EFFECT OF REPEATED TREATMENT WITH LOW DOSES OF SPIRONOLACTONE ON ELECTROLYTES AND OSMOLALITY

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ABSTRACT:
Spironolactone, a mineralocorticoid antagonist is widely used clinically in the treatment of essential hypertension, congestive heart failure, hepatic ascites, primary aldosteronism and other edematous states. The present study was designed to investigate the role of spironolactone on serum electrolytes and osmolality in male and female rabbits. Test rabbits were given spironolactone at a dose of 50mg/kg body weight in 0.5ml of sesame oil subcutaneously for five days. Control groups were treated identically with sesame oil. The results show that serum levels of sodium were significantly decreased in both sexes. Serum chloride and osmolality were also significantly decreased in both sexes. Serum calcium was slightly decreased in both male and female rabbits. The increase in serum concentration of potassium and magnesium was more significant in male as compared to female rabbits, whereas the concentration of phosphorus in serum remained unaltered. The results reported in present study suggest that the drug inhibit aldosterone biosynthesis to a variable degree. The antihypertensive effect of spironolactone has been considered to be produced by an increasing effect on the urinary sodium due to the antagonism between spironolactone and aldosterone in the renal tubules. The drug acts on the basolateral side of the tubule cells, competing with aldosterone for cellular receptors and in this way the effect of the hormone (Aldosterone) is diminished. Our findings imply that unwanted electrolyte disturbances such as hypokalemia and hypomagnesemia associated with the administration of other classes of diuretics can be minimized with spironolactone.

INTRODUCTION
Spironolactone is a mineralocorticoid antagonist used clinically in the treatment of essential hypertension, congestive heart failure and other edematous states (Mantero and Lucarelli, 2000; Michaud and Strickberger, 2001; Rajagopal and Pitts, 2003; Haller, 2000). Some of the side-effects of the drug include hepatic-enzyme induction and inhibition of adrenal and testicular steroidogenesis (Decker et al., 1986; Tuck et al., 1981) Spironolactone antagonises the binding of aldosterone to receptors in the cellular cytosol and nucleus thereby inhibiting sodium resorption in the distal tubule and increasing urinary excretion of sodium while retaining potassium. Furthermore, the drug inhibits aldosterone biosynthesis (Brest, 1986) to a variable degree. Although the antihypertensive effect of spironolactone is considered to be mainly through its antagonism of aldosterone in the kidneys, it has been recently reported that the drug may act directly on the vascular wall and exhibits its antihypertensive effect by inhibiting vasoconstriction due to vasoactive substances (Jiang et al., 2003; Mendlowitz at al., 1968).

Despite the advent of major new antihypertensive therapeutic classes such as β-blockers, calcium channel blockers and Angiotensin converting enzyme inhibitors, a well-defined role for the use of spironolactone continues to exist. The aim of the present study was to investigate the antihypertensive effect of spironolactone and its influence on metabolic parameters by repeated
administration to normal healthy male and female rabbits.

**MATERIALS AND METHODS**

Male and female rabbits weighing 0.9-1.2 kg body weight were taken for the study. The animals were caged individually and given free access to food and water. Animals were randomly divided into control and test groups. The test groups were injected with spironolactone 50mg/kg s.c. in sesame oil for five days. Control groups of both sexes received an equal volume of sesame oil through the same route. The animals were killed by cervical dislocation on the sixth day post injection. Blood samples were collected and analyzed for serum sodium, potassium, calcium, magnesium, phosphorus, chloride, glucose and osmolality. Concentrations of serum sodium, potassium and calcium were determined by flame photometer (Corning-400), serum chloride by the method of Schales and Schales (1941), serum magnesium by procedure of Hallry and Skypeck (1964) and serum phosphorus by the method of Gomorri (1942) and Dryer and Tammes (1957). Serum osmolality was calculated in terms of mOsm/kg.

**STATISTICAL ANALYSIS**

Results are presented as means ± S.D. The effect of sex, treatment and the interaction on serum sodium, potassium, calcium, magnesium, phosphorus, chloride, and osmolality were analyzed by two-way ANOVA. All post-hoc analysis were accomplished by using Newman-Keuls test.

**RESULTS**

Table-1 shows the changes in the serum concentration of sodium, potassium, calcium and magnesium after repeated spironolactone treatment. Two-way ANOVA showed a significant treatment effect for all the parameters. Interactions between sex and treatment were non-significant for serum sodium, potassium, calcium and magnesium. Sex effect was significant for serum potassium only (P<0.01).

