Development of hepatocellular carcinoma after long-term immunosuppressive therapy including danazol in a dog

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Danazol is a synthetic androgen, which is used for the treatment of endometriosis in humans. It also has an immunosuppressive effect and stimulates hepatopoesis [13, 17]. Many reports have documented the effectiveness of danazol in treating several immune-mediated diseases in humans [5, 10]. However, such reports are limited in veterinary medicine [12, 18], and hence, its utility remains unclear. In humans, danazol has been shown to have adverse effects, such as reversible liver damage and virilization [4, 5]. Although a causal relationship has not been conclusively established, some patients have developed hepatocellular carcinoma (HCC) after the administration of danazol [3, 6, 7, 15, 19, 22]. This report describes the development of HCC in a dog after chronic administration of danazol in addition to other immunosuppressive drugs, to induce remission of non-regenerative immune-mediated anemia (NRIMA).

A 2-year-old spayed female beagle, weighing 7 kg, was referred to the Yamaguchi University Animal Medical Center for evaluation of anemia. Laboratory tests, including bone marrow cytology, revealed non-regenerative immune-mediated anemia (NRIMA). Although initial immunosuppressive multi-drug therapy was not effective, additional administration of danazol was successful in treating the anemia. However, hepatocellular carcinoma (HCC) developed about 20 months after the administration of danazol. In humans, several cases of development of HCC after the administration of danazol have been reported. The present report describes a case of HCC development in a dog after chronic administration of danazol in addition to other immunosuppressive drugs.

KEYWORDS: danazol, hepatocellular carcinoma, immune-mediated anemia, immunosuppressive therapy

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The dog showed good progress for more than a year after the administration of danazol, but had to return to the hospital because of vomiting following the consumption of a towel on day 784. We performed abdominal radiography that revealed hepatoenlargement and calcification of the spleen, which had not been detected on day 531 (Fig. 2a). Moreover, a mass on the left lateral lobe, as well as diffuse nodes in the liver, was detected on abdominal ultrasonography.
Computed tomography confirmed the observations (Fig. 2b). Although symptoms, such as vomiting, diminished after eliminating the towel in the dog’s feces, a surgical procedure was performed to excise the hepatic mass, and biopsies of the hepatic nodes and spleen were performed on day 846. Histopathological examination (IDEXX Laboratories, Tokyo, Japan) revealed that the excised hepatic mass was HCC, the hepatic nodes had nodular hyperplasia, and the spleen was calcified. Administration of prednisolone and mycophenolate mofetil was continued, but danazol and cyclosporine were discontinued, and the dog was monitored carefully for the recurrence of anemia and HCC. Although severe recurrence of anemia was not observed, a mild recurrence was suspected because of a mild decrease of PCV (from 40% to 36% over a month) and aggressive reproduction of red blood cells on a blood smear on day 965. Therefore, the dose of prednisolone was increased from 0.5 mg/kg once every three days to 0.5 mg/kg once every two days, and the PCV was elevated to 41% in a month. Thereafter, the dose of prednisolone was maintained at 0.5 mg/kg once a day or 0.5 mg/kg once every two days, and the level of ALT remained stable between about 40 U/l and 100 U/l. However, abdominal ultrasonography performed on day 988 revealed a hepatic mass on the left medial lobe, without any clinical symptoms. Since the previous mass was on the left lateral lobe, this seemed to be a newly developed mass. A surgical excision was performed again on day 1007, and this mass was identified as HCC on histopathological examination. Thereafter, the dog was monitored every 4 to 6 weeks until day 1481. Its condition has since remained stable.

Aggressive immunosuppressive multi-drug therapy may be necessary for the treatment of canine NRIMA; however, the treatment response time can be very high compared to that of immune-mediated hemolytic anemia [23]. We decided to use a glucocorticoid and two other immunosuppressive drugs for the induction therapy in this case, because treatment time was expected to be long because of the peculiar bone marrow cytology. However, in general, the combined usage of more than two immunosuppressive agents should be avoided, because of the risk of infections. Although prednisolone, cyclosporine and mycophenolate mofetil were administered for almost 6 months, they had no effect. After the administration of danazol, the dog showed a favorable response, suggesting the drug's effectiveness. However, these immunosuppressive drugs could not have been completely discontinued, because of the recurrences of mild anemia, which necessitated chronic administration.

