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Endothelial Lesions Associated with Gas Bubble Disease in Fish

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Summary

Two groups of healthy chinook salmon (*Oncorhynchus tshawytscha*) were experimentally exposed to gas supersaturated groundwater. Gross lesions consistent with gas bubble disease (GBD) developed. Vascular lesions associated with intravascular gas bubbles were examined with light and scanning electron microscopy. Dermal blood vessels containing gas bubbles were severely dilated. Additionally, the gas bubbles were spatially associated with endothelial lesions ranging from cellular degeneration to exfoliation. The resulting regions of exposed subendothelial connective tissue were sparsely covered by small unidentified adherent cells and strands of fibrin. In the light of these findings, the similarities in vascular pathology between GBD in fish and decompression disease in man are discussed, particularly with respect to the initiation of haemostatic disorders in both conditions.

Introduction

Gas bubble disease (GBD) is a commonly cited cause of morbidity and mortality of aquatic organisms (Weitkamp and Katz, 1980). It is a condition associated with prolonged exposure to water supersaturated with gases (D'Aoust and Smith, 1974). Recent studies (Machado, Garling, Kevern, Trapp and Bell, 1987; Smith, 1988; Speare, 1991) have redefined earlier reports (Pauley and Nakatani, 1967; Hoffert, Fairbanks and Fromm, 1971; Nebeker, Stevens and Stroud, 1976; Bell, Trapp, Machado and Garling, 1986) of the range of systemic morphological lesions associated with GBD in fish.

Gas bubble disease of fish, specifically salmonids, has been proposed as an animal model for the study of decompression disease of man (D'Aoust and Smith, 1974). Large intravascular gas emboli and cellular thrombi develop in both diseases (D'Aoust and Smith, 1974; Philp, 1974; Levin, Stewart, Lynch and Bove, 1981; Tanoue, Mano, Kuroiwa, Suzuki, Shibayama and Yamazaki, 1987; Speare, 1991). The clinical expression of various aspects of decompression disease is mechanistically linked to the formation and effects of gaseous and cellular thrombi (Levin *et al.*, 1981; Tanoue *et al.*, 1987; Francis, Pezeshkpour and Dutka, 1989). Similar inferences are drawn from the descriptive pathology of GBD (Smith, 1988; Speare, 1991).

Endothelial damage has been implicated as a trigger for the formation of cellular thrombi during decompression disease (Warren, Philp and Inwood,

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1973). Whether this process occurs during GBD in fish is unknown. Therefore, the similarities between decompression disease in man and its proposed animal model, with regard to mechanistic aspects of the thrombogenic event, are unknown. By examining sites of intravascular bullae formation, during experimental GBD, the hypothesis that endothelial damage occurs during GBD in fish was examined.

Materials and Methods

Sampling Protocol and Experimental Induction of GBD

Two groups of approximately 75 healthy chinook salmon (*Oncorhynchus tshawytscha*) fingerlings were used. They were housed in 500 l fibreglass circular tanks. These were supplied with pumped groundwater from which excess nitrogen was stripped by means of a standard desaturation column. Six fish were arbitrarily selected from each tank as controls, 2 days before the induction of GBD, and processed as will subsequently be described. To induce GBD in the remaining fish, the water supply was diverted from the desaturation columns and entered the tanks directly. Agitation of the water surface, in the form of artificial aeration, was manipulated to achieve an optimal sub-lethal supersaturation condition. This allowed for the development of a high rate of morbidity, but a low rate of mortality, over a 2-week test period. Gas bubble disease was diagnosed by gross examination for the presence of intradermal gas bubbles and exophthalmia.

Water Quality Evaluation

Total gas saturation of the water was calculated from measurements of total dissolved gas pressure (Bouck, 1980). The latter was measured twice daily with a Weiss saturatometer (Eco Enterprises, Seattle, Washington). Mechanical agitation of the tank water was periodically adjusted to maintain saturation levels of 110 to 124 per cent during the exposure period.

Histopathological and Ultrastructural Procedures

A total of twelve affected fish were sampled for study from each tank during the 2-week exposure. Specifically, fish were selected at intermittent periods when they developed a combination of grossly visible gas bubbles within fin rays, the roof of the mouth and periorbital areas. Fish in extremis were killed but not further evaluated to avoid the confounding influence of perimortem tissue changes.

