

Antibody changes in paragonimiasis patients after praziquantel treatment as observed by ELISA and immunoblot

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Abstract: To observe antibody changes after praziquantel treatment in paragonimiasis, a total of 46 serum samples from 13 serologically diagnosed patients was collected for 4~28 months. The specific antibody (IgG) levels were measured by enzyme-linked immunosorbent assay (ELISA). All but one patient who needed retreatment became symptom-free within a week. Antibody levels were dropped near to or below a cut-off absorbance (abs.) of 0.25 in varying intervals from 4 to 18 months. Of 9 patients who were retested within 3 months, 5 revealed temporary elevation of antibody level. After the elevation, the levels began to decline slowly to negative ranges. If treated earlier after symptoms developed, the temporary elevation did not occur and intervals to negative conversion were shorter. By sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)/immunoblot, antigen-antibody reactions in individual patient faded gradually without significant changes in reacting antigen bands.

Key words: *Paragonimus westermani*, human paragonimiasis, praziquantel, serologic test, ELISA, SDS-PAGE/immunoblot

INTRODUCTION

The rationale of serologic diagnosis in human paragonimiasis is that serum antibody returns to its negative ranges after a successful treatment. Yokogawa (1965) described that positive antibody titers of complement fixation test (CF) in either egg-positive or egg-negative patients of paragonimiasis turned to negative within 7 months after bithionol treatment. This was quite different from results of intradermal test for human paragonimiasis in which positive reaction persisted up to 5~30 years (Tsuji, 1984). The persisting positive reaction after treatment has been the main cause of low specificity in intradermal test. Actually the specificity

was reported as low as 16% in a previously endemic area in Korea (Cho *et al.*, 1983).

As ELISA is increasingly applied in antibody test for paragonimiasis (Cho *et al.*, 1981; Yokogawa *et al.*, 1983; Kojima *et al.*, 1983; Lee and Choi, 1983; Knobloch and Lederer, 1983; Imai, 1987), the interval between treatment and negative conversion of specific antibody (IgG) becomes important. Because treatment failure rate of praziquantel is about 5% (Rim *et al.*, 1981), those who need retreatment should be differentiated from respiratory diseases of other causes. Knobloch *et al.* (1984) reported that the antibody (IgG) levels by ELISA in paragonimiasis patients elevated temporarily up to 2 months, then declined to normal in 3~6 months after praziquantel treatment. In experimental parago-

nimiasis in cat, Choi *et al.* (1986) reported that high antibody levels abruptly turn to negative within 50 days after praziquantel treatment. These differently reported intervals made it necessary to evaluate again the changing patterns of the specific antibody (IgG) in human patients for sufficiently long periods.

By SDS-PAGE/immunoblot, antigenic protein bands of *Paragonimus westermani* have been examined. Through these observations we can obtain basic information for preparation of purified antigen and evaluate immunoblot itself as a diagnostic test (Sugiyama *et al.*, 1987; Itoh and Sato, 1988; Slemenda *et al.*, 1988; Kim *et al.*, 1988). As Slemenda *et al.* (1988) and Kim *et al.* (1988) showed, sera from individual patient exhibited different combination of reacting antigenic bands. This finding suggests that after specific treatment each patient may produce various new antibodies to hitherto non-reacted bands. In order to facilitate the removal of dying/dead worm, new antibody formation may be quite possible to occur. If it does really occur, new antigen-antibody reactions would be reflected in the immunoblot.

This study was undertaken to observe the changing patterns of specific antibody levels and of reacting antigenic bands after praziquantel treatment in sera of human paragonimiasis.

MATERIALS AND METHODS

1. Antigen

Saline extract of 13 week-old adult *P. westermani* was prepared as Cho *et al.* (1981) and Kim *et al.* (1988) described. The protein content in the crude extract was 2.0mg/ml as measured by Lowry *et al.* (1951).

2. Patients and sera

From May 1982 to September 1988, we diagnosed serologically 210 human paragonimiasis by specific antibody (IgG) test by ELISA. Of them, only 13 patients undertook once or more serologic follow-up after 4 months of praziquantel treatment (75mg/kg body weight/day for 2 days, Rim *et al.*, 1981). These patients were not

selected cases but those who voluntarily complied the physicians follow-up recommendations.

