1985).

The absolute and relative weights of both the right and left kidneys in the treated groups were also decreased, with subsequent decrement in the number of nephrons per mg of the kidney tissue. Donta et al., 1982 showed lowered organ weights in propranolol treated rats except for the kidneys of both sexes and the heart and testes of the males. Such differences may be related to the time of treatment and the age of used animals.

Renal Na-K-ATPase activity was reduced by propranolol and/or verapamil. Such results can explain the raised sodium (Na) concentrations in

the urine of these groups, where a close correlation has been found between the reduction in Na-K-ATPase activity in post obstructive rat kidney and the changes in filtered Na load, and tubular reabsorption (Wilson et al., 1974). Subsequently the elevation in the urinary Na concentrations can explain its decrement in the sera of these groups. Moreover propranolol was found to diminish mineralocorticoids specially aldosterone and impair sodium excretion (O'Connor and Richard, 1982).

Potassium levels were increased in the sera of the animal treated with propranolol and/or verapamil. Such increment may be attributed to decreased glomerular filtration rate (GFR) which was recorded previously in propranolol treated hypertensive patients (O'Connor and Richard, 1982), besides the reducing action of the drug on aldosterone which is responsible for K excretion by the distal convoluted tubules. Moreover these informations may also explain the declined urinary K concentration and the elevated urinary Na/K ratios in these

groups.

Calcium was found to be increased in the sera of all treated groups, and was higher in rats receiving P.+V. than in those receiving other drugs. The profound increase in serum calcium may be related to inhibition of calcium transport from blood through its specific channels (Kandil et al., 1983). The rise in serum calcium levels lead to their elevation in urine.

Serum concentrations of urea and creatinine were elevated also in propranolol and/or verapamil treated animals. Such results which reflects the disturbance in the renal function as shown in the work of Ibsen and Sederberg-Olsen, 1973; Falch et al., 1978; and O'Connor and Richard, 1982, who found declines in (GFR); renal blood flow (RBF); and renal plasma flow (RPF), but without any change in the usual serum indices of renal function (BUN and creatinine) (O'Connor and Richard, 1982).

The histopathological changes in P, V and P+V groups were mainly

tubular, in the form of tubular degeneration (cloudy swelling and vacuolations). Moreover fatty deposition was present in both P and P+V groups only. These renal changes may be attributed, to decrease in renal Na-K-ATPase under the influence of propranolol and/or verapamil (Williams and Fanstil, 1970). The additional fatty change in renal tubules found in group P and group P+V may be related to disturbed phospholipid metabolism induced by propranolol (Pappu and George, 1982).

Glomerular damage was also detected, in addition to tubular change, in group P+V only. It was in the form of focal atrophy with periglomerular fibrosis, associated by focal interstitial nephritis and fibrosis. Some glomeruli appeared, on the other hand, hypertrophic and large sized. The detected glomerular atrophy and periglomerular fibrosis may, be part of the focal nephritis demonstrated in the same group of rats. The large sized glomeruli found in rats of this group may represent a form of compensatory hypertrophy of these glomeruli in response to

atrophic ones.

A striking feature in this study was that addition of calcium to P.+V. prevented the previously mentioned changes, this finding represent a support to previous studies recording that P., V. interaction is induced via blockage of calcium channels (Kandil et al., 1981).

In conclusion, the authors suggested that simultaneous administration of propranolol and verapamil should be used cautiously, and recommended that calcium can be used to reduce their synergistic toxicity.

SUMMARY

The results showed decreases in the percentage change of the mean body weight gain, absolute and relative kidney weights, and the number of nephron in both the right and left kidneys of propranolol (P.) and/or verapamil (V.) treated rat groups. Moreover significant decreases were shown

in serum Na, urinary K, and renal Na-K-ATPase activity; while significant increase was illustrated in serum K and Ca; urinary Na and Ca; and serum urea and creatinine of the same groups. The changes were more obvious in the group treated with (P.+V.) in combination. Administration of calcium chloride (CaCl₂) minimized these effects.

Histopathological and histochemical examination revealed tubular epithelial degenerations in either P or V groups and accompanied by glomerular changes and interstitial nephritis in the group given combined P+V. The previous lesions were diminished after calcium supplementation.