Repeated spironolactone treatment decreased serum sodium levels comparably in the two sexes (P<0.05).

Potassium content was increased in both male (P<0.01) and female (P<0.05) rabbits.

**Table-1**

Effect of repeated administration of spironolactone (50 mg/kg; s.c.) on serum sodium, potassium, calcium and magnesium in male and female rabbits

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th></th>
<th>Female</th>
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<th>Two way ANOVA (df 1, 28)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
<td>Sex</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>139.5 ± 1.11</td>
<td>134.87* ± 3.58</td>
<td>139.24 ± 1.79</td>
<td>136.00** ± 3.70</td>
<td>F=0.15 n.s.</td>
</tr>
<tr>
<td>Potassium (mEq/L)</td>
<td>5.62 ± 0.58</td>
<td>5.17 ± 10.05</td>
<td>5.12 ± 10.05</td>
<td>5.845** ± 0.47</td>
<td>F=8.70 P&lt;0.01</td>
</tr>
<tr>
<td>Calcium (mEq/L)</td>
<td>5.47 ± 0.28</td>
<td>4.96 ± 50.62</td>
<td>4.74 ± 50.23</td>
<td>4.74 ± 0.32</td>
<td>F=3.62 n.s.</td>
</tr>
<tr>
<td>Magnesium (mEq/L)</td>
<td>2.02 ± 50.61</td>
<td>2.62** ± 10.43</td>
<td>1.75 ± 0.40</td>
<td>2.23 ± 50.14</td>
<td>F=4.08 n.s.</td>
</tr>
</tbody>
</table>

Values are means ± S.D. (n = 8)
Significant differences by Newman-Keuls test following two-way ANOVA.
* P < 0.01, **P < 0.05 from respective controls.
+ P <0.05 from similarly treated male rabbits.
after the treatment. The levels in spironolactone treated females were significantly (P<0.05) smaller than observed for similarly treated male rabbits, suggesting smaller drug-induced increases of potassium in females. Serum calcium decreased in both sexes but the differences were non-significant. Serum levels of magnesium were not significantly different in the control male and female rabbits. Repeated spironolactone treatment increased serum magnesium significantly only in males (P<0.05) but not in female rabbits.

Table-2 shows the effect of repeated spironolactone administration on serum phosphorus, chloride and osmolality in male and female rabbits. Two-way ANOVA showed a significant drug treatment effect for serum chloride (P<0.01) and osmolality (P<0.01) only. Sex effect, interactions between sex and treatment was found to be non-significant for all the parameters.

Repeated spironolactone treatment decreased serum chloride in both sexes (P<0.01). Basal values of serum chloride were slightly higher in males but the differences were not significant. On the other hand, a significant (P<0.05) decrease in serum osmolality was observed in both male and female rabbits after repeated spironolactone treatment. Decreases of serum osmolality were comparable in the two groups. Changes of serum phosphorus were non-significant.

**DISCUSSION**

Several studies on the mechanism of antihypertensive effects of aldosterone antagonist have been done in human and animals (Mantero and Lucarelli, 2000; Rado, 1988; Saito, 1991; Bourke and Delaney, 1994; Los and Colby, 1994; Fujimura et al., 1994). But these studies sometimes are contradictory and most of the studies available showed the action of these antagonists through various physiological and hormonal mechanism. The antihypertensive effect of antialdosterone drugs are greater in animals with low renin activity and in those with elevated mineralocorticoids including aldosterone. However, it is unclear whether the drug produces satisfactory antihypertensive responses in animals with normal renin activity.

The results showed that spironolactone has a major effect on electrolytes concentration and osmolality. Hyperkalemia as observed in the present study (Table-1) following spironolactone administration in both male and female rabbits is consistent with previous reports (Hu et al., 2002; Aagaard et al., 2002; Soberman and Weber, 2000; Jiang et al., 2003; Abbas et al., 2003). It is well-established that the cationic sodium without an anion creates a lumen–negative electrical gradient that favours the secretion of potassium (through selective K* channels) and hydrogen. Thus inhibition of sodium reabsorption at this site can lead to hyperkalemia and metabolic acidosis due to concurrent decrease in urinary K* and H* loss (Hropot et al., 1985; Greenberg, 2000).