Although there have been some reports on the use of danazol for immunosuppressive therapy in dogs, its effect is not clear [12]. In humans, danazol has been shown to exert an immunosuppressive effect in vitro mediated by the inhibition of lymphocyte proliferation and lowering of IL-1 and TNF-α levels [13]. It also has a stimulatory effect on hematopoiesis [17]. In addition, danazol increases the levels of cyclosporine in the blood by inhibiting cytochrome P-450 3A (CYP-3A), which is the metabolizing enzyme of cyclosporine A and is thought to potentiate the immunosuppressive effect of cyclosporine. Therefore, danazol is considered useful for the treat-
HCC INDUCED BY IMMUNOSUPPRESSIVE THERAPY IN A DOG

The trough levels of blood cyclosporine concentration in the present case were measured before and after the administration of danazol (days 138 and 277), but there was no increase (250 ng/ml and 88 ng/ml, respectively). In contrast, the elevation of the blood levels of ALT and ALP, as well as abdominal distension, was observed only after the administration of danazol, even though prednisolone had been used for nearly 6 months before the administration of danazol. Danazol is known to cause liver damage; hence, the above findings are thought to be the side effects of danazol. Alternatively, since CYP-3A also metabolizes prednisolone, the favorable response to immunosuppressive therapy and the adverse events, such as the elevation of the levels of liver enzymes and abdominal distension, in the present case after using danazol may be due to the potentiation of prednisolone by danazol.

In humans, the development of cancer after the administration of immunosuppressive drugs in patients with organ transplants, such as bone marrow, kidney and heart transplants, has been reported [8, 9, 20]. Two compelling factors relate immunosuppressive therapy and the development of tumors [21]. First, the ability of immune surveillance for detecting developing tumors and viruses associated with malignant tumors, such as Epstein-Barr virus, may be impaired in immunosuppressive states. Second, immunosuppressive drugs themselves have oncogenic effects, such as a reduction in the ability for DNA repair, induced by cyclosporine [1, 8]. Although such reports are limited in veterinary medicine, a few studies have been reported, in which the incidence of development of malignant tumors, especially lymphoma, was increased by cyclosporine-based immunosuppressive therapy after renal transplantation in cats [16, 24, 25]. Although the relationship between administration of cyclosporine and development of HCC was reported in rats [11], it is not clear in humans, felines and canines. More importantly, there have been several reports of human patients developing HCC after administration of danazol [3, 6, 7, 15, 19, 22]. Although the effect of danazol on the liver is not completely clear even in humans, danazol is known to adversely affect liver enzymes, such as ALT and ALP, by elevating their levels [4, 5]. In addition, hepatocellular adenomas and focal nodular hyperplasia induced by danazol have been reported in humans [2]; chronic injury to the hepatic cells may induce these abnormalities. A chronic immunosuppressive state could also contribute to the development of HCC. Cirrhosis, hepatitis and chronic hepatitis B/C viral infection are dominant factors for the development of HCC in humans; however, almost none of the patients with HCC induced by danazol have the abovementioned conditions, despite receiving chronic administration of danazol for more than two years. Hence, chronic administration of danazol is considered one of the factors for the development of HCC in humans.

Table 1. Myelogram on day 3

| Cell type            | Percentage (%) |
|----------------------|----------------|
| Rubriblast           | 1.3            |
| Basophilic rubricyte | 6.4            |
| Polychromatophilic rubricyte | 1.3 |
| Metarubricyte        | 0.0            |
| Myeloblast           | 1.9            |
| Promyelocyte         | 3.2            |
| Myelocyte            | 4.8            |
| Metamyelocyte        | 9.0            |
| Band neutrophil      | 20.1           |
| Segmented neutrophil | 48.9           |
| Band eosinophil      | 0.3            |
| Segmented eosinophil | 0.0            |
| Band basophil        | 0.0            |
| Segmented basophil   | 0.0            |
| Lymphoblast          | 0.6            |
| Lymphocyte           | 1.6            |
| Plasma cell          | 0.0            |
| Monoblast            | 0.0            |
| Promonocyte          | 0.3            |
| Monocyte             | 0.3            |
| Megakaryoblast       | 0.0            |
| Megakaryocyte        | 0.0            |
| M/E ratio           | 9.9            |
| Blast ratio          |                |
| ANC (b)              | 3.3            |
| NEC (c)              | 2.2            |

a) M/E ratio: myeloid cells relative to erythroid cell ratio.
b) ANC: all nucleated cells, c) NEC: non-erythroid cells.

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**Fig. 2.** Imaging findings of (a) abdominal radiography and (b) contrast-enhanced computed tomography (CT) performed on day 784. (a) Significant hepatomegaly is observed. (b) A mass on the left lateral lobe (a shaded arrow) and diffuse nodes (an arrowhead) in the liver are detected. Calcification of the spleen is also observed (a white arrow).
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