Studies on the number and ingesting ability of thrombocytes in sick carps (*Cyprinus carpio* L.)

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ABSTRACT: Carps suffering from acute form of brachiomycosis (age: 2 months), chronic form of brachionecrosis cyprinorum (age: 12 months), acute form of erythrodermatitis (age: 23 and 28 months) or chronic form of erythrodermatitis (age: 23 months) were studied. The level of thrombocytes in carps with acute forms of the diseases was significantly lower than that in healthy carps. The inverse pattern was observed in carps with chronic forms of the diseases. Carps suffering from the acute form of erythrodermatitis (age: 23 and 28 months) demonstrated no differences in the level of thrombocytes which could be related to age or body weight of the animals. Index of thrombocyte ingesting ability and percent of ingesting thrombocytes were lowered in sick carps as compared to carp of control groups. A significant decrease in the parameters was disclosed, however, only in carps with acute forms of the diseases. Similarly to the level of thrombocytes, neither did the level of their ingesting ability demonstrate any differences which could be related to the age of carps suffering from the acute form of erythrodermatitis.

Keywords: level of thrombocytes; phagocytic activity of thrombocytes; sick carps

INTRODUCTION

Mechanisms of specific immunity in fishes, as in animals of lower phylogenetic advancement, are significantly less developed and play a markedly less important role than in birds or mammals (Pastoret *et al*., 1998). On the other hand, fishes manifest a relatively more efficient, as compared to "higher" animals, nonspecific resistance system, which plays the basic role in defense of the organism against pathogenic and environmental factors (Agius, 1981; Anderson and Siwicki, 1989; Ellis, 1999; Ingram, 1980, 1998; Sopińska, 1992; Stosik and Deptuła, 1990, 1991). Therefore, studies on the protective function of thrombocytes represent an important research goal, aimed at clarifying nonspecific resistance mechanisms in fishes. Recognition and definition of the cell role in organisms of healthy and sick fishes should permit a more complete appraisal of thrombocyte significance as protective cells. Apart from our own studies (Stosik, 1993, 1995) and papers of Yokoyama (1960), Ferguson (1976), McKinney *et al*. (1977), Daimon *et al*. (1979), Morrow and Pulsford (1980), Hunt and Rowley (1986), protective functions of fish thrombocytes have not been subjected to scientific scrutiny. Thus, it seems purposeful to undertake such studies and an additional hint for doing so comes from the fact that thrombocytes are at present included in the group of immune system cells in the man and homothermic animals (Roitt *et al*., 1993) and, most probably, play an identical role in the fish (Stosik and Deptuła, 1992).

The studies were performed in order to obtain data which should permit clinical appraisal and prognosis of development in carp diseases, resulting from the action of pathogenic agents, bacterial, invasive and environmental ones, on the basis thrombocyte levels as well as the ability of cells to ingest bacteria.

MATERIAL AND METHODS

Material for the studies came from 164 sick carps and 100 healthy carps studied in 10 groups (Table 1):
– fry, age: 2 months, fished out from acute branchiomycosis-affected stock – group 1,
control: healthy carps of the same age – group 2;
– older fry, age: 12 months, fished out from stock affected by chronic form of brachionecrosis cyprinorum – group 3,
control: healthy carps of the same age – group 4;
– commercial carps, age: 23 months, with signs of acute form of erythrodermatitis – group 5, or signs of chronic form of erythrodermatitis – group 7,

control: healthy carps of the same age – groups 6 and 8;
– commercial carps, age: 28 months, with acute form of erythrodermatitis – group 9,
control: healthy carps of the same age – group 10.

Blood of carps was studied. Blood samples were taken from the tail vein to tubes containing heparin (50 IU per ml blood) and ACD solution (acid citrate dextrose, B formula) (0.1 ml per ml blood), in field conditions, immediately after taking the fish out of the pond. Laboratory tests were performed 60 to 80 min. after blood sampling, i.e. immediately after delivery of the blood samples to the laboratory.

Studies of water environment (Table 2)

Samples of water for chemical and physical studies were taken using Patallas scoop at the depth of 20 cm below water level, in three sites of the pond: in inflow region, outflow region and at the spot positioned out of the stream. Water testing included determination of temperature, pH, contents of dissolved oxygen, ionised ammonia (NH_4^+), nonionised ammonia (NH_3), total ammonia ($NH_4 + NH_3$) and phosphates. The studies were performed as recommended by the Polish norm, PN-74/CO4620.00 (Water and Sewages).

Diagnosis of diseases

Erythrodermatitis was diagnosed from clinical and anatomopathological signs and the diagnosis was corroborated by microbiological studies and a biological test (Stosik, 1991).

