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Vitellogenin as a Biomarker of Endocrine Disruptor in the Aquatic Environment

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ABSTRACT - A number of chemical released into the environment eliciting their effects by disrupting normal hormonal pathways. Endocrine disrupting compounds are present in the aquatic environment and pose potential health consequences to wildlife and humans. This review are designing for xenobiotic estrogens based on induction of the egg-yolk precursor protein vitellogenin. In fish of aquatic environment, it may result in decrease fertility and egg production in females or lead to reduced gonad size or feminization of genetic male fish. It has been known that male fish exposed to estrogenic compounds show induced production of vitellogenin. Vitellogenin production is normally restricted to adult females, which have elevated estrogen levels during egg production. However, vitellogenin can be induced in males by pollution of environmental endocrine disruptors. Consequently, the presence of vitellogenin in male fish can serve as an indicator of exposure of environmental endocrine disrupting compounds. In immature fish polluted at low levels of environmental endocrine disruptor, vitellogenin can serve as a reliable biomarker for exposure to endocrine disruptor. This review demonstrates the utility of vitellogenin as a biomarker for exposure to estrogenin agents in auqatic environment.

Key words □ Endocrine disruptor, Fish, Vitellogenin, Biomarker

Introduction

Over the past few years, concern has been increasingly expressed over the possible disruption of hormonal systems by environmental chemicals which lead to changes in reproductive health and fecundity. The issue of environmental endocrine disruptors has attracted considerable media attention and this, in part, has contributed to increase the activity of many expert panels, steering committees of governmental organizations, industry and academia.

Concern for the successful development and reproduction of human and wildlife populations has been heightened researches focused on endocrine disrupting compounds. Broadly defined, endocrine disrupting compounds are natural or man-made agents released in the environment that interfere in some was with normal endocrine function.[1] Endocrine disruptor acts by either enhancing or interfering with the actions of natural hormones in the body.[2] Because hormones are especially important in regulating reproduction and development, the effect of endocrine disruptor may be greatest on the processes. A number of industrial, municipal, agricultural, and natural compounds have been shown or are suspected to be estrogenic.[3]

There is vast scientific literature available which clearly shows that the aquatic ecosytem is heavily polluted and that fishes accumulate toxic materials in their tissues.[4-5] It has been reported that xenobiotic compounds such as 1, 1-bis(parachlorophenyl)-2, 2, 2-trichloroethane(DDT), some polychlorinated biphenyl (PCBs), dieldrin, and dioxin are potent disruptors of reproductive activity.[6-7] This fact became common public knowledge in the early 1960s with the publication of Rachel Carson's *Silent Spring*.[8] However, the focus for most studies linking contaminants and wildlife or human health has centered on the occurrence of cancer or mortality as end-points. Recent studies, summarized in Colborn and Clement,[9] suggest that a new set of end-points must be implemented, as evidence is rapidly accumulating that xenobiotic compounds are capable of acting as agonistic and anta-

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gonistic agents of the endocrine system. Ecosystem is typically exposed to lower levels of complex mixtures over a much longer time. Therefore, it can provide a valuable biomarker of environmental pollution.[10]

Fish may show no signs of stress or disease. So there are a multitude of long term effects which provide a warning both of ultimate destruction of economic fisheries and of hazards to human health. The high incidence of disease in fish and the incidences of high motality among aquatic mammals suggest that such low level of long term effects may be much more common than hitherto suspected.[11-12]

The definition of an "endocrine disruptor" is not perhaps as clear as may be first thought. In order to establish a common understanding for the basis on which chemicals can be defined as endocrine disrupters, an acceptable definition of an endocrine disrupter is required.

Kavlock et al.[13] broadly define an endocrine disruptor as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental processes". One which has found extensive favour within Europe was developed at a recent major European workshop on endocrine disruptors[14]: "An endocrine disruptor is an exogenous substance that causes adverse health effects in an intact organ or its progeny, subsequent to changes in endocrine function".

Environmental examples have been identified as a major cause of estrogenic effects on fish in the fresh water environments. In the first, this was detected by measuring vitellogenin as the biomarker blood plasma of male fish.[10]

This was detected by measuring the biomarker vitellogenin in the blood plasma of male fish.[10-11] Vitellogenin is the protein precursor of yolk and is synthesised by the liver exclusively in response to estrogens. In males there is almost no natural estradiol, so vitellogenin induction in these fish is an excellent marker of exposure to exogenous estrogenic materials.

