Therapeutic Trials on Progressive Muscular Dystrophy

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The special medical care in the National Sanatorium prolonged the life span of the patients with progressive muscular dystrophy from 15.8 years to 20.4 years over the last 20 years. Various new drug trials for muscular dystrophy have been implemented in the last 12 years in Japan. Bestatin and Loxistatin, protease inhibitors, showed definite improvement on dystrophic mice or hamsters, animal models of muscular dystrophy. However clinical application of these drugs failed to prove the effects on patients with Duchenne muscular dystrophy. The difficulty of clinical evaluation and judgement of effects in progressive neurological diseases is discussed. (Internal Medicine 31: 841–846, 1992)

Key words: protease inhibitor, animal model, life span of muscular distrophy, clinical effect

In the last several years there has been dramatic progress in the determination of the pathogenesis of the Duchenne muscular dystrophy (1–4). However no effective treatment has been found to date. Here, the therapeutic trials on progressive muscular dystrophy which were undertaken in Japan are reviewed and the results of the new drug trials within the last 20 years are discussed.

Epidemiological studies have indicated that the prevalence rate of Duchenne muscular dystrophy in Japan is 1.7 to 2.6 per 100,000 population (5). This incidence is almost the same as that of other countries. Accordingly, approximately 3,000 patients of Duchenne muscular dystrophy would be expected in Japan. Since 1968 the government of Japan started to provide special beds for the care of Duchenne muscular dystrophy patients (DMD patients) in 26 national sanatoriums located all throughout Japan. Twenty-four hundred free beds for DMD patients were prepared. Each sanatorium has 40, 80 or 120 beds with a special nursing program with PT, OT or other speciality staff. Primary schools, junior and senior high schools for crippled children were also set up annexed to the hospital. The cost of this special nursing care is very expensive; it is calculated to be 14,000,000 yen or 100,000 dollars per patient per year. Accordingly, 240 million dollars is budgeted by the government for the DMD patient care every year. In addition, 1.5 million dollars has been allocated for research grants for muscular dystrophy and related disorders by the Ministry of Health and Welfare and it is distributed through the National Center of Neurology and Psychiatry.

With these special care steps for DMD patients in the national sanatorium, the life span of DMD patients has gradually become prolonged. From 1966 to 1970, the number of DMD patients who died at a national sanatorium was 33 and the mean of the age of death was 15.8 years. In the following 4 years (1971 to 1974), 92 patients died and the mean life span was 18.7 years. In the subsequent 4 years (1975–1979) the mean life span was prolonged to 19.4 years and in the next 4 years (1980–1983), it was prolonged to 19.9 years (Table 1) (6). In one of these sanatoriums, Suzuka Hospital, the life span of the DMD inpatients was compared with that of DMD patients outpatients who lived at home. From 1978 to 1988, the mean life span of 33 inpatients was 20.4 ± 3.6 years, whereas of 19 DMD outpatients it was 18.3 ± 3.8 years (7). Namely with the special care provided at the national sanatorium the life span could be prolonged at least 2 years or more than that of the patients treated at home. Recently respirators and other types of instruments can be applied for the terminal care and with these instruments, the patients can be expected to live longer in the near future.

In 1978 the Ministry of Health and Welfare proposed a new research grant to ellucidate new drugs for the incurable diseases for which no proper drug or treatment has been effective. DMD was selected in this category and very active research groups were organized. The pathogenesis of the progressive muscular dystrophy (PMD), at that time, was considered the following. The primary site of the lesion in PMD at the cellular level is in the plasma membrane which was proved later.

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Table 1. Life Span of the Patients with Duchenne Muscular Dystrophy in the National Sanatorium

Period	Number of Cases	Age of Death
1966–1970	33	15.8 ± 2.47
1971–1974	92	18.7 ± 3.78
1975–1979	126	19.4 ± 3.49
1980–1983	134	19.9 ± 3.75

In muscles of PMD, accumulation of calcium ion and Z-band loss were reported (8, 9). Accumulation of Ca ion brings about the activation of calcium-activated neutral proteinase (CANP) which results in the fragmentation of myofibrils at an early stage of the muscle fiber degeneration. The lysosomal cathepsin also promotes muscle protein degradation (Fig. 1).