Branchiomycosis was diagnosed on the basis of disturbed behaviour of fishes (signs of sultriness), macro-

Table 1. Groups of sick and healthy carps used for the studies

Experimental group	Number of fishes used for the studies	Group of cultured carps and their symbol	Clinical status	Age (months)	Body weight (g ± 10%)
1	32	fry – K_1	sick – branchiomycosis, acute form	2	8
2	20	fry – K_1	healthy – control group	2	9
3	45	older fry – K_2	sick – branchionecrosis, chronic form	12	90
4	20	older fry – K_2	healthy – control group	12	80
5	30	commercial carps – K_3	sick – erythrodermatitis, acute form	23	250
6	20	commercial carps – K_3	healthy – control group	23	240
7	27	commercial carps – K_3	sick – erythrodermatitis, chronic form	23	250
8	20	commercial carps – K_3	healthy – control group	23	250
9	30	commercial carps – K_3	sick – erythrodermatitis, acute form	28	750
10	20	commercial carps – K_3	healthy – control group	28	760

Table 2. Chemical tests of water samples from ponds, in which carps were cultured

Ponds from which water samples were taken	Season of the year (months)	Groups of expemental fishes	Number of tested water samples	Water temperature* (°C)	Chemical tests*					
					pH	O_2 (mg/l)	NH_4^+ (mg/l)	NH_3 (mg/l)	$NH_4^+ + NH_3$ (mg/l)	PO_4 (mg/l)
I	VII	2	33	24.1	7.02	6.85	0.55	nd	0.55	0.20
II	V	4	3	14.3	7.00	8.30	1.76	nd	1.76	0.40
III	IV	6.8	6	12.3	7.43	7.30	1.40	nd	1.40	0.30
IV	IX	10	3	11.3	7.35	7.40	1.52	nd	1.52	0.20
V	VII	1	3	23.3	7.82	5.70	1.94	nd	1.94	0.70
VI	V	3	6	20.1	8.89	4.30	2.60	0.038	2.638	0.90
VII	IV	5	6	10.8	7.47	8.20	1.34	nd	1.34	0.40
VIII	IV	7	3	12.3	7.53	7.50	1.38	nd	1.38	0.30
IX	IX	9	6	10.8	7.28	7.30	1.51	nd	1.51	0.30

nd = no NH_3 was detected
*the established parameters were given in the form of arithmetic means

scopic pattern of gill lesions (necrotic foci, marbled pattern of gills, tissue defects) and the diagnosis was confirmed by microscopic studies (presence of *Branchiomyces sanguinis* mycelia in the lumen of gill blood vessels).

Branchionecrosis cyprinorum was diagnosed by clinical examination and microscopic examination of gills (significant amount of mucus, oedema, blood congestion or tissue ischaemia, extravasations, marbled pattern of gills, necrosis of gill leaflets). The diagnosis was additionally confirmed by chemical water testing (Table 2) since environmental factors are known to play a significant role in the etiology of the disease.

Studies on the level and ingesting ability of thrombocytes

Thrombocyte level was determined by the technique of Deissi, as adapted to fishes (Stosik, 1993).

The ability of thrombocytes to ingest *Staphylococcus aureus* 209P bacteria was determined according to Mantur *et al.* (1986), as adapted to fishes (Stosik, 1993). The results were expressed as the index of ingestion by thrombocytes (Iit) which denoted the mean number of bacteria ingested by 100 thrombocytes capable of doing so, and as the percentage of ingesting thrombocytes (%it), which corresponded to the proportion of consecutively observed thrombocytes which demonstrated ingesting abilities.

Results of the studies were subjected to statistical analysis employing Student's *t*-test at $\alpha = 0.05$. The parameters were expressed by arithmetic means and standard deviations. Significance of differences was tested between results obtained in sick carps and control carps. Results of the studies are presented in Table 3.

RESULTS AND DISCUSSION

Levels of thrombocytes (Table 3) indicate that changes in the parameter remained in close parallelism to the form of the disease. On the other hand, the parameter showed no changes in sick fishes in the trend and direction of changes, which could be related to differences in age. In carps suffering from acute forms of the diseases, i.e. from branchiomycosis (group 1) or erythrodermatitis (groups 5, 9), lowered levels of thrombocytes were observed. The inverse situation was observed in carps affected by the chronic forms of branchionecrosis (group 3) or erythrodermatitis (group 7) in which augmented levels of thrombocytes were observed, pointing in a way to the importance of cells in protective mechanisms. Moreover, in carps affected by the acute form of erythrodermatitis no changes in thrombocyte levels were noted, which could be related to differences in the age or body weight of studied fishes (age of 23 months was accompanied by the body weight of 250 g, while 28 months-old carps weighed 750 g).

Index of ingesting ability of thrombocytes and percent of ingesting thrombocytes (Table 3) showed lowered values in carps suffering from erythrodermatitis (acute form, groups 5 and 9, chronic form, group 7), branchiomycosis (group 1) or branchionecrosis (group 3), as compared to the controls. Nevertheless, a significant decrease in the parameter was observed only in carps with the acute form of erythrodermatitis (groups 5 and 9) or branchiomycosis (group 1). No differences in ingesting abilities of thrombocytes were noted between fishes of different age affected by the acute form of erythrodermatitis (groups 5 and 9).

