Phagocytic and bactericidal activity of blood thrombocytes in carps (*Cyprinus carpio*)

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ABSTRACT: The studies aimed at monitoring development of phagocytic and bactericidal activity in carps in the course of their ontogenetic development. The studies were performed using the techniques described by Mantur *et al.* (1986a, b), adapted to fish. Results were expressed in thrombocyte phagocytic index (Ipt), percentage of phagocyting thrombocytes (%tp), percentage of ingested bacteria (%bp) and in the index of intracellular killing by thrombocytes (Ibt). Number of thrombocytes was also examined using the technique of Dessi. Results of testing thrombocyte capacity to ingest the standard *Staphylococcus aureus* 209P strain showed that Ipt and %tp increased gradually in carps between the age of 3 and 17 months. In older carps, 19 to 29 months of age, as well as in spawners the growth in activity paralelled the ontogenetic development within Ipt values while %tp values remained at a similar level. Phagocytic activity of thrombocytes expressed in Ipt was reflected also by %bp values, which was particularly evident in fish aging 23 months to 5 years. In the case of Ibt, values of the index were not related in any way to stage of ontogeny or sex of the fish. Number of thrombocytes in carps aged 3 months to 5 years was increasing in parallel to their ontogenetic development.

Keywords: carp; thrombocytes; phagocytic ability; bactericidal activity

Fishes exhibit a much less developed system of specific immunity than that seen in higher animals (Stosik and Deptuła, 1990). The principal protective function in the fish, preventing infection and effects of other pathogenic factors, is played by elements which form mechanisms of non-specific immunity (Stosik and Deptuła, 1990). Among the latter mechanisms one should search for reactions which can be employed to evaluate and to protect health of the fish.

Protective functions of thrombocytes continue to represent a relatively neglected field of investigations. Studies performed up to now have dealt, first of all, with qualitative and quantitative appraisal of the cells in healthy and sick fishes (Stosik, 1993; Pulsford *et al.*, 1994; Richards *et al.*, 1994; Klinger *et al.*, 1996; van Erp *et al.*, 1996; Fischer *et al.*, 1998; Kozińska *et al.*, 1999a, b; Matsuyama *et al.*, 1999; Schutt *et al.*, 1999; Stosik *et al.*, 1999, 2001; Somamoto *et al.*, 2000; Schuwerack *et al.*, 2001). The qualitative appraisal has relied on microscopic techniques but also, which is worth stressing, on flow cytometry with the use of monoclonal antibodies (Romano *et al.*, 1996; Rombout *et al.*, 1996; Nakayasu *et al.*, 1997, 1998; Kfoury *et al.*, 1999; Esteban *et al.*, 2000). Origin of thrombocytes (Romano *et al.*, 1996, 1997; Schutt *et al.*, 1999) and their functional properties have been studied, including their protective function (Bielek, 1988; Stosik, 1993; Pulsford *et al.*, 1994; Kozińska *et al.*, 1999a, b; Stosik *et al.*, 1999, 2001). Present study has been focused on evaluation of thrombocyte phagocytic capacity, based on quantitative analysis of bacteria used in the studies and on analysis of bactericidal activity of the cells. The topics still represent a novel field of studies and the investigations have been motivated, i.a., by the documented view (Bielek, 1988; Kozińska *et al.*, 1999a, b; Stosik *et al.*, 1999, 2001), that thrombocytes, thought to represent immune system cells (Stosik and Deptuła, 1998), play important role in mechanisms of non-specific immunity also in fishes.

Present study aimed at monitoring development of phagocytic and bactericidal activities of thrombocytes in healthy carps during their ontogeny.

MATERIAL AND METHODS

Material for the studies included 570 healthy carps, 3 months to 5 years of age. The studies included all culture groups of carps:

the fry, K_1, groups I–IV (240 fishes were studied, i.e., 60 fishes in each group),

older fry, K_2, groups V–X (180 fishes were studied, i.e., 30 fishes in each group),

commercial carps, K_3, groups XI–XIV (120 fishes were studied, i.e., 30 fishes in each group),

spawners-milters, K_m, group XV (15 fishes were studies),

seeders, K_i, group XVI (15 fishes).

In each of the groups, the fish originated from the same parental groups. The carps were fished off the culture ponds of good environmental conditions. The form and health status of the fish were evaluated in clinical, anatomopathological and parasitological studies. Carp stocks, from which the studied carps originated, were free of spring viraemia of carp, which was confirmed by serological tests performed using ELISA tests (TEST-Line Ltd., Brno).

