HEPATOCELLULAR FIBRILLAR INCLUSIONS IN THE LIVER OF EUROPEAN FLOUNDER FROM ESTUARIES CONTAMINATED WITH ENDOCRINE DISRUPTING CHEMICALS

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Introduction

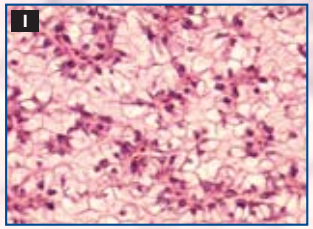
As part of ongoing studies assessing the health status of European flounder (*Platichthys flesus*), we have recorded a specific liver pathology, **hepatocellular fibrillar inclusions** (**HFI**) in fish from several estuaries including the Clyde and Mersey but not from the main reference site, the Alde. HFI have been previously described in flounders from the contaminated Elbe estuary in Germany where their presence was associated with regeneration following removal to clean laboratory facilities (Köhler, 1989). They have also been recorded in flounders from offshore sites in the North and Wadden Seas and from polluted sites in the Scheldt and Ems estuaries (Vethaak & Wester, 1996). More recently HFI was recorded in flounder from several UK estuarine sites, particularly those impacted most heavily by contamination including **endocrine disrupting chemicals** (Stentiford *et al.* 2003). Intense cytoplasmic basophilia reflecting proliferation of endoplasmic reticulum (ER) has previously been associated with vitellogenesis. In addition, PAS-positive hyaline material indicative of vitellogenin has been noted in the liver of male summer flounder exposed to estradiol (Zaroogian *et al.*, 2001). The data presented here provides evidence that HFI's represent a component in a degenerative process associated with induction of hepatocellular vitellogenin production at sites known to contain endocrine disrupting chemicals.

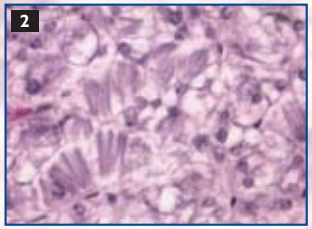
Pathology and ultrastructure

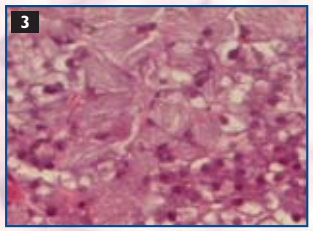
- Normal hepatocytes (Figure 1) are altered to contain conspicuous basophilic fibrillar arrays (Figure 2).
- These arrays occupied most of the cellular volume and often displaced the nucleus and remaining cytoplasm to a peripheral location close to the cell membrane (Figures 2 and 3).
- HFI's were orientated in various directions within the cell and were often associated with nuclear pleomorphism and small eosinophilic droplets (Figure 3).
- In severe cases, most hepatocytes were affected and necrotic foci were observed. Large eosinophilic inclusions replaced HFI's (Figure 4).
- Affected cells show increased osmiophilia and cytoplasmic condensation. Mitochondria show marked degenerative changes including swelling and loss of cristae (Figure 5 and 6).
- HFI's are conspicuous in longitudinal (Figure 5) and transverse or oblique orientation (Figure 6) and appear to be pathologically altered ER.
- Within degenerate hepatocytes, the large hyaline bodies are seen to consist of condensed fragments of membrane-bound ER (Figures 7 and 8).

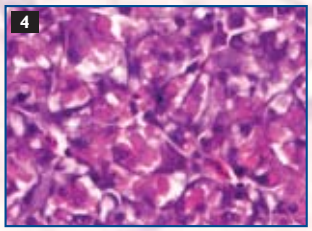
Immunohistochemistry

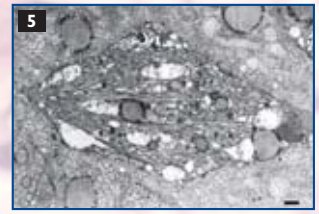
- Labelling with rabbit anti-flounder VTG. Positive control labelling in flounder oocytes (arrow) (Figure 9).
- General labelling of hepatocyte cytoplasm and fibrillar arrays (Figure 10). Specific labelling of HFI's (Figure 11).
- Labelling of condensed HFI's within hyaline bodies in degenerate hepatocytes (Figure 12).

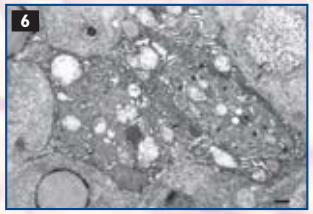


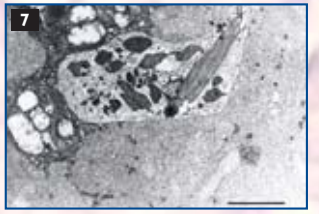


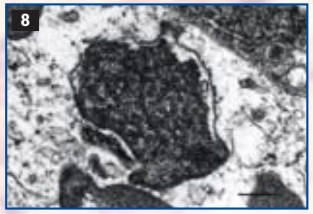


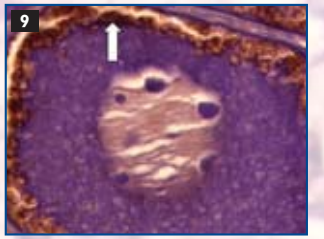


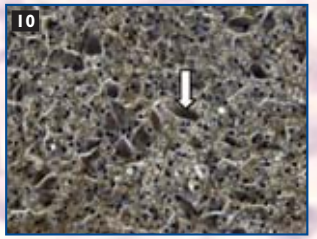


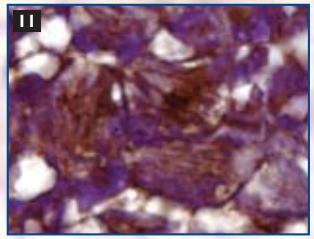


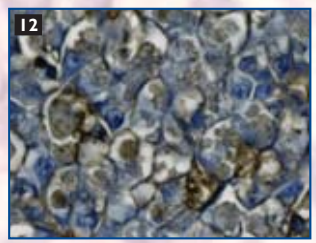








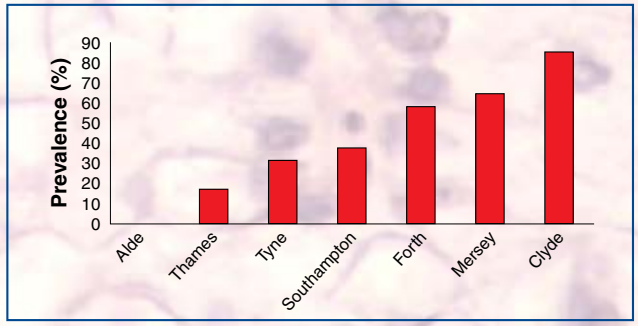




Prevalence

- Flounder sampled from several UK estuarine sites.
- Prevalence of HFI was highest at the Clyde and Mersey sites.
- HFI were absent in fish captured from the reference site, the Alde.

Prevalence of hepatic fibrillar inclusions, by site



Conclusions

- HFI's are prevalent in flounder from estuaries known to be contaminated with endocrine disrupting chemicals.
- The pathology induced appears to be degenerative rather than regenerative as previously described.
- HFI's appear to be associated with the induction of vitellogenin in both male and female flounder.
- This study has linked the HFI's with the formation of hyaline deposits (previously described from the liver of fish exposed to estrogens).
- **Their presence may provide a new and sensitive biomarker for endocrine disrupting chemicals in the marine environment.**

References

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