FACTORS AFFECTING PENETRABILITY OF CHEMOTHERAPEUTICS INTO THE LESION

Presumption from Peroxidase Reaction of Isoniazid-Resistant Tubercle Bacilli

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Introduction

It is required, for chemotherapeutics to be effective in treatment of infectious diseases, that sufficient amount of the drug must come to infecting bacteria in the lesion. Therefore, the ability of the chemotherapeutics to penutrate up to the infected organ is one of qualifications for the selection of chemotherapeutics. Penetrability of a drug into healthy tissue, however, might be different from that into infected tissue or the lesion[1]. Moreover, properties of various lesions are also not identical, thus the penetrability of the chemotherapeutics might also be different to an individual lesion.

It is not easy, however, to measure actual concentration of the chemotherapeutics in the environment where bacteria in the lesion are growing. For the respiratory organs, the concentration of the drug in the lung lesion is conventionally estimated from the concentration of the chemotherapeutics in the sputum. An attempt was made, in the present study, to estimate the concentration of isoniazid in the lesion from the attitude of peroxidase reaction of the isoniazid-resistant tubercle bacilli. It has been known that isoniazid-resistant tubercle bacilli become to be negative to the peroxidase reaction. When initial concentration of isoniazid in the growth environment of the tubercle bacilli was very low, however, the bacilli do not easily lose their peroxidase activity, even when the bacilli become resistant to isoniazid[2]. This phenomenon was utilized in this study.

Materials and Methods

Subjects investigated in this study included 26 patients suffering from the pulmonary tuberculosis with positive sputum, consisted of 12 male and 14 female patients, ranging in ages from 23 to 74 years. All of the patients had been received the administration of isoniazid.

Investigations were made, for each of the cases, on the period between onset of pulmonary tuberculosis and the beginning of isoniazid administration, X-ray findings at the beginning of isoniazid administration, the modes and doses of isoniazid administration for a period of 3 months after the beginning of isoniazid administration and the duration of isoniazid administration.

The isoniazid resistance and peroxidase reaction of the tubercle bacilli isolated from sputum of each patient were tested. The concentration of biologically active isoniazid in serum collected 6 hours after the administration of isoniazid at a dose of 4 mg/kg was also determined by OGAWA method[3].

The peroxidase reaction was carried out in the following manner: A mixture of 1 ml of 0.2 M acetate buffer solution (pH 4.0), 1 ml of 2% catechol aqueous solution and 1 ml of 3% hydrogen peroxide solution was poured onto bacterial colonies on a solid culture medium. The coloration of the colonies was examined after 24 hours. The coloration of the bacteria which were resistant to isoniazid of not less than 0.3 μg/ml was evaluated as the positive. When peroxidase-negative colonies presented in a mixture with the positive colonies, it was evaluated as the positive.

Results

Eleven cases out of total 26 cases were evaluated as the negative and remaining 15 cases were judged as the positive, to the peroxidase reaction, the results being given in Table 1 and 2 respectively.

1) Relation between periods from the onset of tuberculosis to the beginning of isoniazid administration and peroxidase reaction : It should be noted that the term "the onset of the tuberculosis" used

Table 1 Cases expectorating tubercle bacilli resistant* to isoniazid showing negative peroxidase reaction

case No.	Age	Sex	Period from onset to the beginning of isoniazid therapy (months)	Roentgenological type of lesion at the beginning of isoniazid therapy	Modes and doses of isoniazid administration	Duration of isoniazid administration (months)	Blood** level of isoniazid (μg/ml)
1	61	M	8	infiltrating-caseous lesion	0.4g daily	27	1.90
2	50	M	0	cavity in infiltrating lesion	0.3g daily	27	0.13
3	37	F	0	unknown	unknown doses twice a week	26	0.10
4	62	F	9	cavity in infiltrating lesion	0.3g daily	29	0.15
5	38	F	0	unknown	unknown	36	0.28
6	25	M	9	unknown	0.4g dally	11	0.10
7	35	M	0	unknown	unknown	58	0.84
8	32	F	3	multiple cavities in non-sclerotic lesion	0.6g daily	2	0.24
9	50	M	15	unknown	unknown	20	0.38
10	33	M	11	unknown	unknown	21	0.27
11	51	M	0	multiple cavities in non-sclerotic lesion	0.4g daily	12	0.54

* Tubercle bacilli resistant to not less than 0.3 μg/ml of isoniazid

** Blood level at 6 hours after oral administration of 4 mg/kg of isoniazid

in this report means the time of the appearance of the subjective symptoms, but the time of the first diagnosis as the tuberculosis was tentatively identified as the onset in cases in which no subjective symptoms were noticed.