Sampled fish were killed individually with an overdose of tricaine methanesulphonate. The heart was removed and transected sagitally, the roof of the mouth dissected and transected transversely. Pieces of heart, roof of the mouth, and regions of affected skin were fixed in Bouin's fixative for histology, or in 2·5 per cent glutaraldehyde in 0·1 M phosphate buffer for scanning electron microscopy (SEM). Blocks of tissue were routinely processed for histology. Cut paraffin wax sections were stained with haematoxylin and eosin (HE), Masson's trichrome, Mallory's phosphotungstic acid haematoxylin method, Martius scarlet blue and methenamine silver (Luna, 1968). For SEM, tissues were post-fixed in 2 per cent phosphate buffered osmium tetroxide. They were then dehydrated in ascending concentrations of alcohol, critical point dried with CO_2 with a Tousimis Samodri PVT-3 critical point drier, sputter coated with gold palladium in a Technics Hummer 6 coater and examined with a Jeol JSM-35U scanning electron microscope. Close attention was paid to using standard and identical fixation and processing procedures for all exposed and control fish. This was in an attempt to avoid the confounding influence of artefactual changes

which are known to influence the SEM appearance of endothelial cells (Clark and Glagov, 1976).

Results

The surface topography of the endothelium of the heart and dermal blood vessels of the control fish was characterized by a continuous flat sheet of pentagonally or hexagonally shaped cells. Cell surfaces were undulated, with ruffles near cell junctions, and small randomly distributed blebs and pits. Cell junctions were accentuated by trough-like shallow depressions around the perimeter of the cell (Fig. 1). Bulging of the nuclei was not prominent.

Affected fish had translucent gas filled bullae of variable size within the dermis. Particularly large (>4 mm in diameter) bullae characteristically developed in the roof of the mouth (Fig. 2). Examination of affected sites, with light and electron microscopy, indicated that these bullae were located within the dermis and subcutis, and were either intravascular (Fig. 3) or extravascular (Fig. 4).

Blood vessels with large intravascular gas bubbles were grossly distorted and devoid of contents (Fig. 3). The epithelium overlying such areas bulged outwards, appeared thinner than adjacent areas (Fig. 3), and was eroded to a variable extent (Fig. 5).

In comparison with the appearance of the endothelium of control fish and also that of unaffected blood vessels of fish with GBD, the endothelium of vessels distorted by gas bubbles was characterized by several changes. Degenerative changes included marked exocytotic vesiculation and pitting of the apical membrane (Figs 6 and 7). Cells were variably swollen (Fig. 7) and intercellular junctions in some cases appeared to be restricted to attenuated cellular processes (Fig. 6). Endothelial exfoliation produced areas of exposed subendothelial connective tissue (Figs 7 and 8). Some of these denuded areas were sparsely covered by a combination of small round cells which occurred singly or in small groups loosely enmeshed by strands of material which resembled fibrin (Fig. 8).

Inflammatory changes were also observed in association with intra- and extravascular bubbles (Figs 4 and 9). Compression and degeneration of connective tissue adjacent to the bullae was accompanied by oedema and sparse amounts of fibrin. Additionally, a cuff of neutrophils developed around some bullae (Figs 4 and 9).

Discussion

Activation of clotting mechanisms occurs during decompression disease in man (Levin *et al.*, 1981; Tanoue *et al.*, 1987). Similarly, a haemostatic disorder resembling disseminated intravascular coagulation (DIC) has been demonstrated in salmonids with GBD (Casillas, Miller, Smith and D'Aoust, 1975). Several factors acting alone or in concert have been proposed to explain the formation of cellular microthrombi during decompression disease in man. These include the direct effects of gas bubbles on the endothelium and

