SERUM IONISED CALCIUM AND ITS RELATIONSHIP TO PARATHYROID HORMONE AFTER Tibial FRACTURE

J. R. W. Hardy, D. Conlan, S. Hay, P. J. Gregg

From Leicester Royal Infirmary, Leicester, England

The changes in serum adjusted ionised calcium and parathyroid hormone (PTH) were prospectively studied in 32 patients with isolated tibial fractures, treated conservatively. We measured serum albumin, adjusted total calcium, phosphate, pH, adjusted ionised calcium and PTH at intervals until the fractures had healed.

The mean ionised calcium adjusted for pH fell within 24 hours of injury, and then rose to a peak at between four and six weeks. These changes cannot be explained by changes in serum pH or PTH. The restoration of normal ionised calcium levels after fracture coincided with the period when the callus was being calcified. Analysis of the changes in ionised calcium, phosphate and PTH suggests that PTH levels alter in response to changes in ionised calcium levels. PTH is highest immediately after fracture and lowest, often not recordable, at six weeks. The cause of the changes in the ionised calcium level has yet to be elucidated.

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Little has been published on the biochemical changes that follow fractures, and the biologically active moiety of total calcium, ionised calcium, has been largely ignored. There are conflicting reports on the biochemical changes that accompany fracture healing because of the heterogeneity of the fractures studied, the comparison of different species and the sampling intervals which missed important early changes (Speed 1931; Lal et al 1976; Meller et al 1984b). By deducing the optimum sampling interval from pilot studies we have achieved an accurate profile of the changes in ionised calcium and parathyroid hormone (PTH) in a select population after fracture. This is the first time that ionised calcium has been measured serially in a homogeneous group of fractures. These measurements may form a basis for future research into the biochemistry of fracture healing.

MATERIALS AND METHODS

A group of 50 consecutive patients being treated for tibial fractures agreed to supply blood samples on days 1, 2 and 3; weeks 1, 2, 4 and 6; and months 2, 3, 4, 5 and 6 after their injury or until union was complete. Venesection was always performed in the morning, at least four hours after eating, without a tourniquet or stasis. No patient had received intravenous fluids. The blood was collected in three 10 ml Monovette Z tubes for serum separation (Sarstedt, Germany), and 3 ml were collected in a heparinised syringe for immediate analysis of serum ionised calcium, pH and pH-adjusted calcium by a Ciba Corning 634 ionised calcium analyser (Ciba Corning Diagnostics Ltd, Halstead, UK). Quality control and accuracy were ensured by regular monitoring with Industry Standard Electrolytes from the same source. Each 3-ml specimen was sampled three times and an average calculated. Whole blood was centrifuged at 3000 rpm for 15 minutes and separated into aliquots. A sample was sent for urea, electrolyte and bone biochemistry profiles.

Eighteen of the patients were excluded from the series because they had other injuries, or needed surgery for failure of conservative management. The full serum analysis was performed on the remaining 32 patients, all of whom healed after conservative treatment. An ASTRA 8 analyser (Beckman Instruments Inc, Brea, CA, USA) was used to analyse a sample from each attendance for serum total calcium, albumin, adjusted calcium and phosphate. PTH analysis was performed using a whole-molecule assay kit (Ciba Corning Magic Lite Intact PTH Immunoassay) from serum stored at −20°C. Samples from day 1, week 1, and week 6 after fracture were analysed. The results were statistically compared using the paired Student t-test. The power of the test for adjusted ionised calcium was measured retrospectively.

RESULTS

There were 30 men and two women. Their mean age was 30 years (median 29; 18 to 72). Fracture patterns were...
J. R. W. HARDY, D. CONLAN, S. HAY, P. J. GREGG

Mean levels in 32 patients during the healing of a tibial fracture. Figure 1 – Serum albumin. Figure 2 – Adjusted serum calcium. Figure 3 – Serum phosphate. Figure 4 – Ionised calcium. Figure 5 – pH. Figure 6 – Adjusted ionised calcium.

classified according to the AO classification (Müller et al. 1990); they included twenty-four 42A1 to 3, seven 42B1 to 3 and one 42C3.1. All these fractures healed with conservative treatment in plaster casts within 20 weeks.

The mean serum albumin levels (Fig. 1) remained within the normal range of 35 to 55 g/l, but we detected a trend of low initial values which rose to a maximum at month 5. The lowest mean value for serum albumin was recorded at week 1; this was significantly different from the value at month 2 (p = 0.003). Mean adjusted serum calcium remained within the normal limits of 2.1 to 2.6 mmol/l, although a few patients had adjusted calcium levels below 2.1 mmol/l in the first three days. Mean adjusted calcium levels rose from an initial low value at day 3 to peak at week 4 (Fig. 2).

