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Clearance of collodial carbon from the blood of Tilapia (*Oreochromis niloticus* L.)

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ABSTRACT

This study reports the rate of clearance of colloidal carbon from the blood of Tilapia (*Oreochromis niloticus* L.), over a period of 250 minutes following intravenous injection of five different doses. Thirty Tilapias (125-170g) were divided into 6 equal groups (A-F). Carbon doses of 4 mg, 8 mg, 16 mg, 32 mg and 48 mg were injected to fish in 0.15 ml distilled water. The control, group F, was injected with 0.15 ml distilled water. The clearance of carbon was observed to be regular and progressive for all doses. Carbon particles uptake by monocytes was noticed 5 minutes post-injection. However, the phagocytic index was inversely proportional to the dose of carbon injected. The significance of clearance of carbon and other particulate antigens is discussed.

Key words: colloidal carbon, phagocytic index, *Oreochromis niloticus*

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Introduction

Phagocytosis is considered to be the main non-specific defence mechanism in fish. Mononuclear phagocytes or macrophages are central participants in both non-specific and specific immune responses (SORENSEN et al., 1997). Phagocytes are located in most tissues and organs of the vertebrate body and in addition to other activities macrophages govern immunological responses by the recognition and digestion of foreign substances (SELJELID and ESKELAND, 1993). Potentiation of non-specific defence mechanisms may occur during microbial invasion, leading to more efficient clearance and destruction of pathogens or other harmful substances (DALMO et al., 1997). Kidney, spleen and liver are considered to be the main organs in fish where endothelial cells and macrophages eliminate undesirable substances from circulation (DALMO et al., 1997). Of the vast majority of cells circulating in the blood of fish, granulocytes and monocytes are the most important in the cellular aspects of the defence mechanism. During a histological study of blood cells BOOMKER (1981) found a cell resembling a monocyte with a number of intracellular bacteria in bream (*Sarotherodon mossambicus*). During an *in vitro* study, ELLIS (1977) discovered that phagocytosis of yeast particles was performed principally by monocytes. Although conflicting results exist as to the phagocytic ability of granulocytic neutrophils in fish, MACARTHUR et al. (1984) observed that neutrophils in plaice (*Pleuronectes platessa*) were phagocytic for carbon, and under inflammatory conditions these cells were actively involved in the uptake of glycogen and bacteria in the peritoneal cavity.

FERGUSON (1984) demonstrated that up to 99% of *Staphylococcus aureus* was cleared from carp (*Cyprinus carpio*) blood in 30 minutes, while 90% of *Salmonella pullorum* was lost from rainbow trout (*Salmo gairdneri*) blood within 15 minutes, and that carbon similarly cleared from plaice blood with 80% removed over a period of 30 minutes. FERGUSON (1984), MACARTHUR et al. (1984), JORGENSEN et al. (1993), BENNANI et al. (1995) have established that the activities of phagocytes and reticulo-endothelial systems can be quantitatively measured by injecting particulate materials into the blood-stream.

This study presents our findings on the clearance of intravenously injected colloidal carbon at varying doses and subsequent distribution in some organs of *Oreochromis niloticus* L.

Materials and methods

A carbon suspension containing 100 mg/ml carbon was prepared according to BIOZZI et al. (1953). The suspension was diluted using distilled water to make dilutions containing 4 mg, 8 mg, 16 mg, 32 mg and 48 mg carbon/ml. Thirty Tilapia weighing between 125-170 g were divided into six groups (A-F) and were marked based on the dose of carbon (4 mg-48 mg/100g of fish) to be administered. Group F served as the control and received 0.15 ml of distilled water. Carbon dilutions were sterilized by autoclaving, and cooled. A baseline blood sample was collected from representatives of each group before administration of 0.15 ml of the allotted carbon concentration. To minimize quantitative bleeding error, 0.1 ml of blood was obtained by venous puncture from two fish from each group at the following times post injection: 5, 10, 15, 20, 25, 35, 50, 75, 100, 150, 200, 250 minutes. 10 µl of blood from each sample was then lysed in 2 ml acetic acid. Using the lysed pre-injection blood as the reference standard, the percentage transmittance of light was read on WP S105 spectrophotometer at a wavelength of 540 nm. The mean reading was then recorded for each group. The phagocytic index, which is a measure of the rate of clearance of carbon particles, was then calculated according to BENACERRAF et al. (1959).