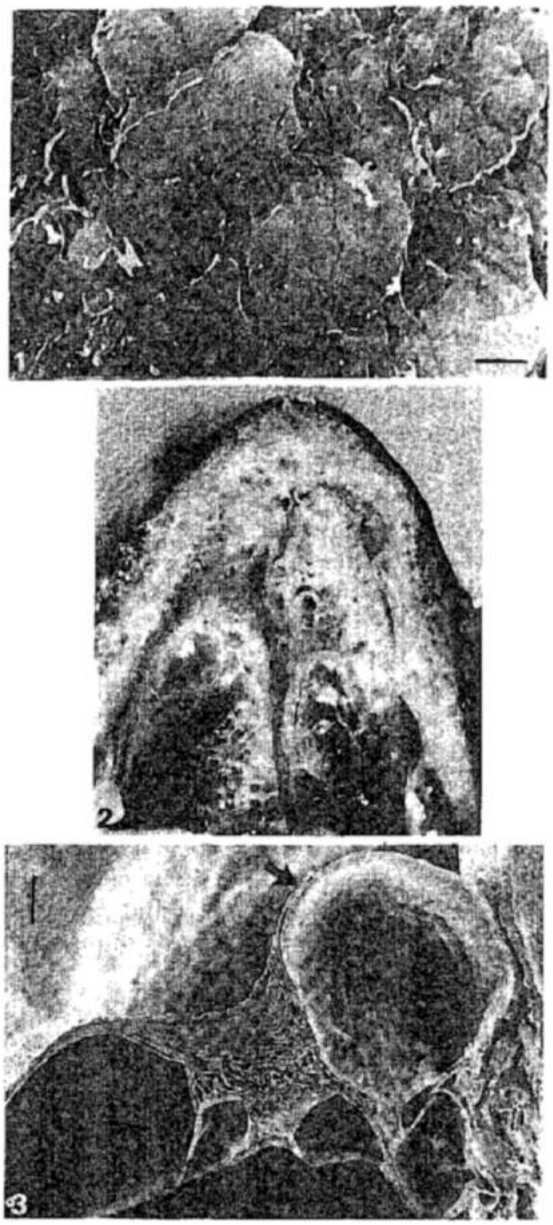

Fig. 1. Surface topography of several endothelial cells lining a dermal blood vessel from the roof of the mouth of a healthy control fish. SEM, bar = 6·3 µm.

Fig. 2. Gross appearance of the numerous gas bubbles which developed in the roof of the mouth of fish affected with GBD.

Fig. 3. Distortion of dermal blood vessels by gas bubbles. In some cases the overlying epithelium is markedly bulged (arrow) and appears thinner than at adjacent sites. SEM, bar = 130 µm.

Fig. 4. Oedema accompanied by a mild cellular inflammatory response surrounding an extravascular intradermal gas bubble. HE, bar = 86 μm.

Fig. 5. Eroded skin surface overlying a gas bubble. Degenerate epithelial cells (arrows) at the margin of eroded site. SEM, bar = 5·7 μm.

Fig. 6. Endothelium of a dermal blood vessel distended by a gas bubble. Surface pitting is pronounced on some degenerate endothelial cells (arrows). Intercellular junctions, in some cases, are restricted to attenuated cellular processes. SEM, bar = 12 μm.