The goal of this review is to present the state of the science of endocrine disruption as it pertains to fish problem with focus on assessing the ecological ramifications of the tissue.

Fish as a biomonitor of environmental endocrine disruptor.

The words 'aquatic environment' hide the fact that this is a highly variable environment. Not only does it include freshwater and seawater, but both of these are themselves variable. For instance, freshwater includes lakes, river, reservoirs and groundwater. Even within a single category of freshwater, there will be considerable variability. An upland reservoir situated in mountains is a very different environment from a lowland reservoir supplied primarily from a river. Because most of the estrogenic chemicals are man-made, waters in urban areas are more likely to contain significant concentrations of the chemicals than are waters in rural areas.[15-16]

Most of the estrogenic substances are lipophilic and hydrophobic. They have strong tendencies to bioaccumulate in aquatic organisms, and are often transferred through the food chain. The first visual sign of a grossly polluted aquatic ecosystem is usually the appearance of dead fish, but although the presence of apparently healthy fish is commonly used as an indicator of good quality water, this can be misleading. Fish accumulate pollutants preferentially in their fatty tissues, such as liver and gonads immune system,

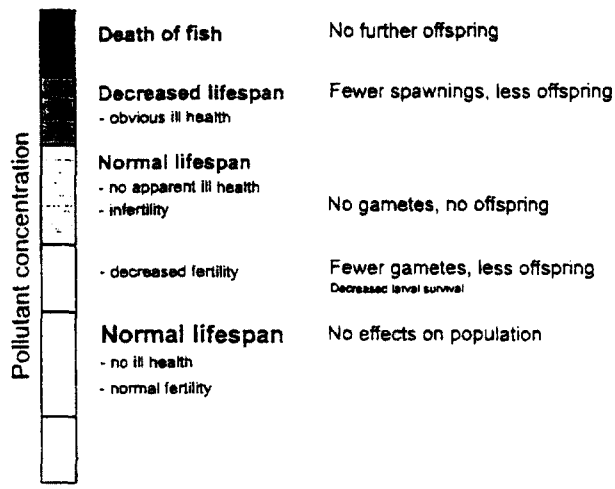

Fig. 1. Effect of pollution on fish of their production of offspring.[5]

tumours, respiratory problems etc, which shorten the lifespan and will decrease the population both by the premature mortality and by the decreased number of spawning seasons in which the fish is productive. It may also affect the reproductive system and decrease the fertility healthy fish (Fig. 1).

Fish is particularly well suited as long-term biomonitors since they can be exposed to either a controlled quantity of pollutant in the diet or water in the laboratory, or to polluted ecosystems. They are unique amongst the vertebrates in that many species to produce very large numbers of eggs during each reproductive cycle. Their fertility is therefore very easily measured by the numbers and viability of their eggs and sperm, the fertilisation rate and the survival of their offspring. The essential preliminary to the imposition of such limits is to determine whether present levels of a specific chemical are harmful and whether current unaffected. This requires evidence on accumulation of pollutants by fish from the waters and sediments,[17] whether the concentrations in their tissues are such that serious malfunction will occur and whether this will decrease either their viability and reproductive capacity or that of their offspring. An examination of such effects must progress stepwise (Fig. 2). Firstly the pollutant must be identified and its site of action or localisation within the fish determined. Accumulation in muscle of edible fish has a clear implication for human health, while high levels in the gills will indicate respiratory problems.

Fish possess a similar physiological system to mammals, including humans, and many of the pollutant induced disturbances in fish are likely to have similar effects on humans. In particular, the reproductive endocrine system has a great deal of similarity within the vertebrates and effects found in fish may be readily applicable to other vertebrate systems.[18]

Fish provide a suitable model for monitoring such as transgenerational effects both in the wild and in the laboratory. Any such effects found in fish might be expected to have much more serious consequences in humans in which the period of accumulation between generations is so much greater.

Thus aquatic environment can serve as important sentinels of ecosystem health and human risk. An ecoepidemiological approach to identify causation may provide the best model for predicting human reproduction.