The administration of isoniazid was found to be started within less than 12 months in 10 cases out of 11 cases of the peroxidase-negative group. On the other hand, 10 cases out of 15 cases of the positive group were given later than 12 months after the onset.

2) Relation between X-ray findings at the begining of isoniazid administration and peroxidase reaction : X-ray pictures of 5 patients in the peroxidase-negative group and 8 patients in the positive group were able to be obtained. All cases in the peroxidase-negative group showed the non-sclerotic lesion, while 7 cases out of 8 cases in the positive group had the sclerotic lesion (Table 1 and 2 respectively).

3) Relation between modes and doses of isoniazid administration and peroxidase reaction : Seven cases of the peroxidase-negative group and 10 cases of the positive group had records of the modes and doses of isoniazid administration at the beginning of isoniazid treatment. The majority (13 cases out of the 17 cases) had been given 0.3~0.4 g of isoniazid daily. One case of peroxidase-negative group and 2 cases of the positive group had been given the similar dose twice a week. Only one case of the negative group was administered with 0.6g of isoniazid daily. Therefore, there was no marked difference in the modes and doses of isoniazid administration between the two groups.

4) Relation between durations of isoniazid administration and peroxidase reaction : Average duration of isoniazid administration was 24.5 months for the peroxidase-negative group and 30.5 months for the positive group, these being not significantly different.

5) Relation between the concentrations of biologically active isoniazid in serum and peroxidase reaction : Biologically active isoniazid concentrations in serum 6 hours after the administration at

Table 2 Cases expectorating tubercle bacilli resistant to isoniazid showing positive peroxidase reaction

Case No.	Age	Sex	Period from onset to the beginning of isoniazid therapy (months)	Roentgenological type of lesion at the beginning of isoniazid therapy	Modes and doses of isoniazid administration	Duration of isoniazid administration (months)	Blood level of isoniazid (μg/ml)
12	55	M	10	fibrocaseous lesion	0.3g daily	42	0.78
13	60	F	19	cavity in sclerotic lesion	0.3g daily	48	0.25
14	50	F	14	unknown	unknown	34	0.28
15	45	F	26	multiple cavities in sclerotic lesion	unknown	24	0.30
16	23	F	12	multiple cavities in sclerotic lesion	unknown	19	0.94
17	48	M	24	unknown	unknown	57	0.10
18	46	F	17	multiple cavities in sclerotic lesion	0.3g daily	23	0.26
19	47	F	19	fibrocaseous lesion	0.3g twice a week	5	0.27
20	35	F	0	multiple cavities in sclerotic lesion	0.4g twice a week	50	0.14
21	74	F	2	unknown	0.4g daily	36	0.13
22	46	F	48	unknown	0.4g daily	31	0.16
23	30	F	0	unknown	0.4g daily	6	0.16
24	43	M	72	unknown	0.4g daily	9	0.56
25	40	M	12	unknown	unknown	63	1.30
26	37	M	1	multiple cavities in non-sclerotic lesion	0.3g daily	10	0.20

4mg/kg were found not to be significantly different for the two groups, *i.e.*, mean levels being 0.90 μg/ml and 0.93 μg/ml for the peroxidase-negative group and for the positive group, respectively. Values of the median were also about the same, *i.e.*, 0.26 μg/ml for the negative group and 0.27 μg/ml for the positive group. Five cases in which peroxidase reaction was positive in spite of beginning of isoniazid therapy within less than 12 months, showed low concentrations of isoniazid in serum such as 0.13~0.20 μg/ml except for the 2 cases which were shown to be sclerotic type by X-ray diagnosis. Therefore, in these 3 cases the rate of the inactivation of isoniazid in the body was thought to be very rapid.

Discussion

Isoniazid resistant tubercle bacilli tend to lose their peroxidase activity when they become to be resistant to isoniazid higher than 0.1 μg/ml. Some of the bacilli, however, may still exhibit peroxidase activity even if they are resistant to isoniazid at a level of about 10 μg/ml. Extremely low concentration of isoniazid with which isoniazid-sensitive tubercle bacilli first contacted is considered to be attributed to the production of such the bacilli, *i.e.*, the bacilli with high resistance and peroxidase activity.

It was consequently considered that the isolation of tubercle bacilli resistant to isoniazid and positive to peroxidase reaction might indicate very

low initial concentration of isoniazid in the lesion in which such the bacilli had been grown.