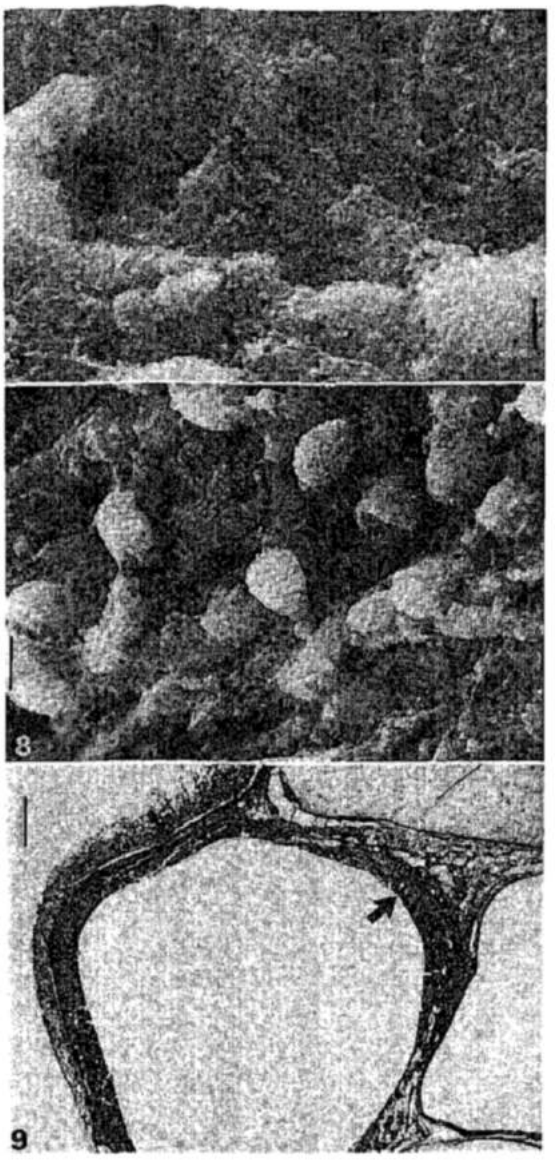

Fig. 7. Exposure of subendothelial connective tissue in a dermal blood vessel distended by a gas bubble. Several swollen and vesiculated endothelial cells (bottom of figure) remain. SEM, bar = 6 μm.

Fig. 8. Exposed subendothelial connective tissue sparsely covered by small round cells and strands of fibrin-like material. SEM, bar = 9·4 μm.

Fig. 9. A perivascular cuff of inflammatory cells (arrow) around an air-distended dermal blood vessel. HE, bar = 172 μm.

platelets, as well as the indirect effects of factors released from both types of damaged cells (Warren *et al.*, 1973; Levin *et al.*, 1981; Tanoue *et al.*, 1987). The present study demonstrates that endothelial damage is also a feature of GBD. This change may be the trigger for the haemostatic disorders which occur during GBD as reported by Casillas *et al.* (1975) and Speare (1991). It is well known that an intact endothelial layer inhibits several key thrombogenic steps. Furthermore, extensive tissue damage, particularly widespread endothelial damage, can be a trigger for DIC either indirectly through exposure of subendothelial collagen, or directly by activation of the clotting cascade.

Endothelial damage during decompression disease may reflect the systemic effect of inflammatory mediators released by damaged tissues (Levin *et al.*, 1981; Catron, Flynn, Yaffe, Bradley, Thomas, Hinman, Survanshi, Johnson, and Harrington, 1984). Alternatively, the interaction may be direct and local (Warren *et al.*, 1973). In the present study, structural endothelial lesions were apparently restricted to sites occupied by large gas emboli. This suggests a local (direct or indirect) effect of gas bubbles on the endothelium during GBD. This conclusion is supported by experimental gas embolization studies which have shown that the pressure exerted by intravascular gas bubbles can directly damage endothelium (Warren *et al.*, 1973; Mason and Balis, 1980). The dramatic size of the intravascular gas bubbles, noted during the present study, would enhance this mechanism. Bubble growth during GBD probably results from the systemic redistribution of gases to existing bubble foci. This mechanism for bubble growth has been proposed for the phenomena observed in decompression disease (Strauss, 1979).

Locally acting indirect effects of the gas bubbles may also be the important mediators of endothelial damage. The interaction of adherent leucocytes and platelets to the endothelium, during decompression disease, has been shown to cause endothelial damage (Flick, Perel and Staub, 1981; Levin *et al.*, 1981). Additionally, leucocytic emigration has been shown to be a general mechanism for endothelial damage (Stewart, Ritchie and Lynch, 1974). The extensive perivascular cellular inflammatory response, noted in this study, suggests the potential for this mechanism of endothelial damage to occur in GBD.

An alternative, locally acting, indirect effect of the gas bubbles on the viability of the endothelium is also plausible. The large bubbles appeared to completely inhibit blood flow. Anoxic injury to the endothelium is possible since the bubbles which develop in cases of GBD due to supersaturated groundwater are usually composed of nitrogen (Weitkamp and Katz, 1980). Furthermore, the apparent sequence of endothelial cell damage and exfoliation, which is inferred from our findings, has been frequently reproduced through experimental endothelial anoxia as reviewed by Mason and Balis (1980). However, the nature of the cellular damage noted in this study, on its own, should not be used to infer any specific mechanism of injury since the range of endothelial changes to a variety of injurious stimuli is limited (Mason and Balis, 1980).

In brief summary, this study provides preliminary morphological evidence to support the hypothesis that endothelial damage occurs during GBD in salmonids at sites where blood vessels are occluded by intravascular bubble

formation. The usefulness of further studies on GBD as a model to investigate rheologic pathophysiological mechanisms and consequences of decompression disease in man, is thus enhanced.

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