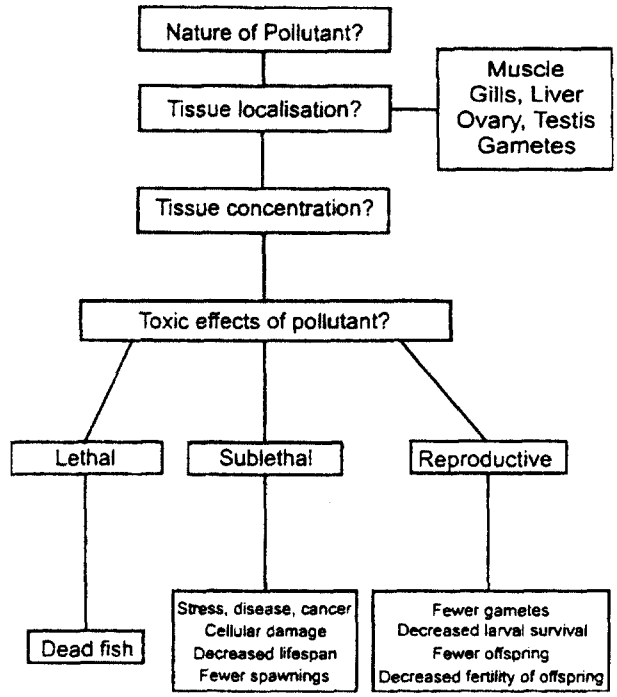

Fig. 2. The levels for examination of the effects of pollutions on fish.[9]

Vitellogenin as a monitor of environmental estrogens

Compounds such as the organochlorine pesticides and PCBs are particularly interesting since they are known to disrupt both ovarian and pituitary function, and some are estrogenic in mammals. Their action within the intact fish may therefore be a combination between toxic effects on the gonad, pituitary and liver, and estrogenic activity resulting in the direct stimulation of hepatic vitellogenesis at these different levels .[18-19]

Livers of both male and female fish have receptors for estrogens and are both capable of producing vitellogenin. Since estrogens are the only known stimulus for the hepatic production of vitellogenesis, the presence of vitellogenin in male plasma is indicative of exposure to estrogen.[20]

Vitellogenin is potentially an ideal biomarker for the estrogenicity of chemicals. It is a large serum phos-

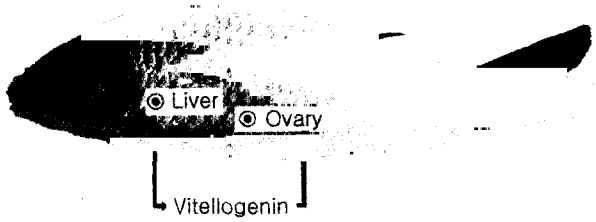

Fig. 3. Hormonal control of vitellogenin synthesis. Endocrine disruptor from granulosa cells of ovarian follicles considered to be the principal hormone that stimulates vitellogenin synthesis in hepatocytes.[10]

pholipoglycoprotein that serves as the major precursor to the egg-yolk proteins of oviparous vertebrates.[18] Vitellogenin is synthesized and secreted by the liver in response to circulating estrogens in maturing females[21-22] (Fig. 3) and is normally undetectable in the plasma of immature animals and males. The presence of this estrogen inducible protein in the serum of an animal can be taken as evidence of exposure to endogenous or exogenous estrogens or estrogen mimics. This potential of vitellogenin as a biomarker has already been explored using several fish species, for which both in vivo and in vitro assays have been developed.[22-23] Thus, the presence of vitellogenin in the plasma of male fish can be used as a biomarker of exposure to estrogenic chemicals.[21]

Recently it was reported that when caged male trout were placed in the effluent of sewage treatment works, they were induced to synthesize vitellogenin. Vitellogenin, the precursor of yolk, is normally synthesize by estrogens, because they have very low (often undetectable) concentrations of circulating estrogens, However, male fish respond to exogenous estrogens, administered by injection of via the water, by synthesizing vitellogenin in the same way that females do.