On the basis of the above consideration, investigation was attempted on the factor affecting concentration of isoniazid penetrating into the lesion, by utilizing the attitude of peroxidase reaction of isoniazid-resistant tubercle bacilli isolated from the patient. It was thus pointed out that the patients in the peroxidase-negative group had begun to receive isoniazid therapy within iess than 12 months after the onset of the tuberculosis, namely 10 cases out of 11 cases in the group beginning to receive isoniazid therapy within 11 months. On the contrary, 10 cases out of 15 cases of the peroxidase-positive group had begun to receive isoniazid therapy later than 12 months from the onset. Therfore, it is conceivable that the longer is the period from the onset of pulmonary tuberculosis to the beginning of isoniazid administration, the more difficult is the penetration of isoniazid into the lesion.

With the lapse of time after the onset of tuberculosis, the lesion is thought to become fibrous and the wall of the cavity also to be sclerotic. Examinations of X-ray pictures of 5 patients in the peroxidase-negative group, which had been taken at the beginning of isoniazid administration, indicated that all of the cases belonged to the infiltrating-caseous type, and that the cavities had non-sclerotic wall. On the other hand, 7 cases out of 8 cases in the peroxidase-positive group were identified to have the fibrocaseous lesion. Five cases out of the 7 cases had the cavities inside the lesion. It is thus considered that the concentration of isoniazid achieved inside the lesion might be low in the lesion of the fibrocaseous type.

It is known that the tubercle bacilli isolated from the patients, who had the lesion of the fibrocaseous type and have been given kanamycin for a prolonged period, may still be sensitive, in some cases, to kanamycin. This phenomenon has also been explained on the basis that kanamycin may not easily penetrate into the lesion of the fibrocaseous type.

The mode and doses of isoniazid therapy are properly considered to affect the concentration of isoniazid in the lesion. It has been reported that isoniazid-resistant tubercle bacilli isolated from patients to whom large does of isoniazid had been administered from the beginning of the treatment were frequently negative to catalase[4] or peroxidase reaction[5]. This fact might indicate that higher concentration of isoniazid is achieved in the lesion by large dose administration than by small dose administration. It was not possible, however, in this study, to study the influence of the modes and doses of isoniazid therapy to concentration of isoniazid in the lesion, because most of the cases studied has been given 0.3 or 0.4g of isoniazid daily.

As isoniazid is inactivated in the body, the concentration of isoniazid in the lesion might properly be affected by the rate of the inactivation. This possibility is suggested to be the case by the fact that administration of isoniazid to rapid inactivator is less effective than slow inactivator[2),6)], and that the bacilli isolated from the rapid inactivators are not as resistant to isoniazid as those from the slow inactivators[7)].

Concentrations of isoniazid in the blood of patients in the peroxidase-negative group were found not to be significantly different from those of patients of the peroxidase-positive group. Three cases out of 5 cases which were positive to the peroxidase reaction though administration of isoniazid had been started within less than 12 months after the onset, were identified as the rapid inactivators, the remaining 2 cases being found to have fibrocaseous lesions. Therefore, the concentration of isoniazid in the lesion may be inferred to be low in rapid inactivators even if they have relatively fresh lesions.

According to the results and considerations described above, the greatest factor, which may affect the concentration of isoniazid in the lesion, is considered to be the age of the lesion. The rate of the inactivation of isoniazid might also affect, to some extent, the concentration of isoniazid in the lesions.

Summary

From the mode of peroxidase reaction of tubercle bacilli in sputum specimens of the patients with pulmonary tuberculosis having been administered isoniazid, an attempt to presume the penetrability of isoniazid into the lesion was made. In case that the initial concentration of isoniazid in the growth environment of tubercle bacilli is very low, even if the bacilli become resistant to isoniazid, peroxidase reaction of the bacilli hardly becomes negative. This phenomenon utilized in this study.

The number of cases in which bacilli resistant to not less than 0.3 μg/ml of isoniazid were negative to peroxidase reaction was 11, and in 10 cases out of them, the period from the onset of the disease to the beginning of isoniazid administration was less than 12 months. Among them, all of which roentgenologic type of lesion at the beginning of isoniazid administration was known were infiltrating-caseous type, without cavity or having cavity with non-sclerotic wall.

The number of cases in which bacilli resistant to not less than 0.3 μg/ml of isoniazid showed positive peroxidase reaction was 15, and in 10 cases out of them, the period from the onset of the disease to the beginning of isoniazid administration was more than 12 months. And the cases in which roentgenological type of lesion at the beginning of isoniazid administration was known were, except one case, all fibrocaseous type, without cavity or having cavity with sclerotic wall.

In spite of the fact that the period from the onset of the disease to the beginning of isoniazid administration was less than 12 months, in 3 cases out of 5 cases of which peroxidase reaction was positive, the blood level of biologically active isoniazid was low.

Therefore, in case that the modes and doses of administration of isoniazid were kept same, it is thought that the most important factor affecting the penetrability of isoniazid into the lesion lies in whether the lesion is new or old, and the rate of inactivation of isoniazid could also be a factor.

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