Japanese Journal of Veterinary Research 57(4): 213–214, 2010

INFORMATION

Hokkaido University confered the degree of Doctor of Philosophy (Ph.D.) in Veterinary Medicine on December 25, 2009 to 1 recipient.
The titles of thesis and other information are as follows:

Development of drug delivery system for erythropoietin as having sustained efficacy with a hydroxyapatite carrier

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For chronic kidney disease (CKD) patients with renal anemia, recombinant human erythropoietin (rhEPO) is a very effective drug; however, the treatment regime is troublesome, requiring multiple administrations each week. In veterinary field, rhEPO treatment has been also introduced; however, multiple administrations each week is necessary like human case. To resolve this problem, the method of sustained release of biologically active rhEPO over a period of two weeks or more should be necessary.

Hydroxyapatite (HAp) is a biocompatible ceramic, widely used in the biomaterial field. HAp particles have been examined for application in the sustained release of various therapeutic agents, such as antibiotics, anticancer drugs and proteins. HAp has the ability to absorb therapeutic agents without deactivation and shows regulated release by biodegradation. The biodegradation speed of HAp can be regulated by calcination temperature. Spray-drying has been shown to be a good fabrication method for spherical porous HAp powder with a large surface area.

In the present study, the efficiency of HAp as a drug delivery carrier for the sustained release of rhEPO was examined to reduce the frequency of administration. Spray-dried HAp microparticles, formed from zinc-containing HAp (Zn-HAp) or Zn-HAp calcined at 400°C, were used as carriers of EPO, and five Zn-HAp formulations incorporating rhEPO were prepared; no formulation, zinc (Zn) formulation, poly-L-lactic acid (PLA) formulation, Zn/PLA formulation, and calcined/Zn/PLA formulation. ICR mice were administered subcutaneously these formulations or rhEPO alone as a control from dorsal neck, and hematological and histopathological analyses, including enzyme-linked immunosorbent assay for plasma EPO concentration, were performed. The efficiency of the sustained release was the lowest in no formulation among the five formulations. For the other formulations, the peak hematopoiesis was delayed and higher hematological values remained until day 14. Further, plasma EPO levels were higher in these formulations than those in control on day 3. A reduction in the initial burst was observed in Zn/PLA formulation, and plasma EPO levels remained high until day 8 in Zn formulation and Zn/PLA formulation. This indicates that sustained EPO release could be achieved by using the Zn and/or PLA formulations on the Zn-HAp microparticles.

The ICGN (ICR-derived glomerulonephritis) mouse is an inbred strain showing the hereditary nephrotic syndrome due to a mutation of the *tensin2* gene. With the deterioration of renal function, ICGN mice

developed a normochromic and normocytic anemia, which is consistent with clinical reports on patients with renal anemia. The expression of EPO mRNA in the kidneys was significantly reduced in ICGN mice. Thus, ICGN mice are an authentic model for human anemia with CKD. In the present study, Zn and Zn/PLA formulations were examined whether these formulations could ameliorate the severe anemia in ICGN mice more efficiently compared with EPO alone.

As a result, the hematological parameters in anemic ICGN mice administered rhEPO alone were not different from that of day 0, indicating that anemic ICGN mice might be resistant to rhEPO treatment similar to human CKD patients. As a matter of fact, rhEPO should be administered to patients three times a week to maintain suitable levels of serum rhEPO. The hematological parameters in ICGN mice administered both formulations increased and the peak of hematopoiesis was observed on day 7 and slightly decreased after day 14; however; they were always higher than that of rhEPO alone until day 21. These data suggest that both formulations are useful for sustaining the release of rhEPO *in vivo*.

Although macroscopic observation showed that both formulations still remained in the subcutaneous tissue on day 21, these formulations did not cause any significant inflammatory reactions. The biodegradability of HAp microparticles injected subcutaneously was known; however, more detailed examinations for degradation of both formulations remaining in the subcutaneous tissue are necessary as well as the adverse long-term effects and the excretion mechanism to be studied.

In conclusion, HAp was recognized as a novel drug carrier to achieve sustained release of rhEPO. According to *in vivo* release test of rhEPO from HAp in ICGN mice, elevated hematopoiesis were maintained for 21 days. There was no adverse effect during and after the administration. Further optimization study is necessary to achieve longer sustained release of rhEPO to establish curative DDS for anemia.

The full text of this thesis (PDF) appears at http://eprints.lib.hokudai.ac.jp/dspace/bitstream/2115/42590/1/nagasaki_thesis.pdf

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Original papers of this thesis appeared in *J. Vet. Med. Sci.,* **71**: 729–736 (2009) and *J. Vet. Med. Sci.,* **71**: 1365–1371 (2009).