In the present study changes in the concentration of serum sodium and chloride (Table-1 and 2) can also be related to the Na-KATPase activity. It can be proposed that the decreased concentration of sodium and chloride observed after repeated spironolactone administration was due to the action of spironolactone through inhibition of Na-K-ATPase (Kleeberg and Belzi, 1974). The results are in agreement with Nielsen et al. (2002). Previous studies suggested that high physiologic levels of aldosterone stimulate a segment specific increase in Na-K-ATPase activity in the cortical collecting tubules of rats and rabbits (Mujais et al., 1985). It can be suggested that the decreased concentration of sodium and chloride observed during the present study might be due to the action of spironolactone through the inhibition of Na-K-ATPase activity. It can be suggested that spironolactone, a mineralocorticoid receptor antagonist may inhibit the epithelial sodium channel (ENaC) and thereby reduce Na+ reabsorption to a point at which blood pressure
Effect of repeated treatment with low doses of Spironolactone

decreases (Pratt et al., 2001). Moreover, the decrease in the chloride reabsorption produced by spironolactone is undoubtedly a sequel of the decreased reabsorption of sodium as a result of which serum chloride concentration decreased during this study.

After repeated administration of spironolactone, a competitive antagonist of mineralocorticoid, a slight decrease in serum calcium concentration was observed (Table-1) in both sexes. This hypocalcemic effect might be due to the result of (1) an effect associated with inhibition of sodium reabsorption causing a decrease in calcium reabsorption, (2) a result of inhibiting an aldosterone-dependent calcium transport system, (3) a direct inhibitory effect of spironolactone on a calcium reabsorptive mechanism in the renal tubule or (4) a result of increased calcium loads by virtue of an action of spironolactone on bone or the gastrointestinal tract (Prati et al., 1971). There are evidences supporting the view that spironolactone may depress the calcium loading of intracellular stores by reducing the filling of the stores.

Hypermagnesemia as observed in the present study in male rabbits (Table-1) may also occur due to magnesium retention (Crippa, et al., 1999; Soberman and Weber, 2000; Aagaard et al., 2002). The mechanism by which potassium-sparing diuretics are magnesium-sparing is unknown but reduction of the transepithelial potential difference could tend to favour reabsorption of a divalent cation. In general, there is a tendency for urinary magnesium conservation with spironolactone (Saito, 1991).

Repeated administration of spironolactone at dose used in the present study caused no significant effect on serum phosphorus concentration (Table-2). Because firstly, none of the potassium-sparing diuretics (triamterene, amiloride and spironolactone) has been shown to inhibit renal tubular phosphorus reabsorption (Johny et al., 1969; Walker et al., 1971). Secondly, phosphorus reabsorption occurs mainly in the proximal tubule therefore distal inhibitors (spironolactone and triamterene) do not alter phosphate excretion (Sonntag and Gaude, 1998). It can be proposed that spironolactone have little or no effect on phosphate transport in the distal tubule. Thus resulting in no adverse effect on phosphorus homeostasis observed during the present study.

Table-2
Effect of repeated administration of spironolactone (50 mg/kg; s.c) on serum phosphorus, chloride and osmolality in male and female rabbits

<table>
<thead>
<tr>
<th></th>
<th>Male</th>
<th>Female</th>
<th>Two-way ANOVA (df 1, 28)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>Phosphorus (mEq/L)</td>
<td>2.79 ± 10.46</td>
<td>2.86 ± 10.33</td>
<td>2.61 ± 30.36</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>116.28 ± 13.36</td>
<td>95.48* ± 10.78</td>
<td>111.79 ± 16.46</td>
</tr>
<tr>
<td>Osmolality (mOsm/kg)</td>
<td>285.57 ± 12.27</td>
<td>275.97** ± 17.02</td>
<td>284.88 ± 13.57</td>
</tr>
</tbody>
</table>

Values are means ± S.D. (n = 8)  
Significant differences by Newman-Keuls test following two-way ANOVA.  
* P < 0.01, **P < 0.05 from respective controls.
The effect of repeated administration of spironolactone on electrolytes concentration and osmolality found in the present study (Table-2) may also be due to the inhibition of aldosterone synthesis which is important in the regulation of renal hemodynamics (Lauler, 1990; Los and Colby, 1994) thereby altering secretion and reabsorption of various electrolytes.

The present study revealed that serum magnesium and potassium levels are greater in males than in females (Table I). Erythrocytes of female rabbits have been shown to have higher Na-K-ATPase activity (Lasker et al., 1985) or may be due to a difference in the rate of absorption of the drug in two sexes (Aaron et al., 1989).

It can be concluded that male genders were important predictors of development of hyperkalemia and hypermagnesemia.

REFERENCES


