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UTILITY OF LIVER MACROPHAGE AGGREGATES AS AN INDICATOR OF FROG EXPOSURE TO DEGRADED ENVIRONMENTS. A STEREOLOGYCAL ANALISYS

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ABSTRACT

Numerous studies have shown an increase in number of macrophage aggregates (MAs), size or pigment content in fish collected at contaminated sites. For this reason, MAs have been suggested as potentially sensitive biomarkers of contaminant exposure. Although they are structures observed histological, it has also been suggested they may be immuno-toxicologic biomarkers.

To determine possible relationships between MA formation and macrophage function, we examined data from two field studies. One end point we are investigating is the accumulation of macrophage aggregates in the liver of frog association with contaminant concentrations.

On the basis of our findings, it can be suggested that these evidences, when coupled with toxicology data (levels in water heavy metal and pesticide analyses) can facilitate characterization of stresses that are impinging on native amphibian population.

These findings are also important in light of evidences showing decrease of amphibian populations worldwide. Many environmental contaminants may influence frog populations through different ways, and one of them could be macrophage aggregation, as cells of immune system.

KEYWORDS:
frog liver, macrophage aggregates, bioindicator,
degraded environment, stereological analyses

1. INTRODUCTION

Macrophage aggregates (MAs) are believed to be functional equivalents of germinal centers, active in storage of exogenous and endogenous waste products, the immune response, and iron storage and recycling □1,2,3□. Numerous studies have shown an increase in their number, size or pigment content in fish collected at contaminated sites. For this reason, MAs have been suggested as potentially sensitive biomarkers of contaminant exposure. Although they are structures observed histologically, it has also been suggested they may be immunotoxicologic biomarkers. To determine possible relationships between MA formation and macrophage function, we examined data from two field studies. One end point we are investigating is the accumulation of macrophage aggregates in the liver of frog association with contaminant concentrations.

2. MATERIAL AND METHODS

The livers of frogs *Rana ridibunda* captured on two different localities at the end of summer and at the beginning of autumn, September and Octomber 2001 were examined. First locality was Kamenyar, small residential area situated at the left bank of the river Danube around 3 km upstream of Novi Sad and very near the bridge which is, during NATO bombardment heavily destroyed. Second locality was Begechka Jama, the old meander of the river Danube some 15 km upstream of the Novi Sad city, which was used as control locality.

After sacrificing of five frogs from each locality, removed livers were fixed in Bouin's solution. Paraffin-embedded livers were cut serially in four-micrometer thick sections and were stained with hematoxlyline and eosin (H.E). These sections were used for both histological and stereological analysis. Stereological analysis was performed with stereological multipurpose grid M42 (placed in the ocular of a light microscope) on upon 60 test fields per animals, and the following stereological parameters, the volume density of sinusoids (Vvs) and volume density of macrophage aggregations (Vvm) were determined. The results obtained were expressed as mean± SE. Statistical significance of difference between animals from control locality and locality Kamenyar was determined by Student's *t*-test while difference between animals from same locality during three years of examination (1999, 2001 and 2002) was determined by analysis of variance.

3. RESULTS AND DISCUSSION

Stereological analysis of the liver sinusoids (Vvs) of frogs, which were, capture at locality 1 showed statistically significant ($p<0{,}01$) increase of its volume density compared to the animals from control locality (Fig. 1).

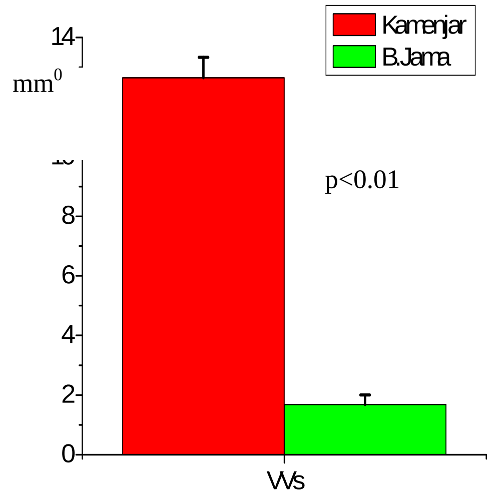
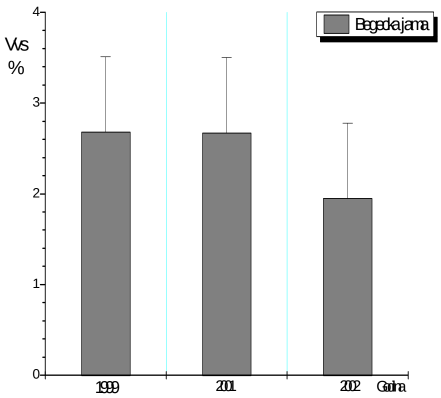


Fig. 1. Volume density of liver sinusoids (Vvs)
of frogs from two examined sites. Mean± S.E. are given.

