Hematologic and Immunologic Studies in Dogs Given Nitrogen Mustard (HN3)

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Summary. Hematologic and immunosuppressive effects of single doses of nitrogen mustard (HN3) were evaluated in 20 dogs. HN3 caused profound depression of peripheral blood counts in all animals. Recovery of total white blood cell counts in dogs surviving the acute gastrointestinal toxicity of HN3 was complete by day 15. Recovery of platelet and lymphocyte counts to initial levels took a more prolonged course.

Granulopoietic progenitor cells (CFU-C) in the bone marrow were assayed by an in vitro culture system. Concentrations of CFU-C were markedly decreased one day after HN3-treatment, but showed at near-lethal doses rapid restoration to normal values within 4 to 7 days.

Cellular immune function of HN3-treated dogs was impaired for prolonged periods as indicated by the reduced capacity of canine lymphocytes to proliferate in vitro in response to stimulation with mitogens. Humoral immune function was similarly affected as determined by the depressed and delayed antibody formation against an intravenous challenge with sheep red blood cells.

In conclusion, our results suggest a different effect of HN3 on lymphoid and myeloid precursor cells.

Key words: Nitrogen mustard–Granulopoietic progenitor cells–Immune reactivity


Granulopoetische Vorläuferzellen (CFU-C) im Knochenmark wurden durch in vitro Kulturen bestimmt. Die CFU-C-Konzentrationen waren einen
Tag nach der Behandlung mit HN3 deutlich vermindert, zeigten aber selbst nach nahezu letalen Dosen schnelle Regeneration zu Normalwerten innerhalb 4 bis 7 Tagen.


Zusammenfassend lassen unsere Ergebnisse eine unterschiedliche Wirkung von HN3 auf lymphatische und myeloische Vorläuferzellen vermuten.

Hematopoietic toxicity is a common limiting factor in the use of chemotherapy for the treatment of malignant disease. Severe marrow toxic effects of nitrogen mustard in rodents have been documented by Host [5]. Rapid recovery from mustard-induced marrow hypoplasia in rats [4] and dogs [9], however, suggested relative insensitivity of hemopoietic stem cells to this agent. The present study has been undertaken to investigate quantitatively the long term effects of various single doses of nitrogen mustard (HN3) on the survival of bone marrow granulopoietic progenitor cells (CFU-C) in dogs. In addition, the influence of HN3-treatment on cellular and humoral immunity was studied.

Materials and Methods

Dogs: Beagles 6 to 12 months old and weighing 5 to 13 kg were obtained from two different breeding colonies. They were immunized against distemper, canine hepatitis and leptospirosis, dewormed and observed for disease for 4 weeks before use.

Nitrogen mustard: Crystalline Methyl-tris (β-chlorethyl) amine hydrochloride (HN3) was dissolved in appropriate volumes of physiologic saline and administered within 15 min of reconstitution as a single intravenous injection.

Hematologic studies: White blood cell (WBC) and platelet counts and hematocrits were performed at least three times weekly. For in vitro determinations of granulopoietic progenitor cells (CFU-C) marrow samples of 1 to 2 ml were aspirated from the iliac crest of HN3-treated dogs. $1 \times 10^8$ marrow cells were suspended in McCoys medium containing 0.3% agar and plated in 1 ml aliquots in 35 mm standard plastic Petri dishes (Greiner, Nürtingen). As a standard source of colony-stimulating activity (CSA) 0.2 ml of dog serum collected on day 12 after irradiation with 1200 R was added to each dish. Cultures were prepared in triplicate and incubated at 37°C in a fully humidified atmosphere flushed with 5% CO$_2$. After 9 days the dishes were removed and examined for colonies (aggregates of 50 or more cells) with a Leitz Diavert microscope. CSA in serum samples of HN3-treated dogs was assayed by its effect on cultured normal dog bone marrow cells.

Immunologic studies: The method for the determination of the in vitro reactivity of canine lymphocytes in response to stimulation with the mitogens Phytohemagglutinin (PHA), Concanavalin A (ConA) and Pokeweed Mitogen (PWM) has been described previously [1].

To assess the effect of HN3 on humoral immune function, dogs were injected intravenously with 2 ml of a 10% suspension of thrice washed sheeps red blood cells (SRBC) 24 h after HN3 administration. Sera for antibody determinations were collected serially and stored at $-20$°C. All sera were tested simultaneously for hemagglutinin titres after inactivation for 30 min at 56°C.