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□ **Brief Communication** □

Activities of scavenging enzymes of oxygen radicals in early maturation stages of *Paragonimus westermani*

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Abstract: In early maturation stages of *Paragonimus westermani* (metacercariae, 4-, 8-, 12-week old worms), activities of antioxidant enzymes, such as superoxide dismutase, catalase, peroxidase and glutathione peroxidase, were examined. Specific activity of catalase was the highest in metacercariae and decreasing with age. That of superoxide dismutase was higher in metacercariae and 4-week worms. Specific activity of peroxidase was at its peak in 4-week worms while that of glutathione peroxidase was in 8-week worms. Specific activities of all these antioxidant enzymes were decreased to their lowest in 12-week old adults.

Key words: *Paragonimus westermani*, oxygen radical, superoxide dismutase, catalase, peroxidase, glutathione peroxidase, developmental stage

Paragonimus westermani is a trematode parasite that causes chronic inflammatory lung disease in carnivorous mammals and man. After an oral infection, excysted metacercariae penetrate intestinal wall and migrate peritoneal cavity to reach lung in 4~12 weeks of infection. In the lung, adults are surrounded by granuloma wall. In the long processes of the parasite infections up to about 5 years, the significance of parasitic proteinases and antioxidant enzymes are increasingly regarded to be important in pathogenesis and parasite survival(Callahan *et al.*, 1988; McKerrow, 1989; Chung *et al.*, 1991).

Oxidant stress is a phenomenon of damaging cellular components by reactive oxygen species, such as superoxide radical (O_2^-), hydroxyl radical (OH•), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) which are generated in oxygen metabolism (Fridovich, 1975). The potential lethal toxicities of oxygen radicals are well illustrated (Kellogg and Fridovich, 1975; Brawn and Fridovich, 1985; Davies *et al.*, 1987). To prevent oxidant stress, aerobic organisms are equipped with a series of defense mechanisms such as superoxide dismutase (EC 1.15.1.1, SOD), catalase (EC 1.11.1.6), peroxidase (EC 1.11.1.7) and glutathione peroxidase (EC 1.11.1.9). In studies of Murray *et al.* (1979) and Murray (1981), the roles of antioxidant enzymes had been proved to be a defense mechanism of parasitic protozoa against oxidant stress of hosts. It has also been postulated that those enzymes in parasites were an evasion mechanism against oxygen-mediated damages from hosts (Callahan *et al.*, 1988). In this connection, antioxidant systems in adult *Schistosoma mansoni* were more active than those in schistosomula stage. By adding exogenous antioxidants to schistosomula, they could protect themselves from toxic oxygen

radicals in cell-free oxygen radical generator, that is, xanthine/xanthine oxidase system (Mkoji *et al.*, 1988a & b). Microorganisms differ greatly in their susceptibility of oxygen-mediated damages because the levels of endogenous scavenging enzymes are different each other (Thomas *et al.*, 1988). We have previously purified and characterized Cu, Zn-superoxide dismutase in adult *P. westermani* (Chung *et al.*, 1991). However, very little information is available on oxygen scavenging enzymes and their role in host-parasite interactions in paragonimiasis. We presumed that early maturation stages of *P. westermani* are challenged by different extents of oxidant stresses. To resist to different degrees of oxidant stress, each stage may be equipped with different activities of antioxidant enzymes. To examine the assumption, we measured the activities of oxygen radical scavenging enzymes in the early maturation stages of *P. westermani*.

Metacercariae of *P. westermani* were obtained from naturally infected crayfish (*Cambaroides similis*) collected in Cholla Nam Do, Korea. Dogs were fed with 200 metacercariae and killed sequentially on the 4, 8, 12 weeks after the experimental infection. The worms of each age were collected, washed with sterile cold physiological saline and stored at −70°C. For the preparation of crude enzymes, worms of each age were homogenized with a tissue grinder in 50 mM potassium phosphate buffer (pH 7.8). Metacercarial homogenate was sonicated additionally at 4°C with 20 Watt for 30 seconds, 3 times each in 30 seconds interval (Branson Sonifier, U.S.A.). Homogenates were clarified at 20,000 *g* for an hour at 4°C (Sorvall RC 5B). Supernatants were saved and used as crude enzymes. Activity of SOD was assayed by the method of McCord and Fridovich (1969); that of catalase by Aebi (1974); glutathione peroxidase by Floehe and Guenzler (1984); peroxidase by Puetter (1974). Protein contents were measured by Lowry *et al.* (1951). Results were shown in Tables 1-4.

