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Effect of High Hydrostatic Pressure on Muscle Larvae of *Trichinella spiralis*

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Abstract

The present study examined the effects of treatment with high hydrostatic pressures on the viability and infectivity of *Trichinella spiralis* larvae. Infected muscle, obtained from mice 6 to 28 weeks postinfection, was pressurized at less than 300 MPa at 25°C for 10 min. After artificial digestion, both motility and infectivity of the released muscle larvae (ML) were examined. Over 99% of ML pressurized at 100 and 125 MPa were as active as untreated larvae. The majority of ML pressurized 175 MPa were less active and did not wander. All ML pressurized at higher than 200 MPa were immobile and tightly coiled as those within mouse muscle. Infectivity of the pressurized ML was monitored by the number of adult worms recovered from intestines and ML from carcasses of inoculated mice. After inoculation with 200 ML pressurized at lower than 125 MPa, the number of adult worms recovered after 5 or 7 days, and of ML recovered after 7 weeks, were the same as those recovered from the untreated controls. When the hydrostatic pressure was raised to 150 MPa, only a few adults but no ML were recovered. After pressures of 175 MPa or higher, neither adults nor larvae were recovered. ML pressurized at higher than 200 MPa were neither motile nor infective. Therefore, pressurization of meat at higher than 200 MPa can prevent trichinellosis and can be considered an effective substitute for heating.

Key words: *Trichinella spiralis,* Muscle larvae, Mice, High hydrostatic pressure

Introduction

Trichinellosis is an important foodborne zoonosis traceable to the eating of raw or undercooked meat of swine or wild animals infected with the parasite *Trichinella spiralis.* Infection of man, domestic, and wild animals has been reported from almost every country in the world. In Japan, three outbreaks of human trichinellosis have occurred following the ingestion of bear meat during the years from 1974 to 1981 (Yamaguchi, 1989). Recently, the importation of meat into Japan has been liberalized, so there is apprehension that the incidence of trichinellosis may increase.

Methods for the prevention of trichinellosis include the following: Heating, freezing, irradiation with ultraviolet or gamma rays, salting and drying (Childers, *et al.*, 1982; Kayfus *et al.*, 1982; Terrell, *et al.*, 1982; Kotula *et al.*, 1983; Zimmermann, 1984; Brank *et al.*, 1985; Zimmermann *et al.*, 1985; Takahashi *et al.*, 1986; Uno *et al.*, 1986; Lin, *et al.*, 1990). The U.S. Department of Agriculture (1988) has also described numerous methods for killing *Trichinella* larvae in pork and pork products. All have merits and drawbacks. Treatment of meat by boiling or intense heating has been the most widely used method for protecting against *Trichinella* infection to date.

High hydrostatic pressure recently has been applied as a method for food processing and preservation in Japan (Hayashi, 1989). Unlike heat, Pascal's law indicates that hydrostatic pressure is transmitted instantaneously and evenly

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throughout a medium. Moreover, pressurization can sterilize and preserve food products without loss of their flavor and/or taste. The present study was carried out to examine the effects of high hydrostatic pressures on the motility and infectivity of larval worms recovered from the muscles of mice experimentally infected with *T. spiralis*.

Materials and Methods

The Polish strain of *Trichinella spiralis* was used throughout the present study. This strain has been maintained by serial passage in mice in our laboratory. Male, six-week-old mice of the BALB/c strain bred under SPF conditions, were purchased from Japan SLC, Inc., Hamamatsu, Japan. The mice were infected orally with 200 muscle larvae (ML) of *T. spiralis* and maintained under conventional conditions throughout the experiments. At 6 to 28 weeks postinfection, ground muscles were prepared from the carcasses of infected mice, placed in polyethylene bags, sealed in a vacuum, and pressurized at 100 to 300 MPa at 25°C for 10 min in a hydrostatic pressure vessel (Hikari Koatsu Co., Hiroshima, Japan) as previously described by Shigehisa *et al.* (1991). As controls, similar preparations were left untreated. The experiments were repeated 5 times with larvae of different age, because the encapsulated *Trichinella* larvae may possess different degrees of resistance according to the stage of larval development. In addition, ground muscle was inserted into the center of pork meat blocks 5 cm thick to determine whether hydrostatic pressure could penetrate to the center and be effective even with the short treatment of 10 min.