Studies on the index of ingesting ability of thrombocytes and percent of ingesting thrombocytes in carps with the acute form of branchiomycosis, erythrodermati-

Table 3. Number and ingesting ability of thrombocytes in sick and healthy carps

Experimental group	Type and form of the disease	Age (months)	Thrombocytes $\times 10^9$/L	Index of ingestion by thrombocytes	Percentage of ingesting thrombocytes
1	branchiomycosis – acute form	2	18.24 ± 4.82*	0.98 ± 0.18*	11.43 ± 1.96*
2	healthy	2	23.46 ± 2.34	1.48 ± 0.32	15.24 ± 2.98
3	branchionecrosis – chronic form	12	34.63 ± 5.27**	1.69 ± 0.24	17.13 ± 2.19
4	healthy	12	28.68 ± 3.31	1.78 ± 0.26	17.43 ± 3.11
5	erythrodermatitis – acute form	23	22.32 ± 4.81*	1.24 ± 0.21*	9.24 ± 2.07*
6	healthy	23	31.48 ± 5.51	2.00 ± 0.24	17.16 ± 3.14
7	erythrodermatitis – chronic form	23	49.23 ± 5.92**	1.86 ± 0.29	16.97 ± 2.86
8	healthy	23	31.48 ± 5.51	2.00 ± 0.24	17.16 ± 3.14
9	erythrodermatitis – acute form	28	24.98 ± 6.12*	1.22 ± 0.19*	10.17 ± 3.11*
10	healthy	28	31.63 ± 7.25	1.87 ± 0.21	17.09 ± 2.41

*statistically significant decrease in value as compared to the control group
**statistically significant increase in value as compared to the control group

tis or chronic form of branchionecrosis cyprinorum or erythrodermatitis represent the first investigative approach of the type. The observed tendencies and directions of changes could be related only to results obtained in healthy carps (Stosik, 1993, 1995), to control carps used in this experiment and to values of the index of neutrophilic granulocyte ingesting ability and the percentage of such granulocytes manifesting ingesting ability determined in an identical experimental system (Stosik *et al*., 2001). The neutrophilic granulocytes, tested in the same experimental setup (Stosik *et al*., 2001), manifested significant differences as compared to thrombocytes. Thrombocytes of carps with acute forms of the diseases (branchiomycosis, erythrodermatitis), in contrast to neutrophilic granulocytes (Stosik *et al*., 2001), showed lower ingesting ability (index of ingesting ability of thrombocytes and percent of ingesting thrombocytes) than that noted in healthy fishes. On the other hand, in carps with chronic forms of the diseases (branchionecrosis, erythrodermatitis) the cells maintain their ingesting ability on the level observed in fishes of control groups. This differs them from neutrophilic granulocytes which, similarly like in fishes in the acute stage of the disease, have manifested increased ingesting ability (Stosik *et al*., 2001). The observed change in thrombocyte phagocytic activity in carps at the acute stage of the disease is reflected most probably by the lowered number of the cells in blood. This may be linked to a lower or slower release of the cells to circulation and a more intense depletion of the pool of mature thrombocytes, which show full functional capacity. The absence of changes in thrombocyte ingesting ability (index of ingesting ability of thrombocytes and percent of ingesting thrombocytes) and the significant increase in the number of the cells in carps with chronic forms of the diseases (erythrodermatitis, branchionecrosis) point to a significant protective role of the cells, particularly at the first stage of the pathological process. The observed in our own studies reaction of blood thrombocytes to biotic and abiotic pathogenic factors (bacteria, parasites, toxic substances), which act on the fish organism, confirms and documents results of studies obtained in healthy carps (Stosik, 1995) and points to a significant role of thrombocytes as cells of the resistance system in the fish affected by the characterised diseases, even if no differences have been detected in reaction of thrombocytes in the fish suffering from chronic forms of the diseases.

CONCLUSION

Thrombocytes of carps form one of the protective barriers and may be regarded to represent elements of the resistance system. In analysis of the obtained results of studies on carps suffering from the acute form of branchiomycosis, chronic form of branchionecrosis cyprinorum, acute or chronic form of erythrodermatitis it should be concluded that the etiological factor involved and age of the sick fishes have failed to affect the level and ingesting activity of thrombocytes (index of ingesting ability of thrombocytes and percent of ingesting thrombocytes) in the carps. The observed differences have seemed to be associated with a chronic or acute course of the disease. The obtained results point to uniform (in the sense of tendency or direction of changes and not absolute values of determined parameters) ability of sick fishes of various age to resistance reactions within the scope of examined indices (thrombocyte level, index of ingesting ability of thrombocytes and percent of ingesting thrombocytes).

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