In this study, we could measure the activities of four antioxidant enzymes in the early maturation stages of *P. westermani* and the changing patterns of the enzyme activities by age were also able to be compared. The activity of catalase of the parasite was comparable to other eucaryotic tissues. Unlike the present result, catalase activity has not been detected in other trematodes such as *Schistosoma mansoni* (Mkoji *et al.*, 1988) or *Fasciola hepatica* (Barrett, 1980). The toxicity of hydrogen peroxide in

Table 1. Activities of superoxide dismutase in early maturation stages of *P. westermani*

Developmental stages	Activity (U/ml)*	Protein (mg/ml)	Specific activity (U/mg)**
Metacercariae	11.10	1.18	9.41
4-week worms	16.10	1.70	9.47
8-week worms	9.20	1.39	6.52
12-week adults	14.33	2.55	5.62

* U : unit

** U/mg : unit per milligram of protein

Table 2. Activities of catalase in early maturation stages of *P. westermani*

Developmental stages	Activity (U/mg)	Protein (mg/ml)	Specific activity (U/mg)
Metacercariae	29.97	1.18	25.40
4-week worms	27.00	1.70	15.88
8-week worms	15.00	1.39	10.79
12-week adults	12.07	2.55	4.73

Table 3. Activities of peroxidase in early maturation stages of *P. westermani*

Developmental stages	Activity (U/ml)	Protein (mg/ml)	Specific activity (U/mg)
Metacercariae	140.00	1.18	118.64
4-week worms	240.01	1.70	141.18
8-week worms	140.00	1.39	100.00
12-week adults	82.70	2.55	32.43

Table 4. Activities of glutathione peroxidase in early maturation stages of *P. westermani*

Developmental stages	Activity (U/ml)	Protein (mg/ml)	Specific activity (U/mg)
Metacercariae	5.80	1.18	4.90
4-week worms	9.50	1.70	5.60
8-week worms	12.10	1.39	8.70
12-week adults	2.80	2.55	1.10

these species may be removed through the action of cytochrome *c*-linked peroxidase and glutathione peroxidase instead of catalase.

The specific activities of antioxidant enzymes of *P. westermani* were higher in metacercariae or 4-week worms. Glutathione peroxidase activity was, however, the highest at 8-week worms (Table 4). Of 4 stages we examined, adult *P. westermani* of 12-week old showed the lowest level of activities of 4 enzymes. These results suggest that metacercariae and juvenile worms (4- and 8-week old) of migrating stage were exposed more to reactive oxygen species released from host immune system. These results are different from antioxidant enzymes of *S. mansoni* which were more active in adults than in schistosomula (Mkoji *et al.*, 1988 a & b). To the point, we think that early stages of *P. westermani*, unlike schistosomula, must generate more antioxidant enzymes to cope with oxidant stress.

It is not yet clearly known that oxygen stress against the lung fluke is evoked more in the early stage of paragonimiasis. In addition, it is also unclear whether these enzymes are really protective to exogenous reactive oxygen species. Activities of these enzymes in secretory-excretory products of *P. westermani* should be studied in the future to confirm the defensive roles of the enzymes. As Mkoji *et al.* (1988a & b) did, it is therefore necessary to undertake *in vitro* experiments to observe the susceptibility of different stages of *P. westermani* in the oxygen radical generation system.

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=국문초록=

산소 라디칼 관련 효소의 폐흡충 발육 단계별 활성도 변화

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Oxygen radical은 생물체의 산소대사 과정의 부산물로 생성되어 세포내 여러 성분을 불활성화시키거나 미생물에 대한 방어기전으로 작용한다. 기생충에 존재하는 antioxidant enzyme은 숙주의 방어기전에서 유리하는 oxygen radical의 독성을 제거하므로 이 효소의 활성도는 기생충의 생존에 영향을 미친다고 생각하고 있다. 폐흡충은 피낭유충이 종숙주에 침입한 다음 숙주내 조직이행 시기를 거쳐서 폐에 도달하여 성충이 되므로 이 과정에서 각 시기별 산소독성과 이에 대항하는 효소의 활성이 다를 것으로 추정하였다. 폐흡충의 피낭유충과 감염후 4주, 8주, 12주에 얻은 충체의 추출물을 조효소(粗酵素)로 하여 SOD, catalase, peroxidase, glutathione peroxidase의 활성도를 측정하였다. 각 효소의 비활성도(specific activity) 중 catalase는 피낭유충에서 최고치였으며, SOD와 peroxidase는 4주 충체에서 가장 높았고, glutathione peroxidase는 8주 충체에서 높았다. 이들 4가지 antioxidant효소의 비활성도는 감염 12주인 성충에서 모두 낮게 측정되어 조직 이행시기의 충체에서 더 높은 효소 활성도를 지니고 있음을 알 수 있었다.

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