Therefore we considered that some of the proteinase inhibitors might delay the degradation of protein or the progress of the dystrophic muscle degeneration. For this purpose several proteinase inhibitors such as Leupeptin, Amastatin, Esterastin, Forphenicine were considered candidates. For chronic progressive diseases oral administration for long-term care is required. Good absorption by the orally administered drug and no side effects during the long-term therapy are desirable. From these points two drugs were selected as good candidates: Bestatin and Loxistatin (Fig. 2).

Bestatin, extracted from Streptomyces olivorecti, inhibits aminopeptidase and leucine peptidase and acts on the cell membrane surface (10). Bestatin binds tightly to the cell membrane of lymphocytes and macrophages. Because of this action, Bestatin is used as an immunomodulator durg (11). Thus, it was speculated that Bestatin might work on the cell surface and might prevent the destruction of cell membrane. Loxistatin (12, 13), extracted from Aspergilles japonicus, is a cystein poteinase inhibitor, CANP inhibitor and cathepsin inhibitor, which may work in the cell to prevent the destruction of protein in the cells.

From 1978 to 1982 extensive pharmacodynamic studies as well as animal experiments were undertaken by members of two research groups and two drug companies. Bestatin research was supported by Nihon Kayaku Company and Loxistatin was supported by the research group of Taisho Drug Company. In the animal experiments using an animal model of hereditary muscular dystrophy, both drugs showed remarkable effects. Most of these results have already been published and are summarized below.

Tsuji and Matsushita (14) found that administration of 0.4 mg per day of Bestatin prolonged the life span of dystrophic mice (dy/dy) of C 57 BL/6J-dy strain (obtained from Jackson Laboratory). Non-treated mice died 129 or 130 days after birth, whereas Bestatin-treated mice lived for 187 or 244 days, when the treatment was started 2 weeks after birth. This difference is statistically

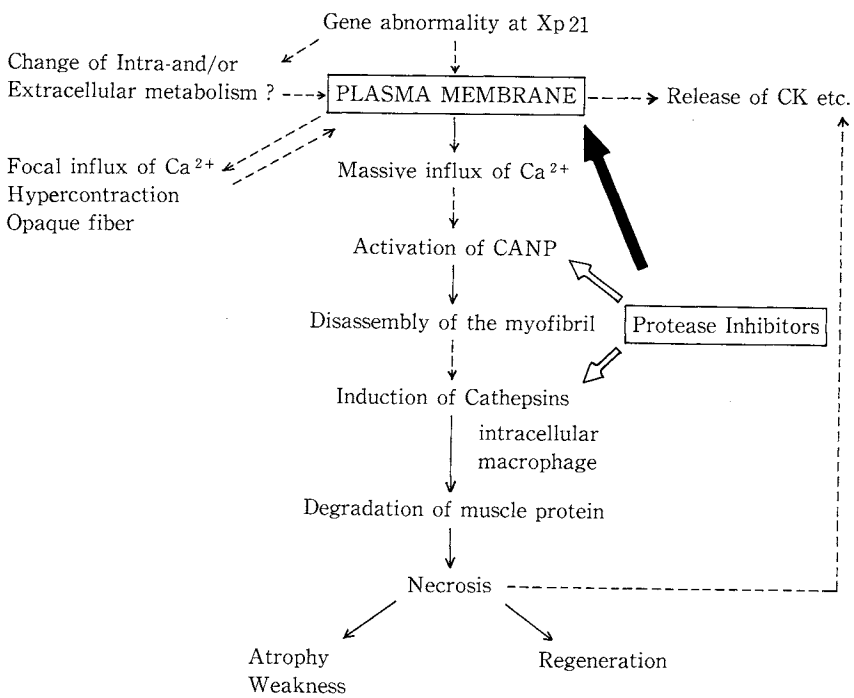
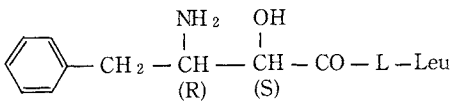


Fig. 1. Hypothesis of the process of muscle degeneration in Duchenne muscular dystrophy and the role of protease inhibitor. Large black arrow indicates the site of action of Bestatin and white arrows indicate the sites of action of Loxistatin. CANP: calcium-activated neutral proteinase, CK: creatine kinase.

