NOTE *Internal Medicine*

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

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ABSTRACT. A 2-year-old female beagle was referred to our hospital 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.

KEY WORDS: danazol, hepatocellular carcinoma, immune-mediated anemia, immunosuppressive therapy

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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 hematopoiesis [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. A complete blood count revealed no abnormalities in white blood cell count (12,200/μl; reference range, 6,000–17,000/μl) and platelets (315 × 10^3/μl; reference range, 200–500 × 10^3/μl), but revealed severe non-regenerative anemia (packed cell volume [PCV] was 10%; reference range, 37–55%). Serum biochemical analysis yielded normal results. A direct Coombs test (4°C and 37°C) and autohemagglutination gave negative results. Bone marrow aspiration was performed after whole blood transfusions on day 3. Cytologic evaluation showed that the subset of granulocytes was normal, but erythroid cell numbers, especially those of polychromatophilic rubricytes and metarubricytes, were remarkably decreased (Table 1). The myeloid/erythroid (M/E) ratio was 9.9 (reference range, 0.75–2.53), and hence, a diagnosis of NRIMA was established.

Figure 1 shows the clinical course. After diagnosis on day 3, immunosuppressive therapy, including administration of prednisolone (2 mg/kg, q12 hr), cyclosporine (5 mg/kg, q24 hr) and mycophenolate mofetil (10 mg/kg, q12 hr) was initiated, and when necessary, whole-blood transfusions (120 m*l* each) were repeated. The PCV could not be sustained without whole-blood transfusions, which were performed nine times until day 166. Administration of danazol (5 mg/kg, q12 hr) was initiated on day 170. Thereafter, the PCV was sustained without whole-blood transfusions, even though an increase in blood alanine transaminase (ALT) and alkaline phosphatase (ALP) levels and abdominal distension were observed. Recurrence of anemia was observed (PCV was 23%) on day 461, but it was controlled by increasing the dose of prednisolone. We also succeeded in gradually reducing the prednisolone dose (Fig. 1).

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 hepatomegaly 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.

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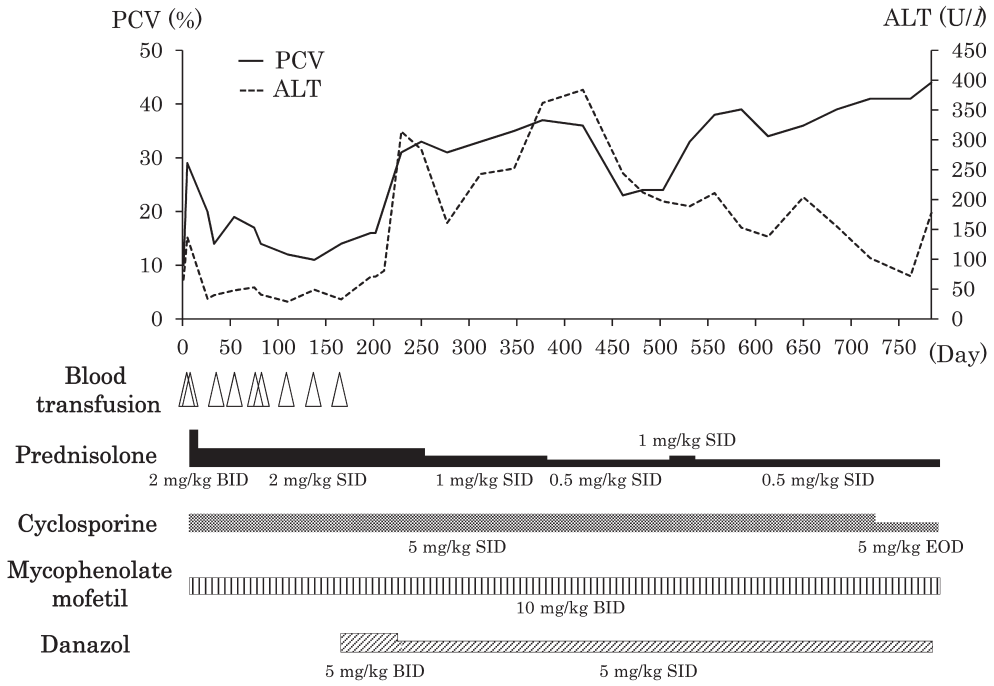

Fig. 1. The clinical course of the present case. Changes in packed cell volume (PCV, a solid line) and alanine transaminase levels (ALT, a broken line) are shown. Performed blood transfusions (120 m*l* each) and administered immunosuppressive drugs are also described below the graph. SID, once a day; BID, twice a day; EOD, once every two days.