The mean serum phosphate level remained in the normal range of 0.8 to 1.4 mmol/l until week 4, after which in 29% of the patients it increased above the normal range. These levels were lowest on day 2, and were then significantly lower than those at week 4.
(p < 0.001), remaining less significantly different until month 4 (Fig. 3).

Mean ionised calcium levels (Fig. 4) were lowest at day 1, then rose rapidly to peak at week 6. There was a significant difference between levels at day 1 and at weeks 4 to 16 (p < 0.001).

The mean pH remained within a physiologically normal range (7.35 to 7.44), but tended to be high in the first week with a fall to 7.37 at week 2 (Fig. 5). There was a significant difference between levels on day 2 and in weeks 2 to 6 (p < 0.001).

The mean pH-adjusted ionised calcium was lowest on day 1 and steadily rose to peak at week 6 (Fig. 6). Serum adjusted ionised calcium levels were significantly different between day 1 and weeks 6 to 8 (p < 0.001). The power of this study was estimated retrospectively; for 21 patients with an expected difference of 0.09 mmol/l and a standard deviation of 0.07 mmol/l a power of 80% is achieved at the 5% significance level.

A pilot study on all samples from two patients showed that the trend in PTH values was for a progressive decline (Tables I and II). The mean PTH from 32 patients all measured at three intervals (day 1, week 1, week 6) fell progressively; the level was undetectable in most patients by week 6 (Fig. 7).

DISCUSSION

Investigations on the changes that take place at the biochemical level during the healing of tibial fractures have been few and have concentrated on the early changes (Frost 1989). The results that have been published are largely conflicting, probably because of the variety of fracture types and long sample intervals.

We studied the changes in mean serum albumin levels to show the typically increased catabolic state seen after injury. The trend of an initial fall and then of an increase in phosphate levels matches that of serum calcium. Speed (1931) observed that serum phosphate levels fell immediately after fracture then rose after 24 hours and remained high until they slowly decreased again, possibly in relation to the period of disability. These changes have been noted in other studies (Lal et al 1976; Meller et al 1984a). Speed also found that operative treatment after fracture caused an almost immediate rise in serum phosphate followed by a rapid fall to normal. Because of this we excluded patients who had operations during the healing period.

The changes in mean pH in our patients may result from respiratory alkalosis due to hyperventilation, a high respiratory rate caused by pain or from some unknown reason. None of our patients received treatment with salicylates, which could have accounted for these changes.

Ionised calcium is the physiologically active moiety of calcium in the blood, and it has been possible to measure this fraction since the 1960s (Moore 1970). The results depend on pH and the electrode response is logarithmic: small electrode potential errors at the extremes of measurement can produce large errors. This applies less to the newer electrodes, but emphasises the need for regular quality-control checks before the use of ion-exchange electrodes. Loss of carbon dioxide from specimens may cause the ionised fraction to fall but this was prevented in our investigation by using stoppered heparinised syringes (Ciba Corning).

Relationship between mean values of parathyroid hormone (PTH) and adjusted ionised calcium in 32 patients after tibial fractures.

Samples from 67 healthy volunteers analysed in one of the first 'flow-through' calcium electrodes, showed that ionised calcium varied only 6% over several months in each individual and remained within the narrow range of 0.94 to 1.33 mmol/l (Moore 1970). In the same group calcium levels were shown to vary inversely with the pH in whole blood. Speed (1931) measured the serum biochemical changes in a group of patients with a variety of fractures undergoing operations at differing periods after fracture. This study showed that the serum total calcium remained within normal limits, probably because of the inaccuracy of the calcium assay of the day and the infrequent sampling intervals. Meller et al (1984b) studied metabolic changes after fracture in 13 dogs, giving results before fracture, at 24 hours, and at 10 and 20 days. The serum total calcium level was significantly reduced immediately after fracture (p \leq 0.05) and rose thereafter, returning to normal during the development of callus. Meller et al (1984a) described the biochemical changes in 13 young patients with a variety of fractures taking measurements after admission and at eight weeks after fracture. In this group the serum total calcium was significantly reduced after admission compared with the mean eight weeks later (p < 0.001). Henderson, Kerr Graham and Mollan (1992) also demonstrated the rapid fall in ionised calcium in the first five days after severe multiple trauma; this fall was related to the fat embolus syndrome. Speed (1931) provided evidence that the level
of ionised calcium in the blood is important for calcification: he found that parathyroidectomised dogs do not calcify callus and go on to delayed union. In addition, Ham, Tisdall and Drake (1938) showed that rats with induced osteomalacia form callus which does not calcify until the osteomalacia is corrected. It is plausible, therefore, that the serum ionised calcium rises and peaks at four to six weeks after fracture to facilitate the process of fracture calcification. Another hypothesis is that the initial fall in ionised calcium is in some way related to the trauma, and there is increasing evidence of the role of fat embolus (Schnaid et al 1987): free fatty acids are believed to be the cause of the initial drop in ionised calcium after trauma (Henderson et al 1992).