BESTATIN



LOXISTATIN

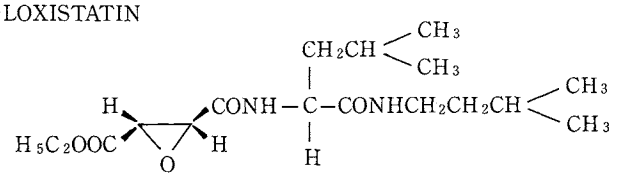


Fig. 2. Chemical structure of Bestatin and Loxistatin.

significant. Body weight of dystrophic mice also increased at 1 month and 2 months in Bestatin-treated mice by 24% and 65%, respectively as compared with those of untreated mice. Histological changes in the muscle became milder with Bestatin and the ratio of urinary creatine to creatinine apparently decreased with Bestatin administration. Serum enzyme activities such as creatine kinase, LDH, GOT or GPT decreased by about 30 percent. A metal cage with a hot plate at 60°C was employed for the endurance test. Bestatin-treated mice showed almost normal recovery of endurance compared with the very poor response of the untreated mice.

Kishi et al (15), using the x-linked mdx muscular dystrophy mice, proved that Bestatin decreases or abolishes myotonic insertion repetitive action potentials. When mdx mice were treated with Bestatin for 7 days, the resting membrane potentials in hemidiaphragm preparation of mdx mice stabilized and improved to the normal value. This data also indicated that Bestatin works on the muscle cell membrane directly to stabilize the membrane irritabilities.

Animal experiments with Loxistatin were reported by Tamai et al (12) in 1986, using the dystrophic hamster, UM−X7, 1. They proved prolongation of the life span of dystrophic hamster with 30 or 300 mg/kg/day of Loxistatin from 214.9 days to 247.8 days or 256.8 days, respectively. Loxistatin also decreased plasma enzyme activities of CK, LDH, GOT or GPT, necrotic changes of skeletal and cardiac muscles and significantly decreased cathepsin B and L activities.

Based on these animal experiments, clinical trials with Loxistatin started in 1982. The fate of the drugs, absorption and proper dosage of drugs to adults, children and infants, and the side effects were carefully studied. Then open trials were undertaken from 1982 to 1985 in various muscular dystrophies such as Duchenne muscular dystrophy, limb-girdle muscular dystrophy, myotonic muscular dystrophy and congenital muscular dystrophy and others. The effects of drugs were evaluated by clinical effects, change of serum enzyme activities, ADL, muscle strength, creatine/creatinine ratio or urinary excretion of 3-methyl-histidine. There were many variable reports of positive or negative results; there were no definite conclusive results.

In the animal experiments, the effects of the drugs were not apparent when the drugs were administered after the clinical sign of muscular dystrophy became evident. This may apply to patients as well. In advanced progressive muscular dystrophy the drugs do not stop or improve the progression of the disease. In animal experiments, when Bestatin was given within 2 weeks of birth, a clinical effect was apparent. Therefore, we decided to use these drugs in the early stage of the muscular dystrophy.

In 1985 a single-blind test of Bestatin on patients with Duchenne muscular dystrophy younger than 7 years of age was undertaken. For this study, 127 cases of DMD were selected from 15 national sanatoriums and a few university hospitals. Of these 52 patients were less than 4 years old and 75 were from 5 to 7 years old. Regarding the younger patients, 150 mg of Bestatin was given daily in three divided dosages. Daily doses of 300 mg of Bestatin was given to the older patients for 52 weeks. As a placebo, lactose was similarly administered. Bestatin was given to 60 patients and placebo to 67 patients. Various clinical observations such as exercise abilities, running time, time required for standing from a sitting position, test for ascending or descending steps, muscle power strength, deep tendon reflexes, ADL, serum enzyme activities, routine blood and urinary studies were evaluated every 4 weeks for 52 weeks.