Motility and infectivity of the larvae recovered from the pressurized muscle preparations were examined as follows: After pressurization, ML were recovered by mildly digesting the muscle in 0.5% pepsin – 0.7% HCl solution at 37°C for 1 hr. The motility of recovered larvae was immediately observed with a light microscope since living ML are active at 35 to 40°C (Takada *et al.*, 1979). Motility of ML was represented as the percentage of motile larvae per 200 ML examined. Next, infectivity of the pressurized ML was determined. A total of 54 BALB/c mice was used in each experiment. The mice were divided into 9 groups of 6 mice each. All mice were inoculated orally with 200 pressurized ML. At 5 or 7 days after inoculation, 3 mice from each group were killed by an overdose of ether inhalation and examined for the presence of the adult worms in the digestive tract. The small intestine was removed, opened longitudinally, incubated in physiological saline at 37°C for 5 hr, then rinsed several times with saline solution. The adult worms in the washing solution were counted with a stereo-microscope and the mean value was calculated from the data of the 3 mice examined. The remaining 3 mice in each group were killed 7 weeks after inoculation to examine for the presence or absence of larvae in muscle. After removing the skin and internal organs from the mice, the carcasses were treated with artificial digestion at 37°C for 5 hr. The number of larvae were counted by the dilution method using 10% magnesium sulfate solution (Takada *et al.*, 1978) and the mean values for each group were calculated from the data of the 3 mice.

Results

The experiments in the present study were replicated 5 times for examining the motility and infectivity of pressurized ML. As shown in Tables 1–3, similar results were obtained in spite of inserting some specimens into blocks of pork meat and differences in the number of days after infection.

As to motility, over 99% of the larvae recovered from muscles pressurized at lower than 125 MPa were as active as those from untreated muscles (Table 1). ML pressurized at 150 MPa were still moving and wandering actively. After pressurization at 175 MPa, the majority of ML were less active and did not wander. All ML pressurized at higher than 200 MPa were immobile and tightly coiled as ML within muscle. In the ML pressurized at 300 MPa, body coloration was dull and some degenerations of the internal organs were observed with a light microscope.

Table 1 Motility of muscle larvae (ML) of *T. spiralis* recovered from pressurized muscles*

Pressure (MPa)	Exp.1 6W†	Exp.2 8W	Exp.3 10W	Exp.4 14W	Exp.5 28W	Total Mean ± SE
Untreated	100	100	100	99.6	100	99.9 ± 0.1
100	98.7	100	99.5	100	100	99.6 ± 0.3
125	100	100	99.5	ND‡	99.6	99.8 ± 0.1
150	98.8	100	99.1	99.6	99.1	99.3 ± 0.2
175	98.7	74.2	93.0	ND	98.3	91.1 ± 5.8
200	0	0	0	0	0	0
225	ND	ND	ND	0	0	0
250	0	0	0	0	0	0
300	0	0	ND	0	0	0

* Muscles were obtained from carcasses of BALB/c mice from 6 to 28 weeks. Tissues were ground, placed in polystyrene bags, vacuum-sealed, and pressurized for 10 min at 25°C. In Exps. 1 and 5, the specimens were inserted into pork meat blocks 5 cm thick. After pressurization, the ML were freed from tissues by mild digestion. Motility was recorded as the percentage of active larvae per 200 ML.
† Age, in weeks, of *T. spiralis* larvae used in each experiment.
‡ ND, not done.

Table 2 The number of adult worms recovered from intestines of mice inoculated with pressurized muscle larvae (ML)*

Pressure (MPa)	Exp.1 6W†	Exp.2 8W	Exp.3 10W	Exp.4 14W	Exp.5 28W	Total Mean ± SE
Untreated	80.0	101.7	97.0	69.3	151.0	99.8 ± 14.1
100	122.5	120.7	110.0	91.0	127.3	114.3 ± 6.5
125	97.7	127.4	66.0	ND‡	133.7	106.2 ± 15.5
150	0.3	0	0	0.3	0	0.1 ± 0.1
175	0	0	0	ND	0	0
200	0	0	0	0	0	0
225	ND	ND	ND	0	0	0
250	0	0	0	0	0	0
300	0	0	ND	0	0	0

* All data represent numbers of adult worms recovered 5 days after inoculation of 3 BALB/c mice with pressurized ML in Exps. 1, 2 and 5, and 7 days after inoculation in Exps. 3 and 4.
†,‡ See legend to Table 1.

