Bile Acid Composition of Dog and Cat Gall-bladder Bile
Tsukimi WASHIZU, Hidenori IKENAGA, Makoto WASHIZU¹, Takuo ISHIDA, Isamu TOMODA, and Jiro J. KANEKO²

Department of Clinical Pathology, ¹Veterinary Medical Teaching Hospital, Nippon Veterinary and Zootechnical College, 1-7-1 Kyonann-cho, Musashino, Tokyo 180, Japan, and ²Department of Clinical Pathology, School of Veterinary Medicine, University of California, Davis, U.S.A.
(Received 26 September 1989/Accepted 28 November 1989)


KEY WORDS: bile, bile acid, gall-bladder.

Bile acids are synthesized in the liver and excreted into the bile and stored in the gall bladder. They are released from the gall bladder following a meal and reabsorbed in the ilium after playing their absorptive physiological role in the intestine [5, 9]. These reabsorbed bile acids are transported back to the liver through the portal circulation, where they are again extracted, conjugated and reexcreted [8]. Serum bile acid concentrations increase above the normal range in various forms of hepatobiliary disease [1-4]. In past years, the fractionation of the individual bile acids has been investigated to understand the metabolic derangement of bile acids in hepatobiliary disease [7]. It is necessary to know the normal bile acid composition of gall-bladder bile when one uses fractionated serum bile acids for the evaluation of hepatobiliary disease. This paper reports the bile acids composition of gall-bladder bile of normal dogs and cats.

Six clinically healthy, mixed breed animals (3 dogs and 3 cats) were used for this study. Their hematological and serum chemistry values prior to the surgery were all within the normal range. Bile samples were obtained from the anesthetized animals by laparotomy. The bile (0.1 ml) was diluted with methanol (0.9 ml), mixed vigorously, and centrifuged at 12000 rpm for 15 min and the supernatant was used as a sample for HPLC.

The equipment used for this study was a Tosoh HPLC system (Tosoh, Ayase, Japan) which included a computerized system controller, SC-8010. TSK gel ODS-80TM (4.6 mm x 15 cm) reverse phase column (Tosoh, Ayase, Japan) was employed. Each sample (5 µl) was applied to this column, which was eluted at 0.6 ml/min with, 1) methanol (CH₂OH): 20 mM sodium biphosphate-10 mM sodium sulphate, pH3.5 (65:35, v/v) for 6.5 min, 2) CH₂OH:20 mM disodium hydrogen phosphate (Na₂HPO₄), pH6.5 (67:33, v/v) for 22.5 min, 3) CH₂OH:Na₂HOP₄, pH6.0 (72:28, v/v) for 11 min. β-Nicotinamide-adenine dinucleotide (β-NAD) solution (0.7 mM) prepared in 0.2 M dipotassium hydrogen phosphate and 1 mM ethylenediaminetetraacetic acid-4H were added to the eluates at a flow rate of 0.3 ml/min before passing through the immobilized 3α-HSD column. Fluorometric measurement of NADH was then performed with the detector, FS-8010, set at a wave length of 345 nm for excitation, and 450 nm for emission.

Fifteen bile acid standards (cholic acid(C), chenodeoxycholic acid (CDC), deoxycholic acid (DC), lithocholic acid (LC), ursodeoxycholic acid (UDC), taurocholic acid (TC), taurochenodeoxycholic acid (TCDC), taurodeoxycholic acid (TDC), tauroliothiocholic acid (TLC), taurosodeoxycholic acid (TUDC), glycocholic acid (GC), glycochenodeoxycholic acid (GCDC), glycodeoxycholic acid (GDC), glycolithocholic acid (GLC), glycursochenodeoxycholic acid (GUDC)) were obtained from Sigma Chemical Co., St. Louis and used as an external standard.

TUDC, TC, TCDC, TDC, TLC, GC, GLC, and C were identified in gall-bladder bile of the dogs examined and the average of total concentration of the bile acids was 51.36 mg/ml, whereas, in the cat, TUDC, TC, TCDC, TDC, TLC, GUDC, GCDC, and C were identified and the average of total concentration of the bile acids was 32.32 mg/ml. TC, TDC, and TCDC were quantitatively the major bile acids in both species and were more than 99% of the total in the dog and 98% in the cat. In humans, GCDC is the most predominant bile acid at 32% and TC is only at 7%. The amounts of other identified bile acids were very small and of the order of μg/ml in both species. Three unidentified peaks were consistently seen in the dog samples and 5 in the cat samples. Some of the unidentified bile acids seen in the cat might have been allocholic acid.
Table 1. Bile acid composition of gall-bladder bile of dogs and cats