All the collected sera were kept at -40°C until used. All sera were tested simultaneously by ELISA and SDS-PAGE/immunoblot to reduce variations between tests.

3. ELISA

Serologic test for *Paragonimus*-specific IgG antibody was performed as described by Cho *et al.* (1981). Briefly, antigen was coated in protein concentration of 2.5 $\mu\text{g}/\text{ml}$ overnight at 4°C . Sera were reacted in dilution of 1:100 and peroxidase-conjugated antihuman IgG (heavy- and light-chain specific, Cappel) was reacted in dilution of 1:10,000 for 2 hours each at 36°C . Substrate, consisted of distilled water (99ml), 1% *o*-phenylene diamine (1ml) and 30% H_2O_2 (10 μl) was reacted at 20°C for 30 minutes. The reaction was stopped by adding 8N H_2SO_4 . Abs. was read at 492nm. To standardize test, a reference positive serum, of which mean abs. was 1.0, was reacted at each test.

4. SDS-PAGE/immunoblot

Methods of Laemmli (1970) and Tsang *et al.* (1983) were applied as Kim *et al.* (1988) described. Briefly, saline extract of *P. westermani* in reducing condition was separated electrophoretically in 10~15% linear gradient gel. The protein bands in the gel were transferred to nitrocellulose paper electrophoretically. After washing, 1:100 diluted sera and 1:1,000 diluted peroxidase-conjugated antihuman IgG were sequentially reacted for 1 hour each. Finally, substrate, consisted of 3,3'-diaminobenzidine (50mg), distilled water (100ml) and 30% H_2O_2 (10 μl), was reacted for 15 minutes. The reaction was stopped by washing paper with distilled water.

RESULTS

1. Changes in specific antibody (IgG) levels by ELISA

Table 1 listed the subjects with main symptoms and duration between the first development of symptoms and specific treatment with

praziquantel. Symptoms were hemoptysis, pleural effusion and migrating masses in subcutaneous tissue. Duration between illness and treatment varied from 1 month to 4 years. Delay in treatment was due mainly to antituberculous therapy in presumption.

As shown in Fig. 1, changing patterns of the specific antibody varied by individual patient. A patient (Case D), who complained of hemoptysis again on 20 months after the first praziquantel therapy, showed still high antibody level of abs. 0.65. The patient was re-treated. Intractable symptoms in other patients disappeared promptly within a week after the specific treatment. Out of 12 improved patients, 5 exhibited elevated levels of the specific antibody while 7 did not when examined in 1~3 months after the treatment. After 4 months, such elevation was no more observed. The antibody levels in early treated patients (Cases E, H, L) declined promptly without the temporary elevation. But in patients of delayed treatment

Table 1. Symptoms and duration of illness in the subjected patients

Case	Main symptoms	Duration of illness before praziquantel treatment (year)
A	H*	4
B	PE**	2
C	PE	1 2/12
D	H	7/12
E	PE	2/12
F	H	?
G	H	3/12
H	PE	1/12
I	H, PE, MM***	2 6/12
J	PE	1 2/12
K	PE	1 6/12
L	PE	2/12
M	H	2

*H : Hemoptysis, **PE : Pleural effusion, ***MM : Migrating subcutaneous masses

(Cases A, C, I, J, K, M), the antibody levels elevated for 3 months and began to decline thereafter.

