Reversal of Digoxin-Induced Changes in Erythrocyte Electrolyte Concentrations by Penicillamine in Children

Bahram MOEZI, Ph.D.,* Roohollah KHŒZI, M.D.,* Fereidoon POOYMÉHR, M.D.,** and Jami G. SHAKI, M.D., F.A.C.C.**

SUMMARY

Previous reports from this laboratory have shown that penicillamine effectively reduces serum digoxin levels and is a clinically useful drug in correcting digoxin intoxication. To elucidate further the antidigitalis effects of penicillamine a prospective study was undertaken in 10 children aged 4–14 years with congestive heart failure. Plasma and intracellular erythrocyte concentrations of sodium, potassium, calcium as well as Na⁺/K⁺ and Na⁺/Ca⁺⁺ ratios were measured before digitalization, 6 days after full digitalization while the patients were on maintenance doses of digoxin (0.02 mg/Kg/day, po, maximum 0.25 mg/day) and 6 hours after 1 Gm of oral penicillamine.

After digitalization RBC Na⁺, Ca⁺⁺, Na⁺/K⁺, and Na⁺/Ca⁺⁺ increased, whereas RBC K⁺ levels decreased significantly. Administration of penicillamine not only reduced serum digoxin levels, but it also caused significant alterations in RBC electrolyte concentrations, toward predigoxin values. All values were significantly changed after penicillamine. Plasma and RBC magnesium levels were not altered significantly, neither after digitalization nor after penicillamine. It is concluded that in addition to RBC Na⁺ and K⁺ levels, intra-erythrocyte levels of calcium are sensitive indicators of digoxin effect; and that penicillamine reverses digoxin-induced RBC electrolyte alterations towards pre-digitalization values.

Additional Indexing Words:
Digoxin Digitalis glycosides Intracellular electrolyte changes Penicillamine

CARDIAC glycosides bind to the erythrocyte (RBC) membrane and inhibit the active transport of Na⁺ and K⁺ across the cell membrane by inhibition of Na⁺, K⁺-ATPase. Administration of digitalis preparations

From the Research Laboratory* and the Department of Pediatric Cardiology,** the Cardiovascular Medical and Research Center, Tehran, Iran.

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Address for reprint: J. G. Shakibi, M.D., Heart Hospital, P.O. Box 33-423, Shemiran, Tehran, Iran.

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in man causes a decrease in red-cell K⁺ concentration and an increase in red-cell Na⁺ level. Loes et al recently reported the usefulness of RBC electrolyte determination in detection of digitalis intoxication. Previous reports showed the effectiveness of penicillamine in counteracting digitalis both in experimental animals as well as in humans. The purpose of this report is to further investigate the effect of penicillamine on digoxin-induced changes in erythrocytes electrolytes with special reference to alterations of intraerythrocyte calcium levels.

**Materials and Methods**

The patient material consisted of 10 children (4 males and 6 females) aged 4–14 years, who were admitted to the Cardiovascular Medical and Research Center for treatment of their cardiac disorder. Informed consent was obtained from all parents, before inclusion of the patients in the study protocol. All but 1 patient were in congestive heart failure and received no cardiotonic drugs at the time of admission. Seven patients had chronic rheumatic mitral regurgitation, whereas congenital mitral regurgitation, idiopathic hypertrophic subaortic stenosis, and post-commissurotomy mitral regurgitation were present in 1 patient each. Blood samples for determination of plasma and erythrocyte electrolyte concentrations were drawn according to the following schedule.

Sample 1—obtained before administration of digoxin, usually at the time of admission of the patient to the floor.

Sample 2—obtained 6 days after full digitalization, while the patients were receiving 0.02 mg digoxin (Burroughs Welcome), po, daily (maximum 0.25 mg/day).

Sample 3—obtained 6 hours after oral administration of 1 Gm of penicillamine, usually on the 6th day following full digitalization. Thus each patient served as his own control.

**Electrolyte determination:**

Sodium and potassium concentrations were measured in triplicate by flame photometry and the average of the 3 readings was recorded as a single value. Calcium and magnesium concentrations were determined by atomic absorption spectrophotometry (Varian Techtron-Model 1000) according to Helbock and Brown method. Standard errors of triplicate electrolyte determinations were: Na⁺ ±0.35, K⁺ ±0.75, Mg⁺⁺ ±0.04, and Ca⁺⁺ ±0.52 mEq/L. Mean ±SD for 20 determinations for plasma trapping was 3.1±1.0.

**Digoxin assay:**

Plasma digoxin concentrations were measured by radioimmunoassay technique using New England Nuclear Corporation kits (Boston, Massachusetts). Each sample was assayed in duplicate and the variance in assay procedure was estimated by addition of known standards with each of digoxin assays. Interference with digoxin assay by penicillamine was excluded in the previous report.

**Statistical analysis:**

The values obtained were compared using paired t-test, p values representing two-tailed probabilities.
Results

Results are summarized in Table I.

Administration of digoxin caused marked alterations in the plasma and intraerythrocyte electrolyte levels. The directions of changes for Na\(^+\), K\(^+\), and Mg\(^{++}\), were similar to those reported before. Thus red cell potassium levels decreased significantly (p<0.005), whereas erythrocyte sodium concentrations increased (p<0.001) after digitalization. Digitalization also led to remarkable increase in red cell calcium concentrations (p<0.005). The RBC Na\(^+/K^+\) and Na\(^+/Ca^{++}\) ratios also increased significantly after full digitalization (p<0.005 for both ratios).