The clinical effects were evaluated in two separate groups: those under 4 years old and those from 5 to 7 years (Table 2). In global judgement, improvement with Bestatin was proven in 43% of those in the younger childrens group; with placebo improvement was seen in 20%. On the contrary, in the 5 to 7 years old, the clinical improvement was the same as that of the placebo group. This study indicated that double-blind studies should be performed in patients who are 4 years old or younger.

From September 1988 to October 1989, double-blind studies with Bestatin were undertaken enrolling 115 cases of DMD from 15 national sanatoriums and 16 university hospitals. There were 55 cases below the age of 3 and 60 patients who were 4 or 5 years old. Trials were done using patients who are able to walk alone. Daily dose of 150 mg of Bestatin was given orally for 52 weeks. After completion of the trial, 14 patients were excluded because of age, inability to walk or inappropriate drug usage; in total 101 patients were evaluated.

The difference between the placebo group and active drug group was not significant. The ten meter running time and step ascending time showed some difference. However, a statistically significant difference was only obtained in the younger children group (3 years); the effect of Bestatin was proved in step ascending time and serum CPK and GOT. The other factors did not prove

Table 2. Global Judgement of Clinical Effect of Bestatin Single Blind Test (117 cases)

Item	Drug	No. of Cases					Improved (%)	χ^2-Test
		Improved	Unchanged	Slightly Agravated	Agravated	Total		
~4 year-old	Bestatin	10	10	1	2	23	43%	$p<0.1$
	Placebo	5	18	1	1	25	20%	
5~7 year-old	Bestatin	3	8	11	11	33	9%	N.S.
	Placebo	5	7	12	12	36	14%	

Table 3. Global Judgement of Clinical Effect

Item	Drug	No. of Cases						Slightly Improved or more (%)	χ^2-Test
		Improved	Slightly Improved	Unchanged	Slightly Agravated	Agravated	Total		
~3 year-old	Bestatin	1	13	8	1	0	23	61%	N.S.
	Placebo	0	11	12	2	0	20	44%	
4 or 5 year-old	Bestatin	1	7	13	3	2	26	31%	N.S.
	Placebo	0	10	13	3	1	27	37%	

any significant differences (Fig. 3, 4).

In the global judgement of the clinical effect, the younger Bestatin treatment group showed a higher ratio of improved or slightly improved cases (61%) compared to the placebo group (44%) (Table 3). On the contrary in the older (4–5 year olds) patients, there was no difference between the Bestatin group and placebo group. Statistical study showed no significant difference between the Bestatin-treated group and placebo group in both age groups.

Concerning Loxistatin, open trials were done including 73 cases from 1984 to 1987 in 4 national sanatoriums; 172 DMD patients admitted to these sanatoriums for 1 to 11 years were employed as a control. In the clinical trials, on improvement of muscle strength and a decrease of enzyme activities or urinary excretion of 3-methylhistidine were observed. The results were not conclusive and final double-blind studies were undertaken from September 1989 to October 1990. However, the clinical effects could not be confirmed.

Discussion

Routine medical special care in the sanatoriums actually proved that careful care can prolong the life span at least 2 years or more at present. Concerning drug treatment with proteinase inhibitors, the clinical effects have not yet been confirmed. In the animal experiments using a dystrophic animal model, both drugs were very effective. However in DMD patients the effect was not striking. The resaon may be related to several factors, dosage of drugs, time of treatment or simply the methods of clinical evaluation.

In the animal experiment, the effect of the drugs was clear when it was used in the early stage before the clinical sign of dystrophy became apparent. In dystrophic mice, when Bestatin was used within 2 weeks of birth, an effect was evident; when it was used after a month or longer, no clinical effects were observed. This fact may apply to humans also. In one family of DMD patients, Bestatin was applied before the patient was 1 year old. The brother of this patient was definite DMD. After 5 years of treatment, his mother noticed that the treated sibling followed a very benign course compared to his brother. This evidence suggested that the time of treatment may be related to the effect. When clinical symptoms become advanced, obviously it would be very difficult to change the genetic factor which was already manifested in the body.

