Biochemical Markers of Bone Turnover in the Domestic Cat: Relationships with Age and Feline Osteoclastic Resorptive Lesions\textsuperscript{1,2}

April DeLaurier,\textsuperscript{3} Brendan Jackson,\textsuperscript{*} Kate Ingham,\textsuperscript{†} Dirk Pfeiffer,\textsuperscript{*} Michael A. Horton and Joanna S. Price

Bone and Mineral Centre, Rayne Institute, University College London, London, UK; \textsuperscript{*}The Royal Veterinary College, London, UK and \textsuperscript{†}The Waltham Centre for Pet Nutrition, Leicestershire, UK

EXPANDED ABSTRACT

KEY WORDS: bone \hspace{0.4em} cats \hspace{0.4em} osteoblasts \hspace{0.4em} osteoclasts \hspace{0.4em} resorption \hspace{0.4em} tooth

Bone is a dynamic tissue that is continuously undergoing turnover throughout life. The activity of the cells that form bone (osteoblasts) and those that resorb bone (osteoclasts) can be monitored using biochemical markers measured in serum and urine using a variety of methods. One marker of bone formation is bone alkaline phosphatase (BAP), which is produced by osteoblasts and required for osteoid formation and matrix mineralization. Specific markers of bone matrix degradation by osteoclasts include deoxypyridinoline (DPD), which crosslinks adjacent collagen molecules, and the carboxy-terminal telopeptide (CTx) region of the type I collagen molecule.

Biochemical markers of bone cell activity are widely used in human metabolic disease research in studies of diseases such as osteoporosis and hyperparathyroidism (1,2). In other animals, markers of bone turnover have been used not only to assess skeletal maturation and the effects of hormones on bone metabolism but also to diagnose bone pathology (3–5). However, to date, there have been no studies of systemic levels of markers of bone turnover associated with specific pathology in cats, and the relationship between marker levels with age is unknown.

The aim of this study was to validate a number of established human assays of markers of bone formation and resorption for their potential use in the cat, and use bone markers to assess the relationship between age and bone turnover. Another objective was to establish the relationship between markers of bone turnover with the presence of feline osteoclastic resorptive lesions (FORL), a common age-related pathology affecting the tooth root and alveolar bone leading to tooth loss. The etiologic factors predisposing for FORL remain unknown, although it has been suggested that abnormalities of calcium regulation associated with changes in bone cell activity may play a role in the pathogenesis of this disease (6).

MATERIALS AND METHODS

Samples

Serum (n = 128) and urine (n = 81) samples were collected from cats between 4 mo and 14 y of age at the Waltham Center for Pet Nutrition (Leicestershire, UK). Cats over 1 y of age were physically examined and radiographically screened while under anesthesia for the presence of FORL, and serum and urine were collected at this time. Serum and urine were collected from cats under 1 y of age undergoing other medical procedures. All cats were fasted overnight prior to the procedure. Urine was collected by manual expression. Serum was separated within 1 h of collection and all samples were stored at \(-20^\circ\text{C}\) until use. For the purposes of this study, cats with at least one lesion were considered positive for the disease and cats with no evidence of resorption were used as controls. Serum and urine samples representing a range of ages and degree of disease (i.e., number of resorptive lesions) were selected for each assay (see Table 1). This study conforms to guidelines of the Waltham Ethical Review Committee. All procedures were conducted under the appropriate Home Office licenses.

Marker of bone formation

BAP. BAP levels were measured in serum by wheat germ lectin (WGL) precipitation, an established, nonimmunological method that has been used to measure BAP in other species, and with a human enzyme-linked immunosorbent assay (Alkphase B ELISA, Metra Biosystems, Great Haseley, UK) (7).
**TABLE 1**

Mean values and standard deviations (SD) of bone alkaline phosphatase (BAP) measured in serum by ELISA and deoxypyridinoline (DPD) measured in urine by high-performance liquid chromatography (HPLC) among cats affected with FORL and controls

<table>
<thead>
<tr>
<th>Bone alkaline phosphatase</th>
<th>Deoxypyridinoline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of resorptive lesions</td>
<td>Number of cats</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>0 (controls)</td>
<td>63</td>
</tr>
<tr>
<td>1–2</td>
<td>20</td>
</tr>
<tr>
<td>3–4</td>
<td>14</td>
</tr>
<tr>
<td>5–14</td>
<td>13</td>
</tr>
</tbody>
</table>

Deoxypyridinoline

<table>
<thead>
<tr>
<th>Number of resorptive lesions</th>
<th>Number of cats</th>
<th>Age range and mean age (y)</th>
<th>Mean DPD (nmol/mmol creatinine)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (controls)</td>
<td>46</td>
<td>1–10 (3.8)</td>
<td>11.3</td>
<td>11.7</td>
</tr>
<tr>
<td>1–2</td>
<td>11</td>
<td>2–10 (5.7)</td>
<td>10.6</td>
<td>8.8</td>
</tr>
<tr>
<td>3–4</td>
<td>5</td>
<td>2–8 (6.9)</td>
<td>11.6</td>
<td>7.9</td>
</tr>
<tr>
<td>5–14</td>
<td>5</td>
<td>4–11 (8.3)</td>
<td>13.0</td>
<td>8.5</td>
</tr>
</tbody>
</table>

1 No significant differences were detected between FORL and control groups.

**Markers of bone resorption**

**CTx.** CTx concentration was measured in serum using an automated analyzer developed for use in the human (Elecsys β-CTx; Roche, Basel, Switzerland), and in urine with a human ELISA (CrossLaps; Osteometer, Herlev, Denmark).