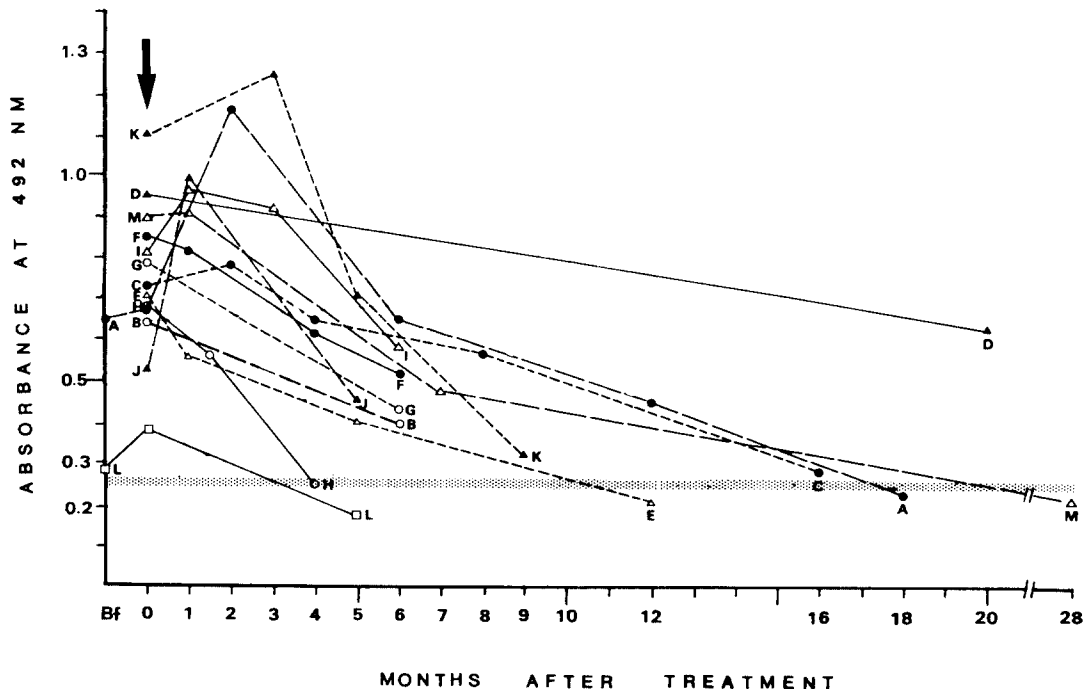


Fig. 1. Absorbance changes before and after praziquantel treatment in paragonimiasis patients by ELISA. A-M : Patient number. Dotted band at abs. 0.25 : Criterion of positive reaction (Bf : Before the praziquantel treatment. ↓ : Praziquantel treatment).