The method of clinical evaluation and term of drug treatment are also important factors. In infants or very young children, the clinical symptoms overlap in the natural course of development of limbs. After 7 to 8 years, the natural course of the dystrophic changes became prevalent. After this stage, evaluation of drug effect may be very difficult because of the definite progressive course of the disease.

In this report, the clinical effect of two proteinase

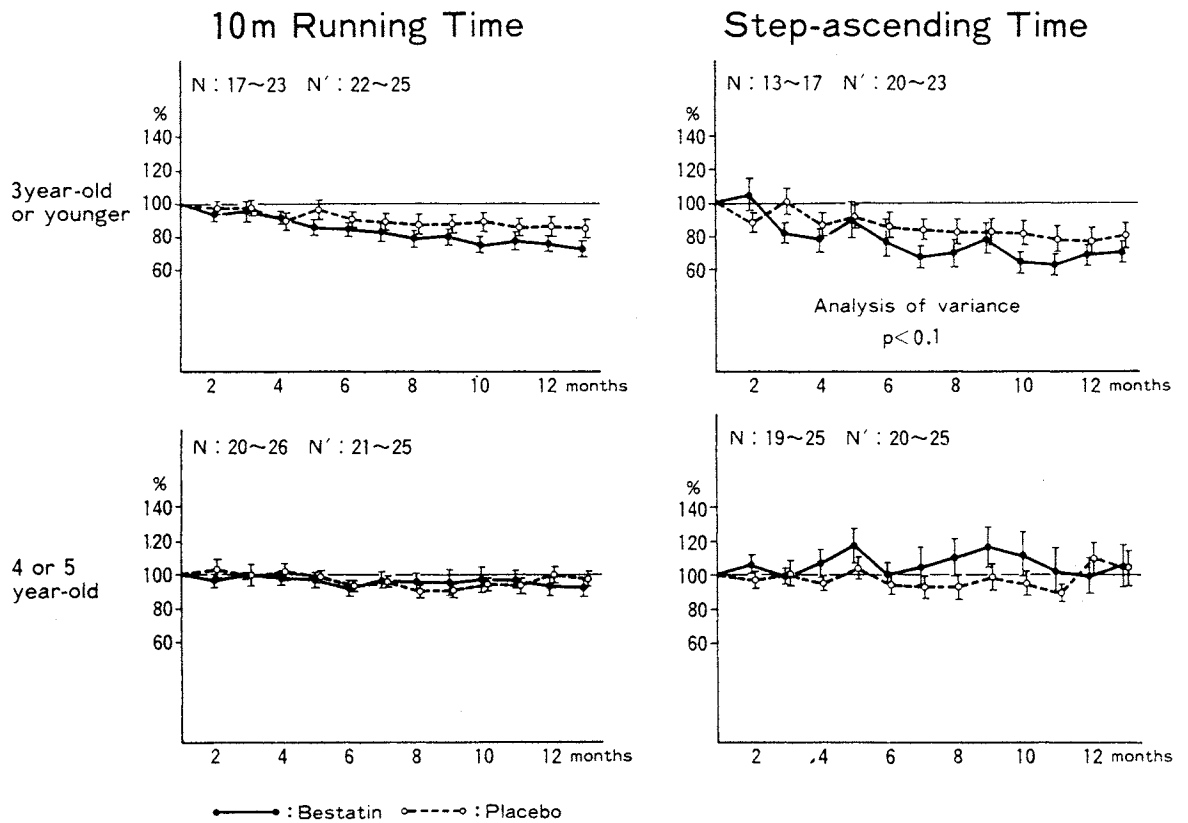


Fig. 3. Change in 10 meter running time and step ascending test. The data before the trial was considered as 100% and the changes were calculated as percent change. The upper two figures indicate the results of the patients with Duchenne muscular dystrophy under 3 years old and the lower two figures, those of 4 or 5 year-old patients with Duchenne muscular dystrophy.