Administration of penicillamine not only lowered the plasma levels of digoxin in a significant manner (p<0.001) but also caused reversal of erythrocyte electrolyte concentrations. Thus RBC potassium increased (p<0.01), RBC sodium concentration decreased (p<0.001), and RBC calcium levels also decreased significantly (p<0.01). Simultaneously RBC Na\(^+/K^+\) and Na\(^+/Ca^{++}\) ratios also decreased remarkably (p<0.01 and p<0.001 respectively).

Alterations of red cell magnesium levels were not significant, neither after digitalization nor after administration of penicillamine. All patients tolerated penicillamine very well and no side effect was noted in any of the patients.

Table I. Plasma and Red-Cell Electrolyte Concentrations* in 10 Children: Pre-Digitalization, Post-Digitalization and Post-Penicillamine

<table>
<thead>
<tr>
<th></th>
<th>Potassium mEq/L</th>
<th>Sodium mEq/L</th>
<th>Magnesium mEq/L</th>
<th>Calcium mEq/L</th>
<th>RBC Na(^+/K^+) ratio</th>
<th>RBC Na(^+/Ca^{++}) ratio</th>
<th>Plasma Digoxin level ng/ml</th>
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<tbody>
<tr>
<td>Pre-digitalization</td>
<td></td>
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<tr>
<td>plasma</td>
<td>4.38 ± 0.13</td>
<td>137.40 ± 1.00</td>
<td>1.92 ± 0.03</td>
<td>4.97 ± 0.18</td>
<td>0.056 ± 0.003</td>
<td>0.68 ± 0.05</td>
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</tr>
<tr>
<td>RBC</td>
<td>102.2 ± 1.26</td>
<td>6.10 ± 0.96</td>
<td>4.94 ± 0.10</td>
<td>9.1 ± 0.27</td>
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<td>Post-digitalization</td>
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<tr>
<td>plasma</td>
<td>4.66 ± 0.12</td>
<td>139.3 ± 0.59</td>
<td>1.95 ± 0.05</td>
<td>5.19 ± 0.20</td>
<td>0.100 ± 0.004</td>
<td>0.96 ± 0.03</td>
<td>1.87 ± 0.08</td>
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<tr>
<td>RBC</td>
<td>92.60 ± 2.51</td>
<td>9.8 ± 0.19</td>
<td>4.98 ± 0.04</td>
<td>10.2 ± 0.28</td>
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<tr>
<td>Post-penicillamine</td>
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<tr>
<td>plasma</td>
<td>4.71 ± 0.11</td>
<td>139.6 ± 0.26</td>
<td>1.93 ± 0.04</td>
<td>5.1 ± 0.17</td>
<td>0.077 ± 0.004</td>
<td>0.80 ± 0.04</td>
<td>1.14 ± 0.07</td>
</tr>
<tr>
<td>RBC</td>
<td>97.50 ± 1.35</td>
<td>7.6 ± 0.31</td>
<td>4.92 ± 0.03</td>
<td>9.5 ± 0.22</td>
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</table>

* All values are given as mean ± SEM.
DISCUSSION

Two aspects of this study deserve attention: 1-Alterations of erythrocyte electrolyte concentrations by digoxin, and 2-Reversal of these changes by administration of penicillamine.

1. Previous studies had clearly shown that cardiac glycosides led to a net loss of K+ and a net gain of Na+ by the affected cells due to specific inhibition of Na+-K+ ATPase in the cell membrane. These digitalis-induced alterations in the exposed cells, such as erythrocytes were used by Loes et al, as an indicator of digitalis effect as well as a useful sign of digitalis intoxication. Thus Loes et al showed that the children intoxicated with digitalis manifested significantly higher ratios of red-cell sodium to red-cell potassium ratios than nontoxic patients. In our study the directions of changes of erythrocyte Na+ and K+ were similar to those reported by Loes et al. In addition to Na+, K+, and Mg++, we also measured RBC calcium levels. Thus it was shown that RBC calcium levels were significantly increased after administration of digoxin. Although we cannot state from this study the mechanism involved in the increased calcium influx in the erythrocytes by exposure to digoxin, however it is noteworthy that the alterations of RBC calcium concentrations were of sufficient magnitude to be used as an additional quantitative indicator of digoxin effect and its reversal by penicillamine.

2. The effect of penicillamine on serum digoxin levels in nontoxic patients as well as its beneficial effects on digitoxic patients were previously reported from this laboratory. In this experiment we further investigated the effect of penicillamine on the changes of digoxin-induced erythrocyte electrolyte concentrations. It was found that oral penicillamine therapy not only significantly reduced the serum levels of digoxin but it also reversed in a significant fashion the changes induced in erythrocyte electrolyte levels. Thus RBC Na+ and Ca++ levels were decreased, whereas RBC K+ levels were increased. Although the exact mechanism of the effect of penicillamine is not yet clear, however based on these results it is clear that penicillamine affects the flux of electrolytes across the cell membrane induced by digoxin. This effect might be direct or most probably indirect, i.e. via its reducing of the serum digoxin levels.

In conclusion this study has demonstrated that in addition to erythrocyte Na+, and K+, RBC calcium levels are also a sensitive indicator of digoxin effect and that penicillamine not only reduces serum digoxin levels but it also tends to reverse the intraerythrocyte electrolyte changes brought about by digoxin.
ACKNOWLEDGMENT

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REFERENCES