**DPD.** DPD was measured in urine by high-performance liquid chromatography (HPLC), which is considered a “gold-standard” method for measuring this marker (B. Jackson, Ph.D. thesis, 1999). Results were corrected for urinary creatinine excretion. DPD was also measured in serum with a human ELISA (Total DPD Serum; Metra Biosystems).

**Analysis**

Species cross-reactivity of the human monoclonal antibodies in the commercial ELISA assays with the markers in the cat was assessed by comparing a serial dilution of feline serum and urine against standards provided with the assay. To assess the relationship between biochemical parameters measured using different assays, and the relationship between age and marker levels, correlation analysis was performed (SPSS, Chicago, IL). To examine whether differences in marker levels exist between control samples and samples from cats affected with FORL, and whether severity of FORL is associated with changes in marker levels, analysis of variance was performed.

**RESULTS**

**BAP**

A curve derived from the serial dilution of cat serum was parallel with the human ELISA standard curve. A significant correlation was established between BAP measured by ELISA and by WGL (r = 0.87, P < 0.01, Fig. 1). BAP measured by ELISA showed a significant inverse correlation with age (r = −0.66, P < 0.01, Fig. 2). Cats ≤ 2 y old had significantly higher serum concentrations of BAP than that of cats over 2 y old (P < 0.01). However, among animals older than 2 y, no significant changes in marker levels were observed between age groups. No significant difference in BAP concentration was detected between cats affected with FORL and controls (Table 1).

**CTx**

A curve derived from the serial dilution of cat urine was parallel with the human CTx ELISA standard curve (data not shown). CTx measured by ELISA was correlated significantly with total urinary DPD measured by HPLC (r = 0.70, P < 0.01). However, CTx measured in serum using the Elecsys autoanalyzer was not significantly correlated with DPD measured by HPLC (r = 0.034).

**DPD**

Serial dilutions of feline serum showed poor correspondence with the human DPD ELISA standard curve, although DPD measured in serum showed a significant interassay correlation with DPD measured in urine by HPLC (r = 0.69, P < 0.01). The intraassay coefficient of variation (CV) for measurement of DPD by HPLC was 3.7% in the concentration range in this study, and the corresponding interassay CV was 6.4%. Urinary DPD measured by HPLC showed a significant inverse correlation with age (r = −0.56, P < 0.01). Cats ≤ 2 y old had significantly higher DPD concentrations than that of cats over 2 y old (P < 0.01). However, among animals older than 2 y, no significant changes in marker levels were
observed between age groups. No significant difference in DPD concentration was detected between cats affected with FORL and controls (Table 1).

DISCUSSION

This study has demonstrated that a number of assays developed for measuring markers of bone cell function in humans can be used in the domestic cat. The measurement of bone markers represents a noninvasive, simple and relatively inexpensive approach for monitoring normal age-related changes in bone cell function as well as those associated with diseases that influence bone metabolism. Bone alkaline phosphatase, a marker of bone formation by osteoblasts, can be measured in cat serum using a wheat germ lectin precipitation method or with a commercially available human ELISA. There was an excellent correlation between results obtained using these two techniques. A commercial human ELISA for measuring the resorption marker collagen C-terminal telopeptide (CTx) in urine did show good species cross-reactivity and displayed good correlation with urinary DPD. This assay has also been validated for use in dogs (4). As expected, deoxypyridinoline (DPD), a very specific marker of collagen degradation in bone, can be measured in cat urine using HPLC. However, the technique is time-consuming and laborious and there can be high assay variability. Unfortunately, measurement of total DPD in cat serum using a commercial human ELISA did not give consistent results, suggesting either a problem with the assay method or a lack of cross-reactivity of the human antibody with cat DPD.

Results show that markers of bone formation and resorption decrease with skeletal maturity in the cat and there was no significant difference among skeletally mature cats of different ages. These results are consistent with findings in other mammals. During growth, when bone is being modeled, turnover occurs rapidly, although once skeletal maturity is attained, osteoblast and osteoclast activities stabilize at a much lower level.

We also examined whether there are changes in the bone markers associated with FORL because it had been suggested that this condition could be associated with alterations in calcium metabolism. However, the presence or severity of FORL does not appear to be associated with systemic changes in bone turnover, suggesting that changes in osteoclast activity associated with this disease are local to the tooth microenvironment. Thus, the presence of FORL, which has a very high prevalence in older cats, would not confound the interpretation of bone marker measurements in studies of other diseases.

LITERATURE CITED