Volume density of macrophage aggregates (Vvm) of livers, also, showed statistically significant ($p<0,01$) increase in frogs from Kamenjar locality compared to animals from control locality.

At the same time volume density of both liver parameters examined: sinusoids and macrophage aggregates showed great level of homogeneity (LSD 5 %) in livers of frogs from control locality during all three years of examination (Fig. 2 and Fig.3).



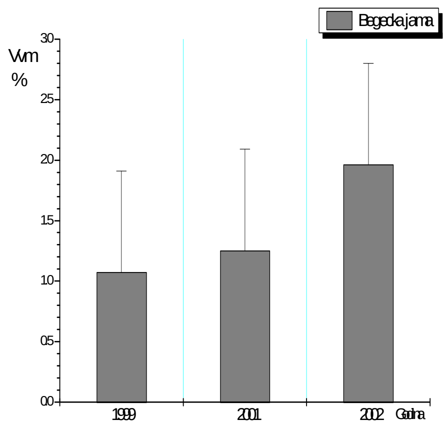


Fig. 2. Mean values of the volume density of liver sinusoids (Vvs)
of frogs from control locality (Begechka jama) during three years examined.
Error bar represents LSD value (5%).
Fig. 3. Mean values of liver macrophage aggregates (Vvm)
of frogs from control locality (Begechka jama) during three years examined.
Error bar represents LSD value (5%).

According to our results MAs in the liver of frog as same as MAs from liver of fish are sensitive parameter well corresponded with environment conditions, and therefore it can be useful indicator of pollution of some region. Volume density of MAs has, as we have shown, great degree of homogeneity in stabile ambient conditions and from this reason investigation of those parameters may be useful in field studies for indicating contaminants of potential concern even more specific then MAs from liver of fish.

It is already well known that macrophage aggregates size, number, and percent of tissue occupied by MAs in different organs of fish have been used in a number of studies as indicators of environmental degradation. Particularly the pigment distribution within MAs is representative of exposure to certain types of contaminants.

Also, it is shown in histochemical analysis with Perl's methods for iron; the pigments hemosiderin, ceroid/lipofuscin, and melanin can be visualized within MA. Laboratory study of WOLKE *et al.* – 4 - indicate significant dose-depend increase in hemosiderin in the arsenic -fed fish, up to 200 ppm while in fish fed deldrin there was an increase in ceroid/lipofuscin within MAs. Also, fish age was significantly and positively correlated with the index assessing the number and size of macrophage aggregates.

MAs are liver pigment cells, which are derive from the Kupffer cells (KCs) (5, 6). KCs synthesize high levels of mRNA for thyrosinase and also they possess dopa oxidase activity: both these data provide that frog KCs is capable of autonomously synthesizing melanin - 7, 8 -.

PURRELLO *et al.*, - 9 - demonstrated that there is an inverse correlation between the amount of tyrosinase mRNA and melanin content, and that populations of terminally differentiated KCs are characterized by high degree of apoptosis. They proposed that differentiating KCs start accumulating eumelanin, as a result of previous expression of high levels of tyrosinase and dopa oxidases activity, acquire the full KC phenotype (characterized by both phagocyte and melanosynthetic ability), and then undergo apoptosis. KCs, also, are able to demolish melanosomes probably by autophagocitocysis – 10 -.

These cells are active in clearing foreign substance from the circulation. Some authors considered - 11 - that pigments cells were formed as a result of erythrophagocytosis and therefore they were the depo of catabolism products.

These findings are in connection with data that under influence of same toxic agents erythrocytes are severely deformed – 2 - what can be reason for invasion of macrophages.

Thus, morphological changes in erythrocytes indicate disturbance in respiration and indirectly it is reason of greater aggregation of MAs. We can speculate that water pollution at Kamenjar locality can be cause of disturbance of respiration or some other process what resulted in changes of observed MAs parameters noticed in livers of frogs from these localities.

On the base of the present findings, it can be suggested that these evidences, when coupled with toxicology data (levels in water heavy metal and pesticide analyses) can facilitate characterization of stresses that are impinging on native amphibian population.

These findings are, also, important in light of evidences showing decrease of amphibian populations worldwide. Many environmental contaminants may influence frog populations through different ways, and one of them could be macrophage aggregation, as cells of immune system.

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