All of the chemicals tested stimulated synthesis of vitellogenin in a dose-dependent manner (at very high concentrations, one of the chemicals was toxic to the cells); all were fairly weakly estrogenic. Nevertheless, some of these chemicals stimulated vitellogenin synthesis at concentrations reported to be present in the aquatic environment (Fig. 4).

Since vitellogenin synthesis is estrogen dependent, induction of the protein, especially in male and juvenile fish, is a clear sign of exposure to environ-

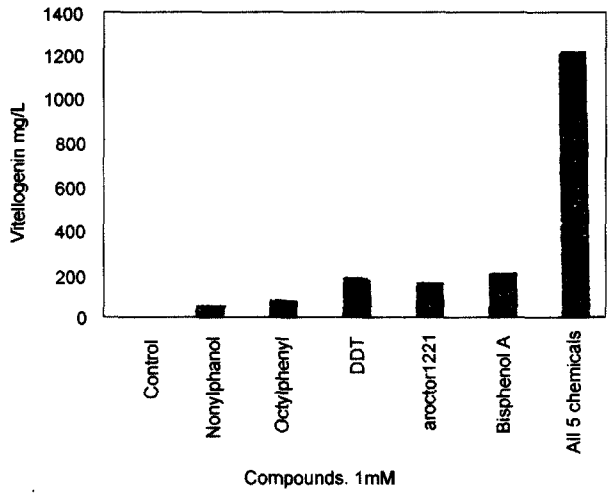

Fig. 4. Estrogenic activity of some environmentally persistent chemicals was investigated by assessing their ability to stimulate vitellogenin synthesis in cultured hepayocytes obtained from male rainbow trout. Nonylphenol and octylphenol are degradation products of widely used surfactants; op'-DDT is a pesticide; Aroclor is used primarily in electrical capacities and transformers, and bisphenol A is a plasticizer. All five chemicals are aquatic pollutants.[16]

mental estrogens and may provide a valuable biomonitor of such pollutants. Vitellogenesis by hepatocyte culture can also be used as a screening procedure for estrogenic activity of suspected pollutants (20). Although elevated plasma vitellogenin provides a valuable biomarker for the exposure of fish to chemicals with estrogenic activity, it should be used only in the first screening. It is then important to clarify what effect this exposure has on the fish itself. Exposure to an estrogen would suggest that the gonads and plasma steroid levels in the affected fish should be examined for signs of reproductive impairment or abnormalities in sexual differentiation.[2-9] There is also some evidence that vitellogenin can feedback to the ovary and inhibit estrogen production, so that an excess of vitellogenin stimulated by exogenous estrogen might disturb gonadal steroidogenic activity in both males and females.[23]

Induced production of vitellogenin in male fish is well known to be a biological indicator for exposure to estrogenic compounds. However, few investigations have attempted to examine the significance of such production.[6] For example, Jobling et al.[12] demon-

strated that elevated vitellogenin levels in developing male fish were associated with decreased testicular growth. The investigators also reported that the estrogenic potency of the chemical was directly related to the inhibition of testicular growth in developing male fish.

Linking biological indicators to reproductive assessment endpoints is considered critical to a fundamental understanding of the biological significance of a number of reported endocrine associated events.

Bioaccumulation of mixture of estrogenic chemicals

Many factors will influence the potential effects of an estrogenic substance on an aquatic organism, including environmental concentration, bioconcentration, bioaccumulation and potency. Although a few of the estrogenic compounds which are known to be, or might be, present in the aquatic environment are very potent estrogens, most are not. Ethinyl estradiol is such a very potent estrogen that, if present in water even in very low concentrations, would produce estrogenic effects.[24]

In the real world, fish are unlikely to be exposed to just one estrogenic chemical, but instead are likely to live in water that contains many different estrogenic chemicals. This is particularly true if the estrogenic activity in the water originated from waste effluent because this is a very heterogeneous mixture of chemicals.[25-26] Thus, ideally we need to know how a fish responds to a mixture of estrogenic chemicals rather than to an individual chemical.[27] However, it is very difficult to mimic the real world, primarily because we do not know at present which chemicals, in what concentrations, contribute to the estrogenic activity of effluent.