These results ensure the adverse effects of the combined use of these drugs on the kidney, and deduce that calcium supplementation may reduce these changes but does not prevent them completely.

Table 1. Absolute (mg), and relative weights (mg / 100g body wt)' and the number of nephrons (number / mg wet kidney) of the right (R.) and left kidneys in the control and treated animal groups.

		Absolute wt.		Relative wt.		Number of nephron	
		R.	L.	R.	L.	R.	L.
Con.	Mean±SE	825.4±5.70	800±2.06	449±2.55	435.6±1.56	25.2±0.52	21.2±0.52
P.	Mean ± SE	810.6±1.08	755.4±1.37	408±2.64	380.2±2.37	23.0±0.63	20.0±0.4
	& of change P- value	- 1.79 <0.05	-5.78 < 0.005	- 913 < 0.005	-12.72 < 0.005	- 8.73 < 0.05	- 5.66 N.s.
V.	Mean ± SE	803.6±1.61	792.2±1.04	396.8±2.46	391.2±3.34	23.6±0.46	20.4±0.46
	& of change P- value	- 2.64 < 0.005	-0.98 <0.005	- 11.63 < 0.005	- 10.19 < 0.005	- 2.35 < 0.05	- 3.77 N.s.
P.+V.	Mean ± SE	796±1.41	755.4±1.37	371.4±2.84	349.4±3.42	18.8±0.33	18.6±0.46
	& of change P- value	- 3.56 < 0.005	-5.78 <0.005	- 17.28 < 0.005	- 19079 < 0.005	- 25.40 < 0.05	- 12.26 < 0.005
P.+V.+ Cach ₂	Mean ± SE	814.2±1.43	748.8±1.15	438.4±3.56	423.6±2.54	24.4±0.46	20.8±0.33
	& of change P- value	- 1.36 M. S	6.4 <0.005	- 2.36 < 0.005	- 2.75 < 0.005	- 3017 N.S.	- 1.89 M. S.

Table 2. Serum and urinary concentrations of electrolytes in (mmol / L); and urinary Na / k ration in control and treated animal groups .

		Serum			Urine			L.
		Na	K	Ca.	L.	R.	L.	
Con.	Mean±SE	137±0.63	3.64±0.08	2.44±0.05	12.6±0.46	16.4±0.46	0.64±0.004	0.77±0.01
P.	Mean ± SE	132.0±0.63	408±0.03	3.44±0.09	17.0±0.63	14.4±0.46	0.76±0.04	1.18±0.04
	& of change P- value	10.95 <0.05	+12.09 <0.005	+ 40.98 < 0.005	+34.92 < 0.005	- 12.20 < 0.05	+ 18.75 <0.05	+53.25 <0.005
V.	Mean ± SE	112.8±0.77	4.0±0.6	3.0±0.06	19.6±0.46	14.6±0.22	0.82±0.03	1.32±0.02
	& of change P- value	-17.66 <0.005	+9.89 <0.005	+ 22.95 < 0.005	+ 55.56 < 0.005	- 10.98 < 0.005	+ 28.13 < 0.005	+ 71.45 < 0.005
P.+V.	Mean ± SE	103.2±1.12	4.74±0.08	4.8±0.17	21.6±0.46	12.2±0.33	0.92±0.03	1.77±0.02
	& of change P- value	-24.82 < 0.005	+ 30.22 < 0.005	+96.72 < 0.005	+71.43 < 0.005	- 25.61 < 0.05	+ 43.75 < 0.005	+ 129.87 < 0.005
P.+V.+ CaCl ₂	Mean ± SE	125.8±1.43	3.96±0.08	2.56±0.05	15.0±0.63	14.6±0.46	0.74±0.05	1.05±0.01
	& of change P- value	- 8.18 < 0.005	+8.79 <0.01	+ 4.92 N. S.	+ 19.05 < 0.01	- 10.98 <0.01	+ 15.63 N. S.	+33.77 < 0.005

Table 3. Serum concentrations of urea and creatinine in (mg / 100 ml); and renal Na - K - ATPase activity (umol pi / g / mi) in control and treated animal groups.