Blood smears were taken just before the first blood sample collection at 5 minutes, prepared and stained by the standard Giemsa staining technique to confirm carbon uptake by monocytes. After 250 minutes post injection, three fish from each group were sacrificed and the organs (spleen, kidney and liver) were weighed and then hydrolysed in boiling sodium hydroxide under a layer of ethyl alcohol to absorb fatty substances, after which they were washed and dried before carbon was recovered from them and weighed.

Histological samples were collected from the spleen and kidney of fish sacrificed at 10 minutes and 250 minutes post injection of the medium strength (16 mg/ml). The samples were fixed in phosphate buffered formalin. Paraffin sections at 5 μm were stained by the standard haematoxylin and eosin technique (ROBERTS, 1978).

Results

The clearance of colloidal carbon particles was regular and progressive in all dose groups (Fig. 1). The log-transformed clearance of carbon plotted against time (Fig. 2) showed a straight line-like pattern for all doses except the two highest (32 and 48 mg), which showed initial flocculation, probably

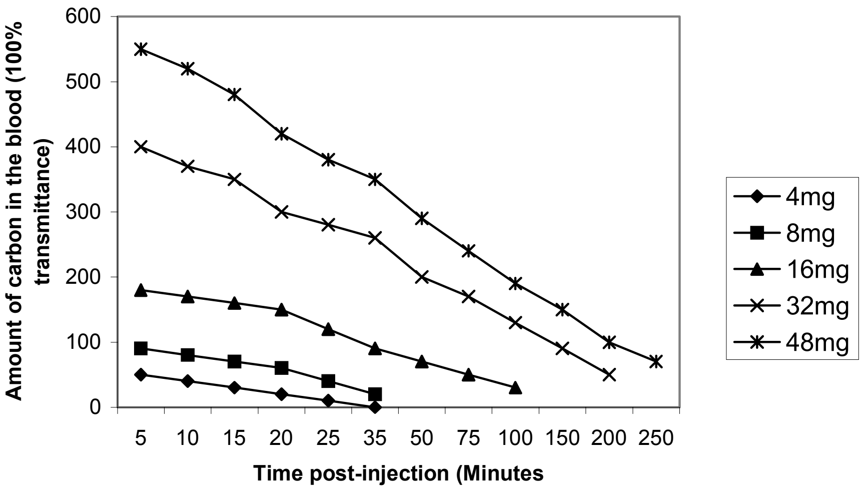

Fig. 1. Clearance curve of carbon from the blood of *Oreochromis niloticus* L. in relation to time following injection of different doses of colloidal carbon (4 mg-48 mg)

due to the high concentration of carbon. Plotting of the mean phagocytic index of each dose of carbon against the corresponding doses of carbon injected is presented as Fig. 3. The curve confirmed the phagocytic index to be inversely proportional to the dose of carbon injected (BIOZZI et al., 1953). The quantity of carbon recovered from organs varied around 80%

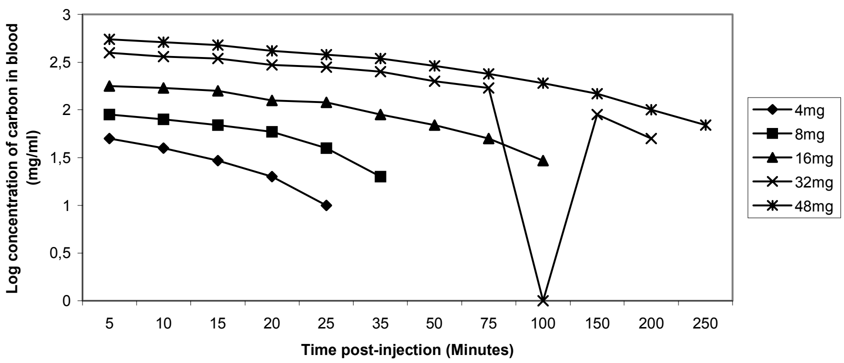

Fig. 2. Transformed clearance rate of carbon from the blood of *Oreochromis niloticus* L.

of the injected quantity and the carbon uptake was most intensive in the spleen (Fig. 4). Blood monocytes phagocytosed carbon particles as early as 5 minutes post-injection (Fig. 5). Carbon was also observed to have