Immunological and haematological tests. Blood for the studies was sampled from the heart and placed in heparin-containing tubes (50 IU per ml blood) in ACD (citric acid, trisodium citrate, glucose) solution. Phagocytic ability (Mantur *et al.*, 1986b) and bactericidal activity (Mantur *et al.*, 1986a), i.e., the thrombocyte ability to kill *Staphylococcus aureus* 209P strain were determined using the techniques described by Mantur *et al.* (1986a, b), adapted to the fish. Results were expressed by the index of thrombocyte phagocytosis (Ipt), percentage of phagocyting thrombocytes (%tp), percentage of ingested bacteria (%bp) and thrombocyte bactericidal index (Ibt). The number of thrombocytes was determined using the method of Deissi (Stosik *et al.*, 2001).

Results of immunological and haematological tests in the form of arithmetic means and standard deviations are listed in Table 1.

RESULTS AND DISCUSSION

Results of testing thrombocyte capacity to phagocytose the standard bacterial strain of *Staphylococcus aureus* 209P (Table 1) demonstrated gradual increase in Ipt and %tp indices in carps with age progressing from 3 to 17 months. In older carps, aging 19 to 29 months and in spawners the gradual increase in activity also paralelled the increasing age of carps but his was noted only in the case of Ipt while %tp in the groups of fish

Table 1. Number, phagocytic capacity and bactericidal ability of thrombocytes in carp

Experimental group		Age of carps (month of testing)	Number of thrombocytes ($\times 10^9$/l)	Ipt	%tp	%bp	Ibt
I (n = 60)	K_1	3 months (VIII)	24.97 ± 2.71	1.29 ± 0.27	13.27 ± 2.91	18.01 ± 2.31	0.17 ± 0.071
II (n = 60)		5 months (X)	24.86 ± 2.31	1.31 ± 0.21	13.68 ± 1.98	18.29 ± 2.18	0.21 ± 0.020
III (n = 60)		7 months (XII)	26.72 ± 2.81	1.46 ± 0.18	14.21 ± 1.78	16.38 ± 1.86	0.27 ± 0.062
IV (n = 60)		9 months (II)	29.36 ± 3.42	1.58 ± 0.30	14.72 ± 2.04	18.01 ± 2.17	0.19 ± 0.084
V (n = 30)	K_2	11 months (IV)	30.21 ± 2.76	1.71 ± 0.19	15.16 ± 2.01	18.29 ± 1.14	0.22 ± 0.073
VI (n = 30)		13 months (VI)	31.27 ± 2.81	1.77 ± 0.18	15.61 ± 1.72	17.34 ± 2.07	0.31 ± 0.067
VII (n = 30)		15 months (VIII)	28.96 ± 3.17	1.82 ± 0.15	16.24 ± 2.57	18.03 ± 3.17	0.24 ± 0.046
VIII (n = 30)		17 months (X)	32.14 ± 3.54	1.87 ± 0.09	17.06 ± 1.86	17.94 ± 1.34	0.18 ± 0.037
IX (n = 30)		19 months (XII)	29.17 ± 3.42	1.94 ± 0.22	16.21 ± 1.72	18.92 ± 1.24	0.16 ± 0.001
X (n = 30)		21 months (II)	31.86 ± 2.92	1.76 ± 0.28	16.76 ± 1.37	17.86 ± 2.76	0.20 ± 0.025
XI (n = 30)	K_3	23 months (IV)	31.91 ± 3.17	1.88 ± 0.10	16.34 ± 3.12	23.46 ± 2.89	0.19 ± 0.017
XII (n = 30)		25 months (VI)	28.29 ± 2.86	1.72 ± 0.31	16.25 ± 1.24	24.02 ± 3.01	0.16 ± 0.033
XIII (n = 30)		27 months (VIII)	29.31 ± 3.21	1.96 ± 0.36	16.99 ± 2.17	27.37 ± 3.17	0.24 ± 0.051
XIV (n = 30)		29 months (X)	30.82 ± 4.24	1.87 ± 0.28	16.37 ± 3.02	26.11 ± 3.24	0.21 ± 0.047
XV (n = 15)	K_m	5 years (IX)	33.27 ± 5.37	2.07 ± 0.31	17.02 ± 2.93	28.91 ± 3.86	0.19 ± 0.036
XVI (n = 15)	K_i	5 years (IX)	34.19 ± 4.18	2.27 ± 0.27	16.57 ± 3.21	31.70 ± 3.42	0.22 ± 0.024

showed similar values. Phagocytic activity of thrombocytes, expressed by Ipt values was reflected also in %bp values (Table 1), which was particularly evident in 23 month- to 5 year-old fish. The highest Ipt values were noted in seeders and the lowest ones in 3 month-old carps. Values of %tp were highest in 17 month-old carps and the lowest in 3 month-old carps. Peak %bp values were noted in seeders while 7 month-old carps demonstrated the lowest values of the index. In Ibt index (Table 1) no relation could be detected between values of the index and stage of ontogeny or sex of the examined fish. Peak values of the index were observed in 13 month-old carps while the lowest values were found in 19 and 25 month-old carps.