		Cont.	L.	R.	L.	R.
Serum Urea	Mean $\pm$ SE & of change P- value	31.6 $\pm$ 0.46	36.4 $\pm$ 0.46 +15.19 < 0.005	42.2 $\pm$ 0.77 + 33.54 < 0.005	48.4 $\pm$ 0.46 + 53.16 < 0.005	33.8 $\pm$ 0.52 + 6.96 < 0.01
Serum Creatinine	Mean $\pm$ SE & of change P- value	0.45 $\pm$ 0.009	0.49 $\pm$ 0.006 +8.89 < 0.005	0.53 $\pm$ 0.009 + 17.78 < 0.005	0.75 $\pm$ 0.01 +66.67 < 0.005	0.47 $\pm$ 0.01 +4.44 N.S.
Renal Na - k - ATPase	Mean $\pm$ SE & of change P- value	10.04 $\pm$ 0.1	9.3 $\pm$ 0.09 - 7.37 < 0.005	8.58 $\pm$ 0.1 - 14.54 < 0.005	7.96 $\pm$ 0.05 - 20.72 < 0.005	9.6 $\pm$ 0.06 - 4.38 < 0.005

P - values indicate significant difference.

N. S. : Not significant. Each group represents 5 male rats.

Fig. 1. Percentage change in the mean total body weight gain of control and treated rats.

Fig. 2. Section of kidney of rat received verapamil showing cloudy swelling and vacuolation of tubular epithelium (Hx & Es, x 400).

Fig. 4. Section of kidney of rat received verapamil and propranolol showing atrophic glomeruli and periglomerular fibrosis. (Hx & Es x 250).

Fig. 3. Section of kidney of rat received propranolol showing mild degree of fat accumulation (oil red O, x 250).

Fig. 5. Section of kidney of rat received verapamil and propranolol showing cloudy swelling of the tubular epithelium (Hx & Es, X 400)

Fig. 6. Section of kidney of rat received verapamil and propranolol showing moderate degree of fat accumulation. (Oil red O, X 250).

Fig. 7. Section of kidney of rat received verapamil and propranolol showing atrophy of the epithelial lining of some tubules (Hx & Es, x 400).

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الملخص العربى

دراسة لدور الكالسيوم فى تأثير التداخل الدوائى للبروبرانولول والفيراباميل على الكلية

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اجرى هذا البحث بهدف دراسة تأثير التداخل الدوائى للبروبرانولول والفيراباميل على الكلية ، والدور الذى قد يلعبه الكالسيوم فى تخفيف اثار هذا التداخل . وقد استخدمت ذكور الفئران البيضاء التى تزن فى المتوسط ١٩٠ جم فى هذه الدراسة، حيث قسمت الى خمسة مجموعات ، اعطيت احداها البروبرانولول فقط (١٠٠ مجم / كجم) ، واخرى الفيراباميل فقط (٨٠ مجم / كجم) وثالثة اعطيت العقاران معا ، ورابعة اعطيت كلوريد الكالسيوم (٣٠٠ مجم / كجم) بالاضافة للعقارين معا ، اما المجموعة الضابطة فقد عولجت بمحلول ملحي (وهو المستعمل لاذابة العقارين) . وقد اعطيت العقاقير عن طريق الفم ، يوميا لمدة ٤ اسابيع . وظهرت النتائج المستخلصة من هذا البحث ان كل عقار على حده له تأثير سىء على الكلية حيث زادت نسبة البولينا والكرياتينين فى مصل الدم ، كما حدث اضطرابات فى تركيز الالكتروليتات فى كل من المصل والبول ، فزاد كالسيوم وبوتاسيوم المصل ، بينما بقص الصوديوم فيه . اما البول فقد زاد فيه الكالسيوم والصوديوم وقل البوتاسيوم . كما نقص نشاط انزيم الصوديوم بوتاسيوم ثلاثى فسفات الادينوزين فى انسجة الكلية . هذا بالاضافة الى تغيرات هستوباثولوجية فى الانابيب الكلوية من حيث الخلايا وترسب الدهون وقد اظهر التداخل بين هذين العقارين نفس التغيرات وشمل ايضا الكبيبات الكلوية مما يدل على التأثير المتزايد لهما . بينما خففت اضافة الكالسيوم لهما من حدة هذا التأثير بدرجة مقبولة .

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