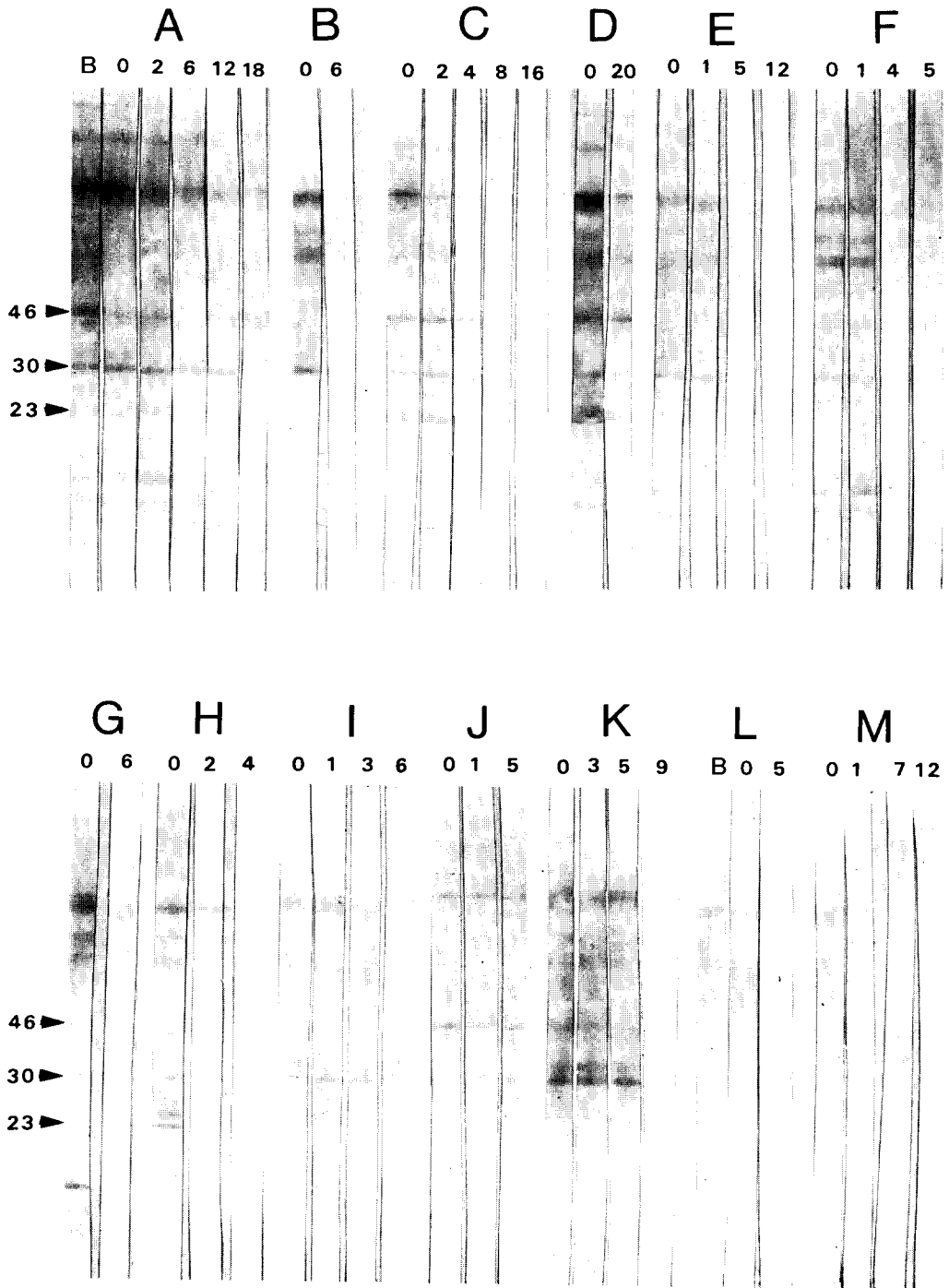


Fig. 2. SDS-PAGE/immunoblot of paragonimiasis patients before and after praziquantel treatment. Saline extract of adult *P. westermani* was electrophoresed in reducing condition. A-M : Patients number. B, 0~28 at top of immunoblot: B: before treatment 0 : At the time of treatment, 1~28 : Months after the treatment. Numerals standing at left column: Molecular weight in kDa.

Later than 5 months after praziquantel treatment, the antibody levels showed declining tendency in all patients. In 2 patients (Cases H, L) the levels dropped below cut-off abs. (0.25) within 4~5 months while in 5 patients (Cases B, F, G, I, J), the levels were declining but not yet to cut-off abs. In other 5 patients (Cases A, C, E, K, M), who were followed until 9~28 months, the levels at the final examination were near to or below the cut-off abs. One patient (Case M), who exhibited similar decreasing pattern of antibody with Cases B, F, G, I and J at 7 month showed abs. of negative range at the 28th month.

2. Findings in follow-up SDS-PAGE/ immunoblot

As shown in Fig. 2, antigenic bands reacted with patient sera varied by individual before treatment. Major reacting bands were 30, 46, 23 and 92 kilodaltons (kDa). In addition, bands of 150, 120, 74, 62, 32 and 16.5 kDa were reacted.

After praziquantel treatment, major findings were changes in intensity in previously reacted bands. New reactions to previously non-reacted bands were rare. For example, in Cases A, F, and K, band of 16.5 kDa reacted stronger at 2-month after the treatment; in Case B, a hitherto unrecognized band of 65~70 kDa was found in 6 months after treatment. As a whole, the reacting bands in each patient faded with time. The sequence of fading bands could hardly be described, but weakly reacted bands disappeared sooner and strongly reacted bands persisted longer. Especially bands of 92 and 30 kDa persisted in most patients.

DISCUSSION

The present study indicated that the interval between praziquantel treatment and negative conversion of anti-*Paragonimus* antibody (IgG) varied from 4 to 18 month by individual. And the interval to negative conversion was shorter in early treated patients. In patients with delayed specific treatment, mostly due to anti-tuberculous therapy, the antibody levels elevated

temporarily for 1~3 months then began to decline in much slower fashion. Another possible factor which may affect the interval was the intensity of infection. In paragonimiasis, however, the intensity is hardly assessable objectively; therefore, not considered in this study. From the present results, we recommend serologic test by ELISA at 18-month to confirm cure. Any patient of paragonimiasis is expected to be serologically negative at that interval.

