RESTORATION OF INNATE IMMUNITY IN FISH AFTER SUPPRESSION INDUCED BY XENOBIOTICS

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OPORAVAK PRIRODNOG IMUNITETA RIBA NAKON SUPRESIJE INDUKOVANE KSENOBIOTICIMA

Tokom procesa evolucije, biološki organizmi su razvili odbrambene mehanizme protiv opasnih bioloških, hemijskih ili fizioloških agenasa. Jedan od tih mehanizama u višim organizmima je kompleksni imuni sistem, čiji se fini detalji funkcionisanja postepeno otkrivaju. Razvoj i održavanje odbrambenih mehanizama je važno za dobrobit, i u nekim slučajevima i za preživljavanje organizama.

Veliki broj sintetički proizvedenih hemijskih materija se ispušta u okruženje. Mnoga istraživanja potvrđuju uticaj polutanata iz okruženja, naročito pesticida, metanih jona, hemoterapeutika i organskih polutanata na imuni sistem životinja, pokazujući da hemijski polutanti rezultuju mnogim imunomodulatornim efektima i na ćelijske i na humornalne imune funkcije, a na kraju i na zdravlje i otpornost na bolesti. Jedan od važnih problema je da imuosupresija indukovana kesnobioticima koji mogu favorizovati prisustvo i rast tumorskih ćelija. Premda još uvek postoji skepticizam u vezi hemijskih materija iz okruženja, istraživanja su pokazala da produžena supresovana imuna reakcija može da poveća opasnost od raka kod ljudi i vodenih životinja.

Prvo, direktno eksponiranje riba mnogim toksičnim materijama koje se ispuštaju u reke, jezera ili bare. Veliki broj ksenobiotika su takođe dodati u okruženje. Ksenobiotici imaju direktan uticaj na ćelijske i humoralne odbrambene mehanizme i zaštitu protiv infektivnih oboljenja.

Zatim, različiti polutanti imunokompetentnih ćelija imaju varijabilnu senzitivnost na ksenobiotike. Mnogi ksenobiotici su indukovali imunospresivne uticaje na nespecifičan imunitet i specifičan odgovor riba. Jedan broj istraživanja se bavio uticajima ksenobiotika na imuni sistem riba. Integritet imunog sistema je fundamentalan za dobru odbranu protiv različitih patogenih agenasa u okruženju, uglavnom parazita, bakterija i virusa. Glavne imune ćelije, limfociti, makrofage i neutrofili, su regulisane različitim

stupnjevitim kontrolnim procesima ćelijskih kooperacija i interakcija. Makrofagi i neutrofili su važni faktori u ćelijskom imunom sistemu riba, s obzirom da su prva linija odbrane i deluju tako da štite domaćina fagocitozom stranog materijala, uključujući i patogene agense. Osim toga, različite populacije imunokompetentnih ćelija imaju varijabilni senzitivitet na ksenobiotike. Ova činjenica je veoma važna u istraživanju uticaja ksenobiotika na nespecifični ćelijski ili humoralni odbrambeni mehanizam i specifične zaštite protiv bolesti. U narednim primerima imuni testovi su generalno grupisani prema specifičnosti odgovora. Neki nespecifični testovi kao što je determinacija lizozimske aktivnosti, C-reaktivni nivo proteina (CRP), cerulopazmini (Cp) i dodatni nivoi su dosta korišćeni u prošlosti za analizu efekata kontaminacije okruženja na zdravlje riba. Lizozimi i Cp aktivnost su važni u inicijalnoj destrukciji invazivnih agenasa i u nekim slučajevima mogu poslužiti kao rani biomarkteri koji indikuju promene u odbrambenim mehnizmima.

Testovi sa humoranim imunitetom su korišćeni u mnogim studijama da se utvrdi izmenjen imuni kapacitet, koji je uslovljen ksenobioticima. U našim istraživanjima demostrirali smo imunosupresiju humoralnih antitela u ribama izloženim teškim metalima. Smanjenje broja ćelija koje produkuju antitela je usledila nakon imunizacione kupke kod riba koje su bile izložene fenolima, rastvorima deterdženata, pesticidima, herbicidima i antibioticima. Takođe su pentahlorfenol (PCP), polihlorovani bifenili (PCB) i dioksini indukovali jaku imunosupresiju ćelijskog i humoralnog imuniteta riba. Antibiotici i drugi hemoterapeutici korišćeni u ljudskoj i životinjskoj medicini imaju sposobnost akumulacije u vodi i direktan uticaj na odbrambene mehanizme vodenih životinja. Čini se da antibiotici menjaju imune reakcije organizama. Neki antibiotici (oksitetraciklin, florfenicol) mogu imati različite efekte na različite faze unutar imunog sistema. Na primer, mogu imati uticaj na povećanje senzitivnosti simultnano sa smanjivanjem ćelijskog imuniteta i proteinskom sintezom.