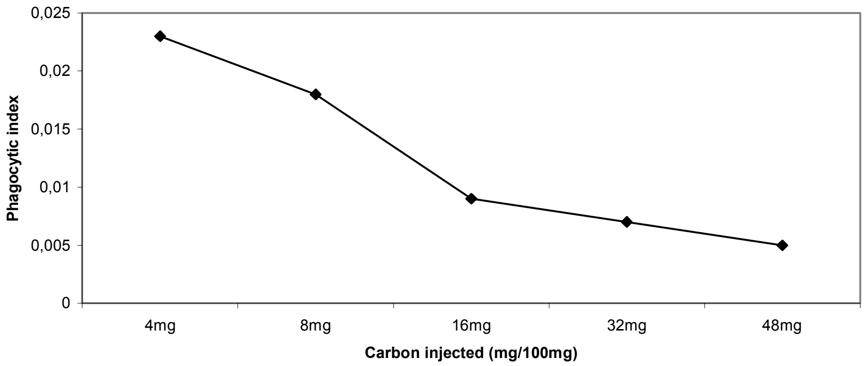

Fig. 3. Relationship between the mean phagocytic index and the dose of carbon injected in *Oreochromis niloticus* L.

accumulated in the spleen of fish injected with 16 mg/ml sacrificed at 10 minutes (Fig. 6) and 250 minutes (Fig. 7) post injection. The spleen was responsible for the greatest part (62-75%) of phagocytic activities in all

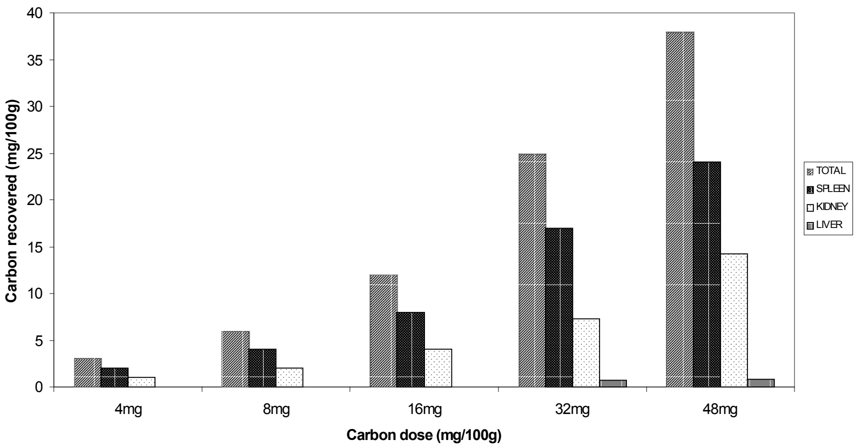

Fig. 4. Distribution of carbon among various organs after injection of doses from 4 mg-48 mg/100g.

experimental groups (Figs. 6 and 7). The kidney was eliminating smaller amount of the carbon (25-37%). There was approximately 20% of carbon in the liver (less than 1mg) in the lower dose (4-16 mg) ones. Although the amount of carbon recovered from the spleen increased in the groups injected

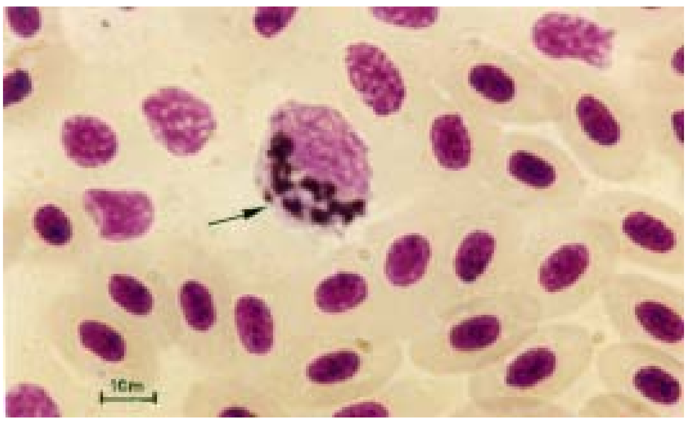

Fig. 5. A monocyte (arrow) containing engulfed carbon particles 5 minutes after injection of 16 mg/ml colloidal carbon, Giemsa, ×1000.

with higher dose, the elimination of carbon by the kidney increased considerably more.