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-

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 [a)]	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.

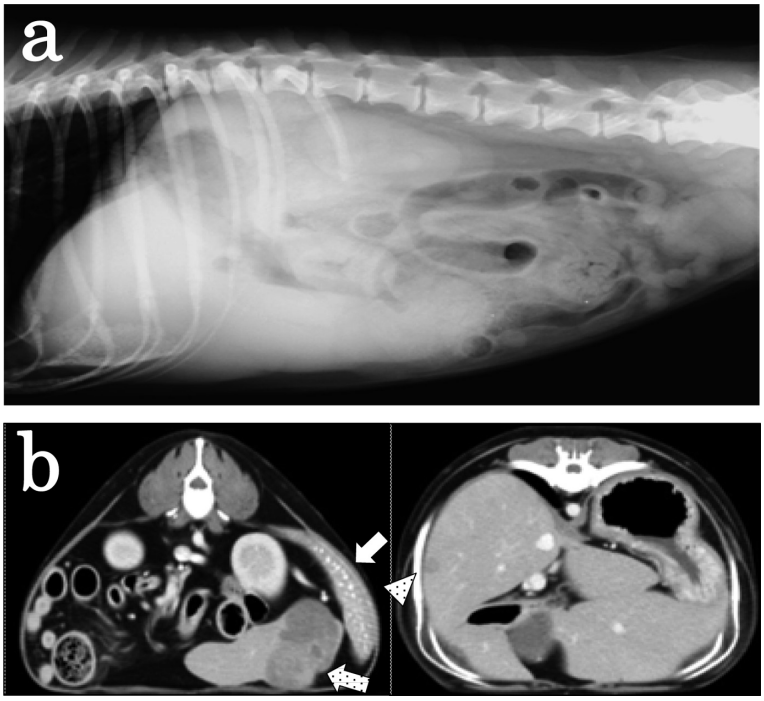

Fig. 2. Imaging findings of (a) abdominal radiography and (b) contrast-enhanced computed tomography (CT) performed on day 784. (a) Significant hepatomegaly is observed. (b) A mass on the left lateral lobe (a shaded arrow) and diffuse nodes (an arrowhead) in the liver are detected. Calcification of the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

ment of immune-mediated cytopenia in humans [5, 10]. 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/m*l* and 88 *n*g/m*l*, respectively). In contrast, the elevation of the blood levels of ALT and ALP, as well as abdominal distention, 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 distention, 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.

In the present case, HCC developed at 4 years and 6 months of age. According to previous reports, the median age of canine HCC is 11 years, and 81% of the dogs are older than 10 years of age [14]; thus, HCC onset in the present case was relatively early. Moreover, multiple hepatic nodular hyperplasias were identified simultaneously with the first detection of HCC, and the second HCC possibly developed as a new lesion. These findings indicate the possibility that these hepatic lesions were induced by chronic administration of the immunosuppressive drugs, especially danazol, which damaged the hepatic cells beyond naturally occurring levels. Indeed, the liver enzymes of the case were maintained high during administration of danazol. Therefore, danazol has been implicated as one of the causes of development of HCC in the present case, even though the relationship between danazol and the development of HCC is not easily proven.

This report documents the successful treatment of NRIMA by danazol administration and the development of HCC after chronic administration of immunosuppressive drugs, including danazol, in a dog. Danazol may be useful for treating canine immune-mediated anemia that is refractory to other treatments. However, although not proven, the development of HCC in the present case is believed to have been caused by the administration of danazol in addition to other immunosuppressive drugs. Thus, regular monitoring for the development of HCC must be performed when chronic administration of danazol is required.

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