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.
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 numerated 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 cryostate 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 explor 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. pre-
vented the previously mentioned changes, this finding represent a sup-
port to previous studies recording that P., V. interaction is induced via block-
age of calcium channels (Kandil et al., 1981).

In conclusion, the authors suggest-
ed 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 rela-
tive kidney weights, and the number of
nephron in both the right and left kid-
neys of propranolol (P.) and/or verapa-
amil ( V. ) treated rat groups. Moreo-
ver significant decreases were shown
in serum Na, urinary K, and renal Na-
K-ATPase activity; while significant in-
crease was illustrated in serum K and
Ca; urinary Na and Ca; and serum
urea and creatinine of the same
groups. The changes were more obvi-
ous in the group treated with (P.+V.) in
combination. Administration of calcium
chloride (CaC12) minimized these ef-
fects.

Histopathological and histochemi-
cal examination revealed tubular epi-
thelial degenerations in either P or V
groups and accompanied by glomeru-
lar 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.

<table>
<thead>
<tr>
<th></th>
<th>Absolute wt.</th>
<th>Relative wt.</th>
<th>Number of nephron</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R.</td>
<td>L.</td>
<td>R.</td>
</tr>
<tr>
<td>Con.</td>
<td>Mean±SE</td>
<td>825.4±5.70</td>
<td>800±2.06</td>
</tr>
<tr>
<td></td>
<td>Mean±SE &amp; of change</td>
<td>810.6±1.08</td>
<td>755.4±1.37</td>
</tr>
<tr>
<td>P.</td>
<td>p-value</td>
<td>-1.79</td>
<td>-5.78</td>
</tr>
<tr>
<td>V.</td>
<td>Mean±SE &amp; of change</td>
<td>803.6±1.61</td>
<td>792.2±1.04</td>
</tr>
<tr>
<td>P.+V.</td>
<td>Mean±SE &amp; of change</td>
<td>796±1.41</td>
<td>755.4±1.37</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-2.64</td>
<td>-0.98</td>
</tr>
<tr>
<td></td>
<td>Mean±SE &amp; of change</td>
<td>814.2±1.43</td>
<td>748.8±1.15</td>
</tr>
<tr>
<td>P.+V.+</td>
<td>&amp; of change</td>
<td>-1.36</td>
<td>6.4</td>
</tr>
<tr>
<td>CaCl2</td>
<td>P. value</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
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</table>

Table 2: Serum and urinary concentrations of electrolytes in (mmol/L) and urinary Na/K ratio in control and treated animal groups.

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Urine</th>
<th>L.</th>
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<tr>
<td></td>
<td>Na</td>
<td>K</td>
<td>Ca</td>
</tr>
<tr>
<td>Con.</td>
<td>Mean±SE</td>
<td>137±0.63</td>
<td>3.64±0.08</td>
</tr>
<tr>
<td>P.</td>
<td>Mean±SE &amp; of change</td>
<td>132.0±0.63</td>
<td>408±0.03</td>
</tr>
<tr>
<td></td>
<td>&amp; of change</td>
<td>10.95</td>
<td>+12.09</td>
</tr>
<tr>
<td>P. value</td>
<td>&lt;0.05</td>
<td>&lt;0.005</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>V.</td>
<td>Mean±SE &amp; of change</td>
<td>112.8±0.77</td>
<td>4.0±0.6</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-17.66</td>
<td>+9.89</td>
</tr>
<tr>
<td>P.+V.</td>
<td>Mean±SE &amp; of change</td>
<td>103.2±1.12</td>
<td>4.74±0.08</td>
</tr>
<tr>
<td></td>
<td>P. value</td>
<td>-24.82</td>
<td>+30.22</td>
</tr>
<tr>
<td>P.+V.+</td>
<td>&amp; of change</td>
<td>125.8±1.43</td>
<td>3.96±0.08</td>
</tr>
<tr>
<td>CaCl2</td>
<td>P. value</td>
<td>&lt;8.19</td>
<td>&lt;8.79</td>
</tr>
</tbody>
</table>

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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.

