

Vincristine

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The vinca alkaloids are biologically-active dimeric alkaloids derived from the Madagascar (or rosy) periwinkle plant, *Catharanthus roseus*. Vincristine, a naturally-occurring vinca alkaloid, were originally characterized phytochemically more than fifty years ago. The diverse biological effects of vincristine have traditionally been attributed to drug-induced disruption of various intracellular microtubules. Microtubules are elongated, tubular cytoplasmic organelles involved in a broad spectrum of cellular processes including chromosomal migration, conduction of cellular secretions, ciliary and flagellar motility, and maintenance of cell shape. Microtubules are composed predominantly of complex helical polymers of the structural protein tubulin. Vincristine binds directly to tubulin, causing both inhibition of microtubule synthesis and disruption of intact microtubules. Microtubular structures susceptible to the effects of vinca-tubulin binding include the mitotic spindle in dividing cells, the neurotubules in neurons, and the cytoskeletal microtubules in platelets. Vincristine may also exert biological effects that are independent of disruption of intracellular microtubules, such as inhibition of RNA, DNA and protein synthesis, and modification of prostaglandin production.

Vincristine is a cell-cycle-specific cytotoxic agent. Vincristine disrupts microtubules within the mitotic spindle of dividing cells, thereby arresting chromosomal separation in metaphase. Vincristine at standard therapeutic doses is minimally myelotoxic, and is therefore commonly used in combination with more myelosuppressive chemotherapeutic agents. Vincristine is frequently used in veterinary cancer chemotherapy, both as a single agent for the treatment of canine transmissible venereal tumors, and as a component of combination protocols for the treatment of acute leukemia, lymphoreticular neoplasms, mast cell tumors, and various carcinomas and sarcomas.

Vincristine is usually administered intravenously as a sulfate salt, which is chemically more stable than its corresponding free base. Inadvertent subcutaneous or intramuscular administration causes severe local tissue irritation and necrosis. Oral absorption of vincristine is poor. Plasma disappearance of vincristine following intravenous administration is markedly biphasic, with a short initial half-life and a prolonged terminal half-life (Castle et al). The short initial clearance phase reflects extensive extravascular drug redistribution due to a combination of both avid binding to intracellular tubulin and rapid biliary excretion. The prolonged terminal clearance phase is due to the gradual release of vincristine bound to circulating plasma proteins and intracellular tubulin. Platelets demonstrate a remarkable ability to concentrate vincristine from plasma, and are therefore the principal circulating cellular carriers of the drug.

The degree of immunosuppression induced by vincristine at intravenous therapeutic doses is minimal compared to that induced by glucocorticoids, cyclophosphamide or azathioprine, and vincristine therefore is not used as an immunosuppressive agent for the treatment of most

immune-mediated or inflammatory diseases in dogs and cats. The one exception is immune-mediated thrombocytopenia (IMT), where vincristine has become a mainstay of treatment.

During early clinical trials in human cancer patients, it was observed that the administration of vincristine was frequently associated significant but transient increases in circulating platelet numbers. A similar phenomenon has since been reported in dogs, both in research animals and in cancer patients. This effect appears to be due to increased megakaryocytopoiesis and thrombopoiesis, although the precise mechanisms of vincristine-associated thrombocytosis are still uncertain. Circulating platelet life-span does not appear to be significantly affected by standard low doses of vincristine in healthy animals.

The serendipitous discovery that vincristine induced thrombocytosis in human cancer patients with normal pre-treatment platelet numbers prompted conjecture that a similar outcome could be obtained in thrombocytopenic patients. Following publication of several anecdotal reports describing prompt, marked increases in circulating platelet numbers after administration of vincristine to people with IMT, vincristine gained favor with some hematologists as the treatment of choice for chronic refractory IMT. Vincristine frequently induces partial or complete remission of thrombocytopenia within one week of commencing therapy, although such remissions are typically transient. Only a relatively small proportion of human chronic refractory IMT patients achieve complete sustained remission with vincristine therapy.

Rapid drug clearance from plasma reduces the therapeutic efficacy of a standard intravenous bolus of vincristine. Several alternate methods of vincristine administration have therefore been used in human IMT patients in order to sustain therapeutic plasma concentrations. Constant intravenous vincristine infusion (over six to eight hours) effectively maintains therapeutic plasma concentrations the drug throughout the period of administration. Alternatively, the ability of platelets to concentrate vinca alkaloids from plasma has been utilized to enhance therapeutic efficacy via transfusion of vincristine -loaded platelets. Incubation of donor platelets in high concentrations of vincristine (vinca loading) prior to transfusion maximizes intracellular vinca-tubulin binding. Following transfusion, circulating vinca-loaded donor platelets gradually release vincristine into the recipient's plasma, thereby sustaining therapeutic plasma drug concentrations. Both constant rate infusion with vincristine and transfusion with vinca-loaded platelets induce sustained remissions in some human chronic IMT patients previously refractory to single intravenous boluses of the drug.