When trying to assess the impact of this contamination of the aquatic environment by estrogenic chemicals, two factors of major importance are the estrogenic potencies of these chemicals and their concentrations in the environment.[16]

Some representative results are shown. Relatively small responses were obtained when hepatocytes were exposed to submaximal concentrations of five different

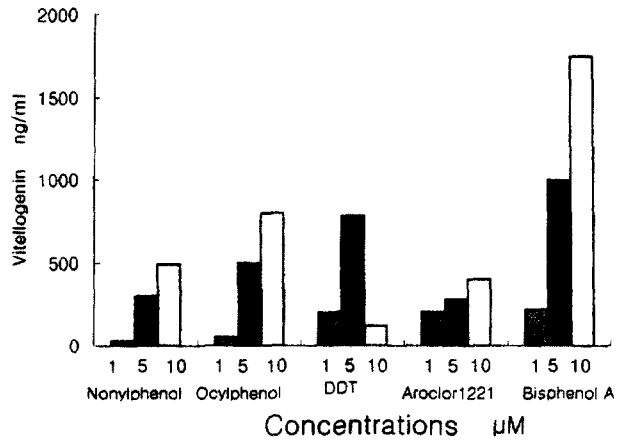

Fig. 5. Enhanced effect of a mixture of weakly estrogenic chemicals. The estrogenic activity of five chemicals was assessed by their ability to stimulate vitellogenin synthesis in cultured hepatocytes of rainbow trout. After 2 days of exposure to the chemicals, either individually or together (all five chemicals, each at a concentration of 1 μm, were present in the culture medium), the vitellogenin concentration in the medium was determined (results are expressed as mean±SEM).[19]

chemicals, but a considerably greater response was obtained when hepatocytes were treated with a mixture of the five chemicals (Fig. 5).

Taking the first approach, chemicals known to be estrogenic and present in the aquatic environment include some organochlorine pesticides, some polychlorinated, bisphenol-A, some phthalates, and many natural and synthetic estrogens.[7-8] The list of estrogenic chemicals known to be present in the aquatic environment is growing rapidly. A recent report found that half of the chemicals selected randomly from a list of those known to be present in sewage effluent possessed estrogenic activity.[9]

This is a complex but very important issue. It is unlikely that fishes are exposed to only one estrogenic chemical at any time rather, the water is likely to contain a mixture of chemicals possessing estrogenic activity. It is possible that the effect of one chemical may be negated by an antagonistic effect of another chemical. Alternatively, the effects could be cumulative, or even synergistic. In an attempt to begin addressing this issue, it has been shown that a mixture of different weakly estrogenic pesticides produces a greater effect than if each is tested individually[7]

It will be necessary to identify not only chemicals of interest in the aquatic environment, but also wuthin the animal. It is also be necessary to understand how these chemicals are metabolized within the animal of interest.

국문요약

자연계에 오염되어 있는 수 많은 화학 물질들은 정상적인 내분비 기능을 교란한다. 내분비 교란 물질은 수계에 오염되면 야생동물과 사람에게 건강에 미치는 영향이 크다. 본 연구는 오염물질이 생체의 내분비계를 교란시키므로서 생성되는 난황 전구체 단백질로서 환경오염의 생물체의 지표로서 비트로게닌의 생성을 유도하는 내분비 교란 물질에 대하여 고찰하였다. 수계의 환경에서 서식하고 있는 물고기는 내분비계 교란 물질에 의하여 암컷에서는 번식률과 난백의 생산이 감소되고 수컷에서는 정소의 왜소화 및 암컷화로 유도되기도 한다. 여성호르몬 작용을 하는 화학물질에 노출된 수컷은 비트로게닌의 생성을 촉진하고, 암컷에 있어서 비트로게닌의 생성은 난백을 형성하는 동안 에스트로겐의 함량을 증가시켜 정상적인 활동을 제한하기도 한다. 따라서 수컷에서의 비트로게닌은 환경에 오염된 내분비교란 물질의 오염에 의하여 생성되게 된다. 결과적으로 수컷의 물고기에서 생성된 비트로게닌은 환경에 오염된 내분비 교란물질이 어느 정도 노출되어 있는가를 결정하는 생물학적 지표로 사용할 수 있다. 특히 치어의 경우 극미량의 내분비 교란물질에 노출되어도 믿을 만한 생물지표로서 이용될 수 있다.

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