<table>
<thead>
<tr>
<th></th>
<th>Cont.</th>
<th>L.</th>
<th>R.</th>
<th>L.</th>
<th>R.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Urea</td>
<td>Mean ± SE</td>
<td>36.4 ± 0.46</td>
<td>42.2 ± 0.77</td>
<td>48.4 ± 0.46</td>
<td>33.8 ± 0.52</td>
</tr>
<tr>
<td>&amp; of change</td>
<td>31.6 ± 0.46</td>
<td>+15.19</td>
<td>+33.54</td>
<td>+53.16</td>
<td>+6.96</td>
</tr>
<tr>
<td>P. value</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>Mean ± SE</td>
<td>0.49 ± 0.006</td>
<td>0.53 ± 0.009</td>
<td>0.75 ± 0.01</td>
<td>0.47 ± 0.01</td>
</tr>
<tr>
<td>&amp; of change</td>
<td>0.45 ± 0.009</td>
<td>+8.89</td>
<td>+17.78</td>
<td>+66.67</td>
<td>+4.44</td>
</tr>
<tr>
<td>P. value</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>N.S.</td>
<td></td>
</tr>
<tr>
<td>Renal Na-K-ATPase</td>
<td>Mean ± SE</td>
<td>10.04 ± 0.1</td>
<td>9.3 ± 0.09</td>
<td>8.58 ± 0.1</td>
<td>7.96 ± 0.05</td>
</tr>
<tr>
<td>&amp; of change</td>
<td>10.04 ± 0.1</td>
<td>-7.37</td>
<td>-14.54</td>
<td>-20.72</td>
<td>-4.38</td>
</tr>
<tr>
<td>P. value</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td>&lt; 0.005</td>
<td></td>
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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.

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

Fig. 3. Section of kidney of rat received propranolol showing mild degree of fat accumulation (oil red 0, x 250).

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


Kida Osamu; Yasuyuki Morotomi; Toshinobu Higa and Kenjiro Tanaka (1985) : Heart Vessels 1 (13) : 158 - 161.


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الملخص العربي
دراسة لدور الكالسيوم في تأثير التداخل الدوائي للبروبيانولول والفيراباميل على الكلية

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أجرى هذا البحث بهدف دراسة تأثير التداخل الدوائي للبروبيانولول والفيراباميل على الكلية، والدور الذي قد يلعبه الكالسيوم في تخفيف اثار هذا التداخل. وقد استخدمت ذكرى الفئران البيضاء التي تزن في المتوسط 19 جم في هذه الدراسة، حيث قسمت إلى خمسة مجموعات، أعطيت إحداهما البروبيانولول فقط (100 مجم / كجم)، وأخرى الفيراباميل فقط (80 مجم / كجم) وثانيثلة أعطيت العقاقير معا، ورابعة أعطيت كلوريد الكالسيوم (300 مجم / كجم) بالإضافة للمقارنين معا، أما المجموعة الضابطة فقد عُرفت ب溶液 ملحي (وهو المستعمل لذاببة العقاقير). وقد أعطيت العقاقير عن طريق الفم، يوميا لمدة 4 أسابيع. وأظهرت النتائج المستخلصة من هذا البحث أن كل عقار على حدة له تأثير سيء على الكلية حيث زادت نسبة البوليتا والكربونات في مصيل الدم، كما حدث اضطرابات في تركيز الأيونات في كل من المصل والبول، فزاد الكالسيوم بوتاسيوم المصل، بينما بقص الصوديوم فيه. أما البرول فقد زاد فيه الكالسيوم والصوديوم وقل البوتاسيوم. كما نقصت نشاط أنزيم الصوديوم بوتاسيوم في النسختين الدائتين في الاتباع الكلوريا من حيث الخلايا وترسب الدهون وقد أظهر التداخل بين هذين العقاقير نفس التغيرات وشمل أيضا الكيماويات الكلورية مما بدل على التأثير المتزايد لهما. بينما خففت اضافة الكالسيوم لهما من حدة هذا التأثير بدرجة مقبلة.