Aquaporin-1 Expression in Canine Peripheral Erythrocytes and Its Relation to Cell Volume

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Abstract: To evaluate the relationship between aquaporin-1 (AQP1) expression and the cell volume of red blood cells (RBCs), canine peripheral RBCs were separated according to specific gravity, and expression of the AQP1 protein on the membrane of RBCs was compared using anti-dog AQP1 polypeptide serum. Western blot analysis indicated that there was no significant difference in AQP1 expression between large and small cell fractions. In addition, the AQP1 expression of inherited high K/low Na RBCs which are known to be 20% larger than normal RBCs, was comparable to that of normal RBCs. These results suggest that AQP1, the major water channel in RBCs, does not determine the cell volume of peripheral canine RBCs.

Key words: aquaporin-1, cell volume, RBCs

Aquaporins comprise a superfamily providing water channels in biomembranes, and aquaporin-1 (AQP1), which is a member of that family, was first detected in red blood cells (RBCs) [2, 12, 13]. Although most mammals possess high potassium (K) and low sodium (Na) content in their RBCs, carnivores such as dogs and cats possess RBCs having low K and high Na. This is due to a lack of the (Na, K)-ATPase (Na-K pump), which is a membrane-bound enzyme playing an active role in transport of Na out of, and K into, cells to maintain the intracellular high K, low Na concentration. However, some dogs possessing the Na-K pump on RBCs were found in the Japanese Shiba dog family, and their cation composition was conversely high K (HK) and low Na as in other mammals [9]. Notably, the volume of HK RBCs is 20% larger than that of LK RBCs [8], and the cause of the larger cell volume is unknown. We determined the cDNA sequence of canine AQP1, the major water channel in RBCs, and investigated the functional role of canine AQP1 [5]. The AQP1 expressions in various tissues of both HK and LK dogs have also been compared [11]. In this report, to evaluate the relationship between the AQP1 expression in canine RBCs and their cell volume, peripheral RBCs from both HK and LK dogs were separated according to their specific gravity, and expression of the AQP1 protein on the membrane of RBCs was compared between cell groups. All experiments met the guidelines of the Laboratory Animal Care Committee of Azabu University. All peripheral blood samples from Shiba dogs possessing normal LK red blood cells were obtained by venipuncture using heparin as an anticoagulant. The light and dense cell fractions were separated according to their specific gravity as described elsewhere [10]. After centrifugation, the top
10% and bottom 10% layers were then harvested as light and dense cell fractions, respectively, and the mean corpuscular volume (MCV) was measured using a microcell counter (Sysmex, Tokyo, Japan). As shown in Table 1, the light cell groups of RBCs were 15% larger than the dense cell groups in both HK and LK RBCs. Membranes of RBCs were prepared for western blot analysis by treating with hypotonic solution and collection by centrifugation. Then, they were solubilized in SDS, electrophoresed into 12% polyacrylamide gels, and immunoblotted by chemiluminescence autoradiography. Anti-dog AQP1 serum was prepared as described previously [11]. Briefly, we used a peptide antigen designed according to the C-terminus amino acid sequence of dog AQP1 (RVKVTSGQVEEYEL; residues 243–257; see Ref. 11). Figure 1 shows the results of the western blot analysis of membranes of RBCs from various animals. All mammalians, including seals and rodents revealed both glycosilated and unglycosilated protein membranes of RBCs, while chickens and snakes did not. This difference among in animal species may be attributable to the difference in the amino acid sequences of their AQP1 C-termini. The relatively few molecules found in sheep was consistent with those in a previous report [15]. Figure 2 shows results of the western blot analysis of the membranes of RBCs from HK and LK dogs with or without N-glycosidase F treatment. A single band at 28 kDa (unglycosilated) and a broad signal from 30 to 50 kDa (glycosilated) were observed. Figure 3 shows the results of the western blot analysis of AQP1 expression on the membranes of HK and LK RBCs separated by specific gravity. There was no significant difference in AQP1 protein expression between HK and LK cells, as indicated in Figs. 2 and 3.

In the present study, to clarify the relationship between cell volume and AQP1 expression on the cell membrane, we focused on the role of AQP1 expression on canine

<table>
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<th>Light</th>
<th>Dense</th>
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<td>HK cells</td>
<td>75.1 ± 2.2</td>
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<tr>
<td>LK cell</td>
<td>67.0 ± 1.7</td>
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Values are mean ± SD of 3 determinations (MCV: fl).

Fig. 1. Immunoblotting of erythrocyte membranes isolated from various animals as indicated. Red blood cells were collected using heparin as an anticoagulant, and washed ice-cold PBS three times. Membrane samples were collected and electrophoresed into 12% polyacrylamide gel and immunoblotted with anti-dog AQP1 serum.

Fig. 2. Immunoblotting of erythrocyte membranes isolated from HK and LK dogs. Samples of membrane protein with (+) or without (−) treatment of N-glycosidase F at 37°C for 30 min. were electrophoresed into 12% polyacrylamide gel and immunoblotted with anti-dog AQP1 serum.

Fig. 3. Immunoblotting of erythrocyte membranes from HK and LK dogs membranes separated by specific gravity. Samples of membrane protein (10 µg) were electrophoresed into 12% polyacrylamide gel and immunoblotted with anti-dog AQP1 serum. a: top; b: middle; c: bottom fractions.
peripheral RBCs. We divided the RBCs of LK and HK cells, which are known to be 20% larger, according to density, and AQP1 protein levels on the cell membranes were investigated. Although AQP1 is known to be a major water channel in RBCs, there was no significant difference in AQP expression between large (light) and small (dense) fractions of both HK and LK RBCs. Recently, it was reported that AQP1 was partially lost during maturation by the release of exosomes containing AQP1 protein from reticulocytes [1]. Moreover, high tonicity suppressed the secretion of AQP1, implying that modulation of medium tonicity regulated the secretion of AQP1 [1]. In general, reticulocytes possess larger cell volumes than mature erythrocytes, and peripheral RBCs gradually reduce their cell volumes during circulation [18]. The results of this study reveal that there was no difference in AQP1 expression in peripheral LK and HK RBCs, and that the AQP1 protein level did not change during circulation. Therefore, we speculate that AQP1 on the membrane is not involved in the determination of cell volume, rather AQP1 is responsible for the response of cell volume to changes in plasma tonicity. HK cells exhibited immature isotypes of pyruvate kinase and hexokinase [7], and the half-life of HK RBCs is about one-half that of LK RBCs, suggesting that an abnormal cell differentiation or maturation may occur at an early stage of erythroid HK. Notably, HK cells had activated Na-dependent glutamate/aspartate transport due to the Na driving force created by the Na-K pump, resulting in abnormal accumulations of three amino acids (Asp, Glu and Gln) and glutathione (GSH) [7, 9]. Elevated levels of these amino acids and GSH may account for the larger cell volume of HK RBCs. Interestingly, HK dogs have only been found in some Japanese and Korean breeds [3], and there have been no reports of HK dogs in other regions. The HK phenotype is inherited in an autosomal recessive mode and the LK phenotype is inherited in a dominant manner [8], though the gene responsible for these phenotypes is still unknown. Analysis of HK dogs may shed light on the evolution of carnivore erythrocytes, and the results of this report may provide further insights into the physiology of water homeostasis in canine RBCs.

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References