This study confirmed that reports of Knobloch *et al.* (1984) and Choi *et al.* (1986) were correct in some aspects. Temporary elevation of the antibody levels (Knobloch *et al.*, 1984) were observed also in this study but not in all. Progressive lowering to negative range without temporary elevation (Choi *et al.*, 1986) was also possible. When followed up by CF after bithionol treatment, Yokogawa (1965) did not mention such elevation, but it may be due to sensitivity differences between CF and ELISA in depicting the antibody levels.

The delayed disappearance of the specific antibody in sera of treated patients may be related with slow resolution of the pathologic lesions. Actually, paragonimiasis patients with infiltrations or nodular lesions at their chest X-ray films revealed temporary aggravation of the lesions after praziquantel treatment (Rim *et al.*, 1981). Resolution of the lesions took 4 months. Possibly, slow resolution of granulomatous lesions which contained antigenic eggs of *P. westermani* (Kim *et al.*, 1986) may be related with slow disappearance of the antibody even after treatment. This presumption explains well the quick decline of the antibody in early treated patients who have negligible amount of eggs in the lesion.

The antigenic proteins specific for *P. westermani* were variously stated as 27 kDa (Sugiyama *et al.*, 1987), 27 kDa and 33~37 kDa (Itoh and Sato, 1988), 8 kDa (Slemenda *et al.*, 1988) and 30, 46 and 23 kDa (Kim *et al.*, 1988). These differences may be due to differences in preparation of antigens, techniques of SDS-PAGE *etc.* Anyway, our results in SDS-PAGE/immunoblot

was identical with those of Kim *et al.* (1988). After treatment, convalescent sera did not contain new antibodies reacting with previously non-reacting antigenic bands in the crude saline extract.

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폐흡충증에서 치료 후 혈청내 특이 항체의 변화

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혈청내 특이 항체를 측정하여 폐흡충증을 진단하는 방법은 폐흡충증 환자가 치료받거나 자연 치유된 후 일정기간 지나면 특이 항체가 음전(陰轉: negative conversion)된다는 것이 그 전제 조건이다. 이 연구는 폐흡충증 환자를 프라지판텔(praziquantel)로 치료한 후 특이 항체가 변화하는 양상과 음전되는 기간을 면역효소측정법(ELISA)으로 관찰하고, 또 치료 후 회복기 혈청에서 항원 구성 단백질 중 반응하지 않던 단백질에 대한 새로운 항체가 형성되는지를 관찰하기 위하여 실시하였다.

1982년 5월부터 1988년 9월까지 혈청학적으로 진단한 폐흡충증 환자 210명 중 13명이 프라지판텔 치료(75mg/kg/일×2일) 후 4개월 이후에 한번 이상 추적검사를 받았다. 이들의 혈청 46개에서 다시 면역효소측정법으로 특이 항체를 측정하고 또 SDS-폴리아크릴아마이드겔 전기 영동후 면역얼룩법(immunoblot)을 실시하였다.

추적검사를 받은 13명 중 1명은 20개월후 증상이 다시 나타났고 또 특이 항체가 양성이어서 치료실패자로 하였다. 그 이외의 환자 12명은 치료 후 1주일 이내에 증상이 사라지고 추적 기간은 다르나 항체가 음전 또는 현저한 저하 경향을 보여 치유된 환자로 판정하였다. 항체의 변동은 치료후 3개월 이내에는 두가지 양상이었는 바 항체가 높아지거나 변동이 없는 경우(3개월 이내에 추적검사를 하였던 환자 9명중 6명)와 그대로 저하하기 시작하는 경우(9명중 3명)로 나눌 수 있었다. 치료 후 항체가 즉각 저하하는 경우에는 계속 저하하여 4~12개월이던 음전하였다. 일시적으로 항체가 상승하였던 환자에서는 4개월 이후 서서히 저하하며 9~18개월에 음전하였다. 폐흡충증의 첫 증상 발현후 치료할 때까지의 기간이 짧을수록 음전 기간은 짧은 경향을 보였고 치료 후 3개월간의 일시적인 항체가 상승 현상은 없었다. SDS-폴리아크릴아마이드겔 전기영동 후 면역얼룩법을 실시한 바 치료 후 환자 혈청은 치료 전의 반응 양상을 그대로 유지하면서 서서히 반응이 약해지는 방향으로 변화하였으며 치료 전에 반응하지 않던 항원에 새롭게 반응하는 항체가 나타나는 경우는 드물었다.