P-23

In vivo effect of Di-*n*-butyl phthalateand Di-2-ethylhexyl phthalate on nonspecific defense mechanism of bullhead catfish (*Pseudobagrus fulvidraco*)

JuChan Kang, Fatima Masroor, YooHwa Keum
Department of Aquatic Life Medicin, Pukyong National University

Introduction:

Phthalate esters, a group of phthalic acid-derived compounds which are commonly used as plasticizers to impart flexibility to a variety of plastics, have been shown to induce estrogen receptor-mediated responses (Jobling *et al.* 1995). Commercial Phthalate esters (PEs), in a priority Di-*n*-butyl phthalate (DBP) and Di-2-ethylhexyl phthalate (DEHP) are used as plasticizers, softeners in many synthetic products, coatings, and different use scenarios in cosmetic industries. Exposure of aquatic organisms to phthalate is associated with developmental and reproductive anomalies. Therefore, there is concern that these compounds may be causing adverse effects on immune system and consequently on fish health. Compared to the large database devoted to influence of these commercially important phthalate on the immune system of mammals (Larsen et al. 2001), there is a paucity of information on aquatic animals. The lack of *in vivo* studies warrant further investigation for screening potential effects of well known estrogen-mimicking phthalate esters on nonspecific immunity of fish. Glutamic Oxaloacetate Transaminase (GOT) and Glutamic Pyruvate Transaminase (GPT) levels were evaluated as measures of the stress response of the fish.

MaterialsandMethods

Experimental fish, bullhead bagrid catfish, *Pseudobagrus fulvidraco* (52.5±0.9 g) were obtained from an Inland Fisheries Research Institute, Chung Cheong, Korea, and were housed in 80-L freshwater flow-through system (water exchange rate: 9 L^{-h}) tanks under controlled conditions of temperature at 22-23^{0}C with a photoperiod (15:9 h light/dark cycle) for acclimatization. Physico-chemical water quality was monitored daily (Table 1).

Stock solutions of DBP (Sigma) and DEHP (Sigma) were prepared in sunflower seed oil. Daily for 3 consecutive days five groups of ten fish were administered with DBP and DEHP (300, 1000 mg DBP or DEHP kg^{-1}bw) via, intraperitoneal injection (i.p.) after acclimatization. Vehicle control group was subjected to the same regime using an equal volume of sunflower seed oil (carrier) injection only.

Five fish per groups were anesthetized following the cessation of dosing (day 4). Blood was collected by puncture of caudal vessel. Thereafter, lymphoid organs were dissected out and weighed. The leucocytes were isolated following the method of Fatima *et al.* (2001) with modification from fish spleen and pronephros. Differential counts were performed to assess the population of leukocytes in the cell suspension of lymphoid organs.

Phagocytic activity and Phagoctic index (PI) were evaluated and calculated by following the method of Ahmad *et al.* (1998) with some modification.

Lysozyme assay was determined according to Ellis (1990). Serum total protein estimation and the activities of Glutamic Oxaloacetate Transaminase (GOT) and Glutamic Pyruvate Transaminase (GPT) assays were performed using a diagnostic kit (Asan Pharm Co Ltd, Korea).

The data were analyzed statistically by one-way analysis of variance (ANOVA). Duncan's multiple range was used to test the significant differences between groups at $p<0.05$.

ResultsandDiscussion

In our experimental conditions, neither DBP nor DEHP showed any detectable changes in the relative organ weights and body weight at $P<0.05$ may be due to too short period of time for treatment. However, both esters caused gradual increasing rate of cellularity in dose-dependant manner. Taken together, the significant findings of DEHP suggest possible higher induction of leukocytic populations in fish pronephros is due to potential proliferative activity of DEHP. The branched chain esters (i.e. DEHP) are much more effective as peroxisome proliferators than the straight-chain esters i.e. DBP in fish like mammals. Results showed tissue specific toxicity of the phthalate esters.

The present *in vivo* data clearly demonstrated the biphasic immune responses of phthalate esters, DBP and DEHP The exposure of catfish to the highest concentration of phthalate esters used (1000mg kg^{-1} bw) influence the potential killing activity of phagocytes and reduced the levels of nonspecific resistance factors viz., lysozyme activity and serum protein.

Thus, DBP-300mg kg^{-1} bw caused no effect on lysozyme activity and significant elevated phagocytic index. Concurrently, no change in GOT and GPT valuesalso conferred the normal hepatic functions of fish treated with low dose DBP. Therefore, it can be implicated an immunostimulatory effect of straight chain phthalate esters at low concentration.

Significant reduction in phagocytosis assay and of total serum protein seemed to be reliable indicators of an immunosuppressive effect of DEHP (a branched chain phthalate ester) at higher concentration. GOT and GPT activities were increased significantly at 1000 mg kg^{-1} bw DEHP-exposed animals which indicated a greater degree of hepatic dysfunctions in immunosuppressed fish.

The significant immunosuppressive effect of DEHP (1000 mg kg^{-1}bw) for pronephros (head kidney) might be a receptor mediated specific induction and disturbed physiological mechanism. This might be explained in part, the phthalate's potency in terms of a xenoestrogenic impact which appearedto be related to a direct estrogen receptor binding in pronephros leukocytes (Hironobu *et al.* 2003). Low potential effects of DBP may be due to its low affinity for estrogen-receptors (Tollefson *et al.* 2000). Present knowledge on *in vivo*effects of DBP and DEHP in fish demonstrated that these chemicals are not only endocrine disruptors but also responsible for the alteration in immuncompetency of aquatic organisms.

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