The results of the experimental infection of mice with pressurized larvae are shown in Tables 2 and 3. In mice inoculated with 200 ML pressurized at lower than 125 MPa, the number of adult worms recovered from the small intestine ranged from 66.0–133.7. This number is almost the same as that from the untreated group. The ratio of adult females to males was 1.36–2.73, while that of the untreated groups was 1.63–2.85. The number of ML recovered was $3.22–4.5 \times 10^4$ in mice inoculated with ML pressurized at lower than 125 MPa. This result is in close agreement with that of the untreated group. In mice inoculated with ML pressurized at 150 MPa, a few adult worms were recovered from the intestine, but no larval worms from the muscles. At pressures higher than 175 MPa, neither adult worms nor ML were recovered from the experimental mice.

Table 3 The number of muscle larvae (ML) recovered from mice inoculated with the pressurized ML*

Pressure (MPa)	Exp.1 6W†	Exp.2 8W	Exp.3 10W	Exp.4 14W	Exp.5 28W	Total Mean ± SE
Untreated	3.19	3.65	4.25	3.37	3.89	3.67 ± 0.19
100	3.35	3.38	3.22	3.57	4.50	3.60 ± 0.23
125	3.48	3.92	3.27	ND‡	4.09	3.69 ± 0.19
150	0	0	0	0	0	0
175	0	0	0	ND	0	0
200	0	0	0	0	0	0
225	ND	ND	ND	0	0	0
250	0	0	0	0	0	0
300	0	0	ND	0	0	0

* All data represent numbers of ML recovered (means × 10^4) 7 weeks after inoculation of 3 BALB/c mice with 200 pressurized ML.
†,‡ See legend to Table 1.

Discussion

Since 1895, it has been observed that many kinds of microorganisms are inactivated by treatment with high pressure (Hayashi, 1989). Shigehisa *et al.* (1991) reported that pressures of 300 to 600 MPa killed 10 species of pathogenic microorganisms inoculated into pork slurries. In the present study, the effects of high hydrostatic pressures on mouse muscles infected with *T. spiralis* were examined. ML pressurized at lower than 125 MPa retained activity and were as infective to BALB/c mice as untreated larvae. Therefore, pressurization at lower than 125 MPa has no apparent effect on ML. Although ML pressurized at 150 MPa were still active, their infectivity to mice was destroyed. The reason for the different effects on activity and infectivity remains unknown. Pressurization at higher than 200 MPa in the present study was lethal to the *Trichinella* ML. The mechanism whereby pressurization kills *Trichinella* ML was not determined in the present study. However, there is a possibility that some substance essential for infectivity might be denatured by pressurization at higher than 200 MPa, because ML pressurized at higher than 200 MPa were immobile and coiled like those within capsules. Only a slight coagulation and discoloration of pork slurries occurred after pressure treatment of 200 MPa (Ohmori *et al.*, 1990; Tujita and Suzuki, 1990; Shigehisa *et al.*, 1991). Ohmori *et al.* (1991) also found that high hydrostatic pressure of 200 MPa destroyed lysosomal membranes and resulted in the leakage of endogenous proteases into the cytosol.

Heating above 49°C was effective for killing *Trichinella* ML in pork meat (Kotula *et al.*, 1983; USDA, 1988). ML were killed after 2 min at 60°C, whereas the lower the heat, the longer were the survival periods of ML. The reason for this might be the difficulty in transmitting heat to larvae surrounded by thick host muscles and calcified capsules. On the contrary, pressure is transmitted instantaneously and evenly throughout a medium, as defined by Pascal's law. This latter is supported by the fact that comparable results were obtained in the two experiments in which test specimens were inserted into the center of blocks of pork meat. This study also demonstrated that, unlike heat, pressure treatment at higher than 200 MPa at room temperature was able to kill *Trichinella* ML within the relatively short time of 10 min. Meat without heat treatment, as "Raw Ham", is considered to have a better taste than cooked meat.

In conclusion, pressurization at higher than 200 MPa effectively kills *Trichinella* larvae, and can be a useful substitution for heating.

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