ofplasmid construct (empty vector, EF-A; G-based DNA vaccine, EF-G; adjuvant,EF-D; and vaccine-adjuvant, EF-GD) in 100 uL PBS, or with 100 uL PBS alone(negative control). Three fishwere collected from three sampling days (day 1, 3 and 14) after vaccination.RNA was extracted from spleen and kidney, cDNA was synthesized then qPCR wasperformed to evaluate expression of IFN-related genes. At 15 days post-vaccination, the fish were infected intraperitoneally(i.p.) with 100 uL of 1 x 10^6 virus particles/mL (W-VHSV150402-P2), and mortality was recorded for 14days.

Results: To assess which typeof IFN was elicited by the immuno-adjuvant, we tested the expression levels ofIFN-1 (type I interferon) and IFN-γ (type II interferon) in kidney and spleensamples. The expression of IFN-1 was significantly up-regulated at day 1post-vaccination in the EF-G group, but not to the degree seen in the EF-GDgroup. In contrast, IFN-γ expression was increased by only ~2-fold in thekidneys and spleens of the EF-D and EF-GD groups. In addition, the expressionof IRF-3 was higher on day 1 versus day 3 post-vaccination in kidney samplesfrom the EF-D, -G and -GD groups which verify that the generated constructs,particularly EF-D and EF-GD, appear to initiate downstream activation of immuneresponses essential for the IFN-1 pathway. Moreover, representativeinterferon-stimulated genes (ISG-15 and Mx) and pro-inflammatory cytokines(IL-1β and IL-6) were also evaluated. The results showed that, in kidney,ISG-15 was highly expressed (150-fold versus the control) in the EF-GD group atday 1 post-vaccination, but only minimally expressed in the EF-D and EF-Ggroups at this point. Mx was significantly up-regulated in the kidney samplesof the EF-G and EF-GD groups at day 3 post-vaccination (45- and 60-fold, respectively).Here, IL-6 expression was not significantly increased; in the kidney, weobserved ~8-fold increases in the EF-D and EF-GD groups on day 3post-vaccination, while the EF-G group showed 5-fold on day 1 post-vaccination. IL-1β expression was higher at day 1 post-vaccination in the kidney samples ofthe EF-D, -G, and -GD groups compared to controls (12-, 13- and 20-fold,respectively). These results suggest that the generated vaccine constructs canstimulate several effector genes that are important to the release of APCsafter a pathogen enters the cell. Our assays revealed that EF-D elicited type IIFN immune responses with a highly up-regulated IFN-1 expression at day 1 postvaccination. IFN-1 and other IFN-related genes were also highly up-regulated inthe spleen and kidney of EF-GD group even greater than the EF-G group. Thesurvival rates were higher in all vaccinated groups compared to controls at 14days after VHSV challenge (76, 71, and 83% for EF-G, -D, and -GD, respectively),compared to 45% in PBS- and 35% in EF-A- treated fish with the highest survivalseen among EF-GD-treated fish.

Conclusions: In summary,these results clearly demonstrate that DDx41 is an effective adjuvant for thisG-based DNA vaccine in olive flounder. Our novel findings could facilitate thedevelopment of more effective DNA vaccines for the aquaculture industry.

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O-013

Pharmacokinetics of Florfenicol-Tylosin Combination after Intravenous and Intramuscular Administration to Beagle Dogs

Abraham Mechesso[1], Seung-Jin Lee[1], Seung-Chun Park[*1]

[1]Laboratory of Veterinary Pharmacokinetics and Pharmacodynamics, College of Veterinary Medicine, Kyungpook National University

Introduction: In spite of the widespread use of the combination of florfenicol and tylosin in dogs in Asia and mainly in Korea, only limited information is available on the pharmacokinetic parameters of each drug following administration of the combined product at different doses.

Materials and Methods: Pharmacokinetic investigation of a commercial florfenicol-tylosin (2:1) combination product was conducted in beagle dogs after intravenous and intramuscular administration at 7.5 mg/kg and 15 mg/kg doses. Serum drug concentrations were determined by a validated high performance liquid chromatography using UV detection.

Results: A rapid and nearly complete absorption of both drugs with a mean bioavailability of 103.9% (florfenicol) and 92.6% (tylosin), prolonged elimination half-life, and high tissue penetration with steady-state volume of distribution of 2.63 l/kg (florfenicol) and 1.98 l/kg (tylosin) were found in dogs treated with 15 mg/kg of the product. However, dogs treated with 7.5 mg/kg of the combination product showed lower bioavailability, reduced volume of distribution, slower clearanceand shorter elimination half-life of florfenicol as compared with the findings at high dose. In addition, administration of 7.5 mg/kg combined product demonstrated high volume of distribution and longer elimination half-life of tylosin which were comparable to the findings at 15 mg/kg dose administration.

Conclusions: Additional studies, including pharmacodynamic and toxicological evaluation are required before recommendations can be made regarding the clinical application of the product in dogs.

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