U našoj studiji smo ispitivali mogućnost oporavka ćelijskog i humoralnog odbrambenog mehanizma i specifičnog imunog odgovora nakon eksperimentalne supresije indukovane atrazinom i pinoksadenom (Axial 100 EC) na šaranu (*Cyprinus carpio*). Takođe smo istraživali mogućenost oporavljanja imuniteta kalifornijske pastrmke (*Oncorhynchus mykiss*) nakon supresiju indukovane deltametrinom i oksitetraciklinom. Zdrave ribe (šaran i kalifornijska pastrmka), težine od 50 do 60g su intoksikovane koncentracijama ksenobiotika koje indukuju imunosupresiju (preliminarno u *in vitro* and *in vivo* studijama). Nakon intoksikacije ribe su imerzijom hranjenje 4 nedelje peletama sa imunomodulatorima: 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) u dozi od 200 mg ili 500 mg po kg hrane i β-hidroksi- β-metilbutirat (HMB, USA) u dozi od 20 mg ili 50 mg po kg mase tela po danu. Krv, slezina i pronefrosi 10 intoksikovanih i kontrolnih riba su uzeti pre i posle intoksikacije i 1, 2, 3 i 4 nedelje nakon hranjenja šarana i kalifornijske pastrmke sa Leiber Beta S. Takođe, nakon dve nedelje hranjenja šarana i kalifornijske pastrmke izvedeni su testovi veštačkih infekcija (challenge tests) sa *Aeromonas salmonicida* u grupama od 20 riba za svaki eksperiment i kontrolne grupe radi utvrđivanja da li je stvorena zaštita protiv furunkuloze šarana i kalifornijske pastrmke primenom dietetskog imunomodulatora. U našem istraživanju razmatrali smo imunotoksični efekat na ćelijski i humoralni odbrambeni mehnizam atrazina ili pinoksadena na šarana i deltametrina ili oksitetraciklina na kalifornijsku pastrmku. Kada su 1,3-1,6-β-D-glukan (Leiber Beta S, Nemačka) i β-hidroksi-β-metilbutirat (HMB, SAD) introdukovani u hranu u različitim dozama, nakon intoksikacije, svi imunološki parametri šarana i kalifornijske pastrmke su značajno porasli ($P<0.05$) u poređenju sa kontrolo-

nom grupom riba. Fagocitarna aktivnost makrofaga i proliferativni odgovor limfocita su statistični značajno porasli u poređenju sa kontrom grupom riba. Osim toga, testovi veštačke infekcije na grupama su pokazale smtnost koja je u korelaciji sa rezultatima testova nespecifičnog odbrambenog mehanizma. Rezultati našeg istraživanja su pokazala da 1,3-1,6-β-D-glukan (Leiber Beta S, Nemačka) i β-hidoksi-β-metilbutirat (HMB, SAD), prirodni imunomodulatori, mogu da se koriste za oporavak ćelijskog i humoralnog odbrambenog mehanizma i zaštite protiv bakterijskog oboljenja (furunkuloze) nakon supresije indukovane različitim ksenobioticima u ribama. Ova eksperimentalna studija ima veoma važnu praktičnu primenu i novi efektivni koncept za redukovanje morataliteta nakon negativnog uticaja kontaminanata iz okruženja ili ksenobiotika na imuni sistem riba, posebno u intenzivnom sistemu gajenju riba.

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During the process of evolution, biological organisms have developed defence mechanisms for protection against assault biological, chemical or physical agents. One of these mechanisms in higher organisms is a complex immune system, the fine details of which are only gradually becoming understood. The development and maintenance of defence mechanisms are important for the well being, and in some cases survival, of the organisms.

A large number of synthetically created chemicals have been added to the environment. Many studies acknowledge the influence of environmental pollutants – with an emphasis on pesticides, metal ions, chemotherapeutics and organic pollutants on the immune system of animals, demonstrating that chemical pollutants result in a variety of immunomodulatory effects on both cellular and humoral immune function and, consequently, on health and resistance to diseases. One of the important problems is that immunosuppression induced by xenobiotics that can favour the existence and growth of tumour cells. Although still open to scepticism in the case of occupational or environmental chemicals, reports have indicated that the prolonged depression of immune responses can increase the hazard of cancer in human and aquatic animals.