Fig. 6. The spleen 10 minutes post injection of 16 mg/ml colloidal carbon showed slight accumulation of carbon particles around ellipsoids (arrows) and scattered within the stroma. H&E, ×250.

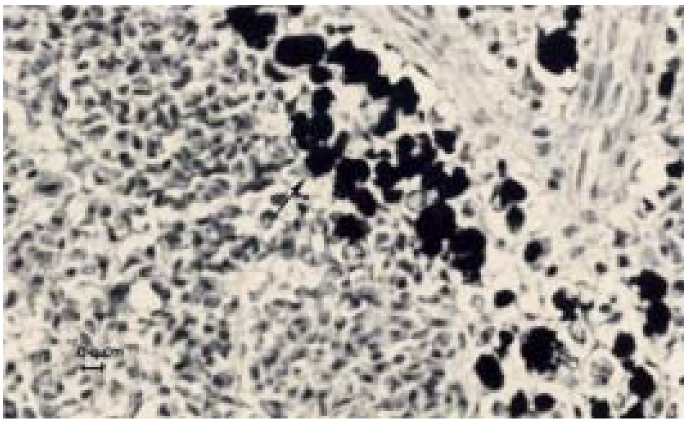

Fig. 7. The spleen from Tilapia sacrificed after 250 minutes post-injection of 16mg/ml colloidal carbon showing heavy accumulation of carbon particles around an ellipsoid (arrow). H&E, ×400.

Discussion

In this study, clearance of injected colloidal carbon for all doses was steady and progressive, as observed by BIOZZI et al. (1953). However, the curve of the mean phagocytic index of the doses showed an inverse proportion when plotted against the dose which confirms that the rate of clearance of a particulate matter (an example is carbon) is reduced with increasing doses. This is also in agreement with the work of BIOZZI et al. (1953) who suggested that reduced clearance rate resulted from saturating effect of the absorbed carbon in the reticulo-endothelial system (R.E.S).

SMEDSROD (1990) observed that the clearance of such substances is an important function of the R.E.S. The R.E.S. cells, located mainly in the kidney and spleen, make these two organs the main scavenging organs of fish (SMEDSROD et al., 1993; DANNEVIG et al., 1994; DALMO and BOGWALD, 1996; DALMO et al., 1996). The ellipsoids in the spleen of teleost have repeatedly been demonstrated to trap various particulate and non-particulate substances (SECOMBES and MANNING, 1980; DALMO et al., 1995), as observed in this study.

In salmonids, blood-borne foreign substances are mainly endocytised by kidney sinusoidal endothelial cells and macrophages (SMEDSROD et al., 1993; DANNEVIG et al., 1994). In this study, carbon was recovered largely from the spleen and less from the kidney, with considerable accumulations in the spleen for higher doses (32 and 48 mg) while less than 1mg was recovered from the liver also in higher doses, and none from the liver in the lower doses.

According to DALMO et al. (1997), one of the major properties of blood monocytes and tissue macrophages is endocytosis/phagocytosis. Clearing the blood of non-particulate matter and cells was also observed in this work.

This study therefore affirms that phagocytosis is indeed a very important aspect of non-specific defence mechanism in Tilapia, an African teleost fish of worldwide importance for aquaculture.

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SAŽETAK

Istražen je stupanj uklanjanja intravenski ubrizganih različitih doza kolodijskog ugljika iz krvi tilapije (*Oreochromis niloticus* L.) tijekom 250 minuta. Trideset riba (125-170 g) bilo je svrstano u šest skupina (A-F). Ugljik u dozi 4 mg, 8 mg, 16 mg, 32 mg i 48 mg bio je ubrizgan s 0,15 ml destilirane vode po ribi. Kontrolnoj skupini je bilo ubrizgano 0,15 ml destilirane vode. Potvrđeno je redovito uklanjanje bez obzira na količinu ubrizganog ugljika. Uklanjanje ugljika monocitima potvrđeno je već nakon 5 minuta iako je indeks fagocitoze bio obrnuto razmjeran dozi ubrizganog ugljika. Raspravlja se o značenju ukljanjanju ugljika i drugih stranih tvari.

Ključne riječi: kolodijski ugljik, indeks fagocitoze, *Oreochromis niloticus*