MacCallum and Voegtlin (1908) first showed that removal of the parathyroid glands reduced serum calcium concentrations. PTH is generally considered to provide a homeostatic mechanism for the control of calcium levels: little is reported on the effect of this hormone on osteoblasts or other cells. It is often considered to be a catabolic hormone with no role in fracture healing (Meller et al 1984a). Simmons (1985) studied the relationship of calcium to PTH. He considered that most of the systemic changes in calcium levels after fracture are secondary effects in response to local haemorrhage, hypothesising that the hypocalcaemia may, in extreme cases only, be due to absorption into the haematoma and that the PTH response to hypocalcaemia is caused by a raised level of catecholamines which increases the parathyroid blood flow and the release of hormone. He further postulated that the increase in PTH, which until now has not been demonstrated, could have an important effect in stimulating proliferation of periosteal and endosteal cells. This theory is based on the work by his own group which showed the effect of PTH on DNA, protein and collagen synthesis in epiphyseal cartilage and bone (Russell, Walker and Simmons 1984). Parathyroid hormone has a vasodilatory effect on bone and may be important in facilitating the early stages of fracture healing (Pang, Janssen and Yee 1980). It may also act by stimulating cartilage metabolism in the newly forming callus (Gunness-Hey and Hock 1984), but little else is known about its role in fracture healing. Parathyroid hormone was first measured in young adults with fractures by Meller et al (1984b); they found no significant differences in the PTH level on the day of fracture and at eight weeks. This is because the patients were a heterogeneous group and some had received Ringer's lactate solution before venesection. In addition, they did not use a whole-molecule assay, and may have detected smaller related molecules as well as biologically-active PTH. Their first samples were taken in the first 12 hours after fracture, while our samples were taken after 24 hours, when the fall in ionised calcium levels had taken place and the resulting physiological rise in PTH was detectable. Our results suggest that PTH is likely to play a role only in the early stages of fracture healing; the reduction in levels up to six weeks after fracture are probably caused by the rise in ionised calcium levels.

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**Table I. Results for patient 1 during healing of a tibial fracture**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Month 2</th>
<th>Month 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/l)</td>
<td>2.13</td>
<td>2.28</td>
<td>2.22</td>
<td>2.32</td>
<td>2.37</td>
<td>2.38</td>
<td>2.36</td>
<td>2.25</td>
<td>2.31</td>
<td></td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>0.93</td>
<td>1.01</td>
<td>0.96</td>
<td>1.35</td>
<td>1.21</td>
<td>1.52</td>
<td>1.2</td>
<td>1.0</td>
<td>1.16</td>
<td></td>
</tr>
<tr>
<td>Ionised Ca2+ (mmol/l)</td>
<td>1.1</td>
<td>1.1</td>
<td>1.14</td>
<td>1.24</td>
<td>1.15</td>
<td>1.33</td>
<td>1.29</td>
<td>1.2</td>
<td>1.28</td>
<td></td>
</tr>
<tr>
<td>PTH* (pmol/l)</td>
<td>2.5</td>
<td>1.8</td>
<td>1.7</td>
<td>1.7</td>
<td>1.0</td>
<td>0.9</td>
<td>&lt;0.8</td>
<td>0.8</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

* parathyroid hormone

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**Table II. Results for patient 2 during healing of a tibial fracture**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Month 2</th>
<th>Month 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium (mmol/l)</td>
<td>2.3</td>
<td>2.28</td>
<td>2.25</td>
<td>2.23</td>
<td>2.16</td>
<td>2.29</td>
<td>2.46</td>
<td>2.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate (mmol/l)</td>
<td>0.95</td>
<td>1.13</td>
<td>1.06</td>
<td>1.42</td>
<td>1.14</td>
<td>1.43</td>
<td>1.19</td>
<td>1.49</td>
<td>1.36</td>
<td></td>
</tr>
<tr>
<td>Ionised Ca2+ (mmol/l)</td>
<td>1.15</td>
<td>1.14</td>
<td>1.13</td>
<td>1.2</td>
<td>1.18</td>
<td>1.18</td>
<td>1.2</td>
<td>1.24</td>
<td>1.1</td>
<td></td>
</tr>
<tr>
<td>PTH* (pmol/l)</td>
<td>2.1</td>
<td>2.0</td>
<td>2.7</td>
<td>1.1</td>
<td>1.0</td>
<td>2.1</td>
<td>0.8</td>
<td>&lt;0.8</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

* parathyroid hormone
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REFERENCES


