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Letter to the editor

Re: "Liposome encapsulated vincristine: preclinical toxicologic and pharmacologic comparison with free vincristine and empty liposomes in mice, rats and dogs"

Bruce R Madewell and Susan A Kraegel
Department of Veterinary Surgical and Radiological Sciences, University of California School of Veterinary Medicine, Davis, CA 95616, USA.

This letter is in regard to the manuscript titled "Liposome encapsulated vincristine: preclinical toxicologic and pharmacologic comparison with free vincristine and empty liposomes in mice, rats and dogs" by Kanter *et al.*, (*Anti-Cancer Drugs* 1994; **5**: 579–90). As veterinary oncologists, we are always looking for new anticancer drug developments or applications, particularly when those studies are done in dogs or cats so that the results can quickly be applied to veterinary practice. After reading this manuscript, however, we have very serious concerns with the conduct of this study and feel that some of the basic tenets for use of live animals in research may have been overlooked.

Specifically, for the dog studies, the authors have included drug doses that would be expected to be lethal in the dog and, indeed, virtually all of the doses selected for evaluation would be anticipated to lead to significant morbidity or mortality. Review of any clinical veterinary textbook reveals clearly that the vincristine dosage for dogs is 0.025 mg/kg (or 0.5–0.7 mg/m^2 body surface area). Indeed, at the high end of the body surface area-determined dose scale, gastrointestinal toxicity and myelosuppression may be dose-limiting. Although these doses have been somewhat empirically established in veterinary medicine, they are based on an important precedent publication whereby vincristine was tested for toxicity in dogs[1]. That study was not referenced in the *Anti-Cancer Drugs* publication, and no explanation is given as to how the drug doses and schedules for drug administration were selected. At the high end of drug dosage used in this study, dogs were given 0.2 mg/kg of vincristine, roughly 10 times the established therapeutic dose for the dog.

Secondly, the wisdom of selecting dogs for this comparative toxicity study is not addressed. The authors' themselves note that the "dog is not considered to be a good model for vincristine-induced neuropathy" (although that statement on p. 588 is not referenced). It is well known that the dog is much more sensitive to vincristine-induced gastrointestinal toxicity than human patients and, overall, the dog is *more* sensitive than man to the toxic effects of vincristine.[1] Therefore, the study was designed using a very toxic or lethal drug dose for the dog, an animal species that would not even be predictive for the important toxic manifestation of vincristine in the relevant human species where neurotoxicity is the issue.

A toxicologic study must use statistically significant numbers of animals to obtain valid results. Statistical assessment is partially influenced by mortality, therefore dose selection is critical if excessive mortality is to be avoided in higher dose groups. In any properly designed study, the goal is to be able to measure biologic effects. In the present study, evaluation of neurotoxicity was precluded by animal deaths and even the question of myelosuppression was difficult to assess because of mortality.

In a manuscript titled "Control of animal pain and distress in cancer and toxicologic research", Montgomery reminds us that "proper use of animals, including the avoidance or minimization of discomfort, distress and pain, is imperative".[2] Having treated many dogs for painful paralytic ileus following a modest 0.7 mg/m^2 body surface area drug dose, we are faced with the realization that the dog deaths in this study due to massive vincristine overdose caused tremendous pain and suffering. We see no evidence in the text of the Methods that dogs were

treated for gastrointestinal signs or sepsis, were observed more than once daily or were even offered the benefit of humane euthanasia when serious clinical signs ensued. In any study using live animals, criteria must be established for euthanasia of moribund animals *before* animals become critically ill and animals having one or more of those criteria should be euthanatized. On p. 581, the author's state that "if animals died before the scheduled necropsy, they were necropsied immediately".

In summary, we are not surprised that the dog studies revealed no "patterns of toxicity of liposomal drug that clearly differentiated it from free drug". The dog study lacked statistical significance, was flawed by high numbers of patient deaths, used an animal species that would not be anticipated to allow detection of subtle neurologic changes, and was clearly insensitive to animal pain and suffering. We are surprised that this study was approved for funding by the United States Public Health Service and passed criteria for animal use by the Institutional Animal Care and Use Committee.

References

1. Folk RM, Peters AC, Pavkov KL *et al.* Vincristine (NSC-67574): a retrospective toxicologic evaluation in monkeys and dogs using weekly intravenous injections for 6 weeks. *Cancer Chemother Rep* 1974; **5**: 17–23.
2. Montgomery CA. Control of animal pain and distress in cancer and toxicologic research. *J Am Vet Med Ass* 1987; **191**: 1277–81.