Number of thrombocytes (Table 1) in carps aging 3 months to 5 years increased in parallel to their ontogenetic development. The highest levels of the cells were encountered in seeders and the lowest ones in 5 month-old carps.

As planned, the studies were performed on carps living in natural conditions, in all seasons of the year. This allowed for an objective monitoring of development of phagocytic and bactericidal activities of thrombocytes, influenced not only by ontogeny but also by the environment. Analysis of tests performed on carps aging 3 months to 5 years demonstrated significant differences related to stages of fish ontogeny but the differences included only some parameters. Values of Ipt, %tp, %bp and numbers of thrombocytes tended to increase in line with the age of carps. No such relation could be detected in the case of Ibt.

Levels of peripheral blood thrombocytes in carps at various stages of ontogeny was noted to vary between 24.86 and 34.19 ($\times 10^9$/l). Reports of other authors (cited by Stosik and Deptuła, 1992) have shown that levels of the cells in various species of fish and even in the same species vary extensively. The data obtained in present studies of our own resemble most closely thrombocyte levels defined in carps by Rijkers *et al.* (1980), Stosik (1993) and by Stosik *et al.* (2001).

Phagocytic activity of thrombocytes, observed in our studies (Ipt, %tp, %bp), has manifested, particularly in the youngest, 3 to 17 month-old fish, a relation with the ontogenetic development. The capacity to phagocytose bacteria has been observed much earlier, during studies devoted to other problems. The phenomenon has been observed in multiple species of fish, including *Pleuronectes platessa* (Ferguson, 1976), *Arius maculatus* or *Pimelodus maculatus* (Ferri, 1982), *Perca flavescens* (Yokoyama, 1960), *Cyprinus carpio* (Daimon *et al.*, 1979), *Lepisosteus platyrhincus* (McKinney *et al.*, 1977), *Scyliorhinus canicula* (Morrow and Pulsford, 1980; Hunt and Rowley, 1986). On the other hand, few studies have attempted to clarify protective functions of thrombocytes (Bielek *et al.*, 1988; Stosik, 1993; Nakayasu *et al.*, 1998; Kozińska *et al.*, 1999a,b; Matsuyama *et al.*, 1999; Stosik *et al.*, 1999, 2001). However, our studies have pointed to a significant role of thrombocytes in protective reactions of carps, in which the low Ipt values are compensated by the high %tp values and by the high thrombocyte levels in the peripheral blood which results in a high percentage of ingested bacteria (%bp). Within Ipt and %tp, values of present results are consistent with the earlier results obtained in healthy and sick carps, studied in a similar experimental system (Stosik *et al.*, 2001). They corroborate also observations of Kozińska *et al.* (1999a, b), who have demonstrated correlation of index values reflecting thrombocyte protective activity in carps (index of bacteria-thrombocyte adherence, percentage of active thrombocytes, index of thrombocyte aggregation) and lower sensitivity of the fish to bacterial infection, particularly at the temperature of 11°C. However, it should also be stressed that participation of thrombocytes in protective reactions has not been confirmed by all authors. Nakayasu *et al.* (1998) have found in their investigations that thrombocytes are incapable to phagocytose while Matsuyama *et al.* (1999) have detected no such cells in an inflammatory focus, which may indirectly negate protective function of the cells. Similarly to studies on the percentage of phagocytosed bacteria, evaluation of thrombocyte capacity of intracellular killing of the bacteria represents a completely new aspect of the problem, not examined before in the fish. In our studies we have demonstrated that the fish thrombocytes manifest this type of activity. Even if weakly expressed, it may play a very important role, since similarly to low Ipt levels it is compensated by the high numbers of peripheral blood thrombocytes.

SUMMARY

Fish thrombocytes exhibit capacity to phagocytose *Stapylococcus aureus* 209P strain and to kill the ingested bacteria.

Results of our studies, performed on a representative numbers of the fish, permit to conclude that specific trends in changes of the number and activity of thrombocytes reflect physiological conditions, linked with the ontogenetic development.

Results of our own studies provide another proof for the importance of fish thrombocytes as one of protective barriers in the animals.

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