Fish, directly exposed to many toxic chemicals from discharges to rivers, lakes or ponds. The large numbers of xenobiotics have been added to the water environment. The xenobiotics have a direct influence on the cellular and humoral defence mechanisms and protection against infectious diseases. Furthermore, different populations of immunocompetent cells have a variable sensitivity to the xenobiotics. Many of xenobiotics induced immunosuppressive influence on the nonspecific immunity and specific immune response in fish. A number of studies have been made on the influences of xenobiotics on the immune system of fish. The integrity of the immune system is fundamental for good defence against a variety of pathogenic agents in the environment, namely parasites, bacteria and viruses. The major immune cells – lymphocytes, macrophages and neutrophils – are regulated by a variety of multistep control processes of cellular co-operation and interactions. Macrophages and neutrophils are important factors in the cellular immune system of fish, since they are the first line of defence and act to protect the host by phagocytosis of foreign material, including pathogenic agents. Furthermore, different populations of immunocompetents cells have a variable sensitivity to xenobiotics. This fact is very important in the study of xenobiotics influence on nonspecific cellular and humoral defence mechanisms and specific protection against

diseases. In the following examples, immune assays are broadly grouped according to the specificity of responses. Some nonspecific assays such as the determination of lysozyme activity, C-reactive protein (CRP) level, ceruloplasmine (Cp) and complement levels have been used extensively in the past for analysing the effects of environmental contamination on the health of fish. Lysozyme and Cp activities are important in the initial destruction of invasive agents and in some cases can serve as an early biomarkers indicating deterioration of some protective mechanisms.

The humoral-mediated immunity assays have been used in many studies to determine altered immune capacity, which is caused by xenobiotics. In our study we demonstrated immunosuppression of humoral antibody levels in fish exposed to heavy metals. A reduction in antibody-secreting cells following bath immunization was observed in fish that had been exposed to phenol, detergent solutions, pesticides, herbicides and antibiotics. Also the pentachlorophenol (PCP), polichlorined biphenyls (PCB) and dioxins induced strong immunosuppression on the cell-mediated and humoral-mediated immunity in fish. Antibiotics and other chemotherapeutics used in human and animal medicine has the possibility of accumulation in water and has direct influence on the defence mechanisms in aquatic animals. Antibiotics seem to modulate the immune responses. Some antibiotics (oxytetracycline, florfenicol) may have different effects on various steps within the immune system. For example, they may possess a sensitising property while simultaneously depressing cellular defence mechanisms and protein synthesis.

In the present study we examined the possibility of restorating cellular and humoral defence mechanisms and specific immune response after experimental suppression induced by atrazine and pinoksaden (Axial 100 EC) in carp **(***Cyprinus carpio*). Also we examined the possibility to restoration of immunity in rainbow trout **(***Oncorhynchus mykiss***)** after suppression induced by deltamethrin and oxytetracycline. Healthy fish (carp and rainbow trout), weighing 50 – 60 g were intoxicated by examined xenobiotics at concentrations induced immunosuppression (preliminary *in vitro* and *in vivo* study). After intoxication by immersion fish were fed 4 weeks pellets with immunomodu**la**tors: 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) at dose 200 mg or 500 mg per kg of feed and β-hydroxy- β-methylbutyrate (HMB, USA) at dose 20 mg or 50 mg per kg of body weight/day. The blood, spleen and pronephros from 10 fish of intoxicated and control groups were separated before intoxication, after intoxication and 1, 2, 3 and 4 weeks after feeding of carp and rainbow trout with Leiber Beta S. Also after 2 weeks of feeding carp and rainbow trout a disease challenge test using *Aeromonas salmonicida* were conducted using groups of 20 fish for each experimental and control groups to determine if protection was provided by the dietary immunomodulators against carp or rainbow trout furunculosis. In the present study, the immunotoxic influence of atrazine or pinoksaden in carp and deltamethrin or oxytetracycline in rainbow trout on the cellular and humoral defence mechanisms was observed. Also challenge test showed that examined xenobiotics decreased protection against furunculosis in carp and rainbow trout. When 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) and β-hydroxy- β-methylbutyrate (HMB, USA) were introduced into the food at different doses after intoxication, all examined immunological parameters in carp and rainbow trout increased significantly ($P<0.05$), when compared with the control groups of fish. The phagocytic activity of macrophages and proliferative response of lymphocytes were statistically increased, compared to the control groups of fish. Also lysozyme activity and Ig levels in serum significantly ($P<0.05$) increased, compared to the control groups. Nevertheless, challenge tests on the groups showed mortality patterns that correlated with the results from the nonspecific

defence mechanisms assays. The results of our study showed that 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) and β-hydroxy- β-methylbutyrate (HMB, USA), a natural immunomodulators, could be used for restoration of cellular and humoral defence mechanisms and protection against bacterial disease (furunculosis) after suppression induced by different xenobiotics in fish. This experimental study has a very important practical application and a new effective concept for reduce mortality after negative influence of environmental contamination or xenobiotics on fish immune system, especially in intensive fish culture.