Vincristine, typically in combination with prednisone, has been reported to similarly facilitate remission of thrombocytopenia in many canine patients with IMT. Original case reports demonstrating an apparent rapid response to vincristine in dogs with IMT have been supported (Greene et al; Jans et al; Williams & Maggio-Price; Wolf), decades later, by evidence obtained from prospective studies (Balog et al; Rozanski et al). Circulating platelet numbers increase markedly within three to five days of vincristine administration in responsive dogs, and the addition of vincristine to standard immunosuppressive therapy in dogs with IMT appears to

shorten hospitalization time by several days (Balog et al; Rozanski et al). Most authors currently recommend an intravenous vincristine bolus dose of 0.02 mg/kg for the treatment of canine IMT (Balog et al). Vincristine boluses may subsequently be repeated weekly if thrombocytopenia recurs. Apparent rapid clinical response to vincristine-loaded platelets has been reported in one dog with refractory IMT (Helfand et al). Vincristine has been used in cats with IMT, although evidence of clinical efficacy is lacking (Tasker et al). One significant advantage of vincristine compared to other therapeutic options for IMT (such as human intravenous globulin) is that vincristine is inexpensive (a 1 ml vial of 1mg/ml vincristine sulfate costs around \$20).

The pathogenesis of vincristine -induced remission of thrombocytopenia in IMT patients is uncertain. Clinicians initially assumed that remissions were due to increased megakaryocyte production and release of platelets, the principal mechanism assumed to underlie the vinca-induced thrombocytosis seen in healthy animals and cancer patients (Mackin et al; Neel et al). However, since IMT patients typically already have high levels of circulating thrombopoietic factors and maximal thrombopoiesis, platelet precursors may be refractory to further stimulation by vincristine. Furthermore, studies in people suggest that the main therapeutic effect of vincristine in IMT patients is not increased thrombopoiesis. Post-treatment average platelet life-spans are significantly prolonged in human IMT patients that respond to vincristine, suggesting that remission is due to reduced platelet destruction rather than increased platelet production. Since platelets are the major circulating cellular carriers of vincristine, researchers have speculated that antibody-coated platelets selectively deliver vincristine to those phagocytes within the mononuclear phagocytic system that are actively involved in platelet destruction. This proposed mechanism explains why, despite being an ineffective immunosuppressive agent for the treatment of most conditions, vincristine can still be very effective for the treatment of IMT.

During electron microscopic studies of platelet ultrastructure, it was discovered that prolonged incubation of platelets in vincristine solutions caused marked disruption of cytoskeletal microtubules. Laboratory investigations have since demonstrated that as well as disrupting platelet structure, exposure to high concentrations of vincristine also significantly impairs platelet function. Based on the *in vitro* evidence that exposure to vincristine impairs platelet function, hematologists expressed concern that using the drug in patients with IMT could similarly induce platelet dysfunction. Subsequent studies revealed that vincristine affected platelet function (aggregation) in dogs with lymphoma (Grau-Bassas et al), but not in healthy dogs (Mackin et al). Since several recent prospective studies showed no significant increase in bleeding in IMT dogs receiving vincristine, the effect of vincristine on platelet function, if it occurs, does not appear to be severe enough to be clinically significant (Balog et al; Rozanski et al).

Neurotoxicity, although uncommon, is the most frequent significant side-effect associated with therapeutic doses of vincristine in dogs and cats (Cho et al; Goldstein et al; Hamilton et al). Reversible vincristine-induced neurotoxicity in the dog has been reported with chronic cancer chemotherapy, but is not likely to be an issue with the single doses used to treat IMT (Hamilton

et al). Other side-effects such as gastrointestinal disorders (including megaesophagus (Selk Ghaffari et al) and gastric hypomotility (Tsukamoto et al)) and alopecia, occur less frequently and are typically mild and temporary. Vincristine at doses used for IMT typically causes minimal myelosuppression in dogs, although dogs with the ABCB1-1Δ (MDR1) gene mutation and some Border Collies have been reported to be more susceptible than other dog breeds to myelosuppression at antineoplastic vincristine doses (Mealey et al). In affected Border Collies, this effect appears to sometimes be independent of the MDR1 gene mutation reported in this breed (Lind et al). Temporary erythrodysplasia of erythroid precursors in the bone marrow and peripheral blood smears, featuring bizarre mitotic figures, abnormal nuclear configurations, and Howell-Jolly bodies, can be observed after administration of vincristine in dogs, but is of little clinical significance (Alleman & Harvey; Collicutt et al). An unusual transient pulmonary toxicity has been reported in a cat receiving chemotherapeutic doses of vincristine (Polton & Elwood). Vincristine has no known mutagenic or carcinogenic potential.

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