<table>
<thead>
<tr>
<th>Bile acid*</th>
<th>Dog</th>
<th>Cat</th>
</tr>
</thead>
<tbody>
<tr>
<td>TUDC</td>
<td>0.06±0.05</td>
<td>0.03±0.04</td>
</tr>
<tr>
<td>TC</td>
<td>37.59±9.08</td>
<td>24.00±6.94</td>
</tr>
<tr>
<td>TCDC</td>
<td>3.07±1.42</td>
<td>3.33±1.24</td>
</tr>
<tr>
<td>TDC</td>
<td>10.33±1.48</td>
<td>4.37±2.52*</td>
</tr>
<tr>
<td>TLC</td>
<td>0.05±0.02</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>C</td>
<td>0.01±0.01</td>
<td>0.09±0.14</td>
</tr>
<tr>
<td>GUDC</td>
<td>—</td>
<td>0.33±0.38</td>
</tr>
<tr>
<td>GC</td>
<td>0.19±0.06</td>
<td>—</td>
</tr>
<tr>
<td>GCDC</td>
<td>—</td>
<td>0.12±0.11</td>
</tr>
<tr>
<td>GLC</td>
<td>0.06±0.05</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>51.36±10.27</td>
<td>32.32±10.34</td>
</tr>
</tbody>
</table>

* Significantly different (p<0.05) from the concentration of dog bile.

T. WASHIZU, ET AL.

Fig. 1. Bile acid chromatogram of dog gall-bladder bile. Peaks without identifying initials are unidentified bile acids. Peaks with retention times of less than 9 are solvent impurities. TUDC, tauroseodeoxycholic acid; TC, taurocholic acid; TCDC, taurochenodeoxycholic acid; TDC, taurodeoxycholic acid; TLC, taurolithocholic acid; C, cholic acid; GC, glycocholic acid; GLC, glycolithocholic acid.

Fig. 2. Bile acid chromatogram of cat gall-bladder bile. Peaks without identifying initials are unidentified bile acids. Peaks with retention times of less than 9 are solvent impurities. TUDC, tauroseodeoxycholic acid; TC, taurocholic acid; TCDC, taurochenodeoxycholic acid; TDC, taurodeoxycholic acid; TLC, taurolithocholic acid; C, cholic acid; GUDC, glycocholsodeoxycholic acid; GCDC, glycochenodeoxycholic acid; GLC, glycolithocholic acid.

because cat bile is known to contain appreciable amounts of allocholic acid, the 5α isomer of cholic acid [10]. In humans, bile acids in bile are predominantly conjugated with glycine and the ratio of glycine conjugated bile acids to taurine conjugated ones (G/T) is 3 [6]. In contrast to the humans, bile acids in the dog and cat are almost exclusively conjugated with taurine. The ratio of trihydroxy bile acids to dihydroxy bile acids (T/D) is approximately 1 in humans, whereas, it was about 3 in dogs and cats [6]. This indicates that trihydroxy bile acids are preferentially synthesized in dogs and cats.

In the present study, fractionation was done to determine the types of bile acids seen in dog and cat gall-bladder bile and to develop methods for HPLC assay of bile acids in bile. The composition of bile acids in dogs and cats was significantly different from that in humans. This suggests that there would be appreciable differences in the intermediates during the synthesis of bile acids and the ratios, such as G/T and T/D, which are used as indicators of liver disease in humans, cannot be used in dogs and cats.

REFERENCES
2. Center, S. A., Baldwin, B. H., Erbk H. N., and

要約

高速液体クロマトグラフィー（HPLC）による犬、猫の胆囊胆汁中胆汁酸分析（短報）：鶴巻月美・池永英紀・藤巻誠・石田卓夫・友田勇・兼野敬一（日本獣医薬品大学獣医臨床病理学教室）——犬および猫の胆囊胆汁中の胆汁酸をHPLCにより分析した。犬胆囊胆汁中に確認された胆汁酸はTUDC, TC, TCDC, TDC, TLC, GC, GLCおよびCで、これら8種類の胆汁酸の総和は平均で51.36mg/mlであった。猫胆囊胆汁中に確認された胆汁酸はTUDC, TC, TCDC, TDC, TLC, GUDC, GCDC, Cの8種類で、その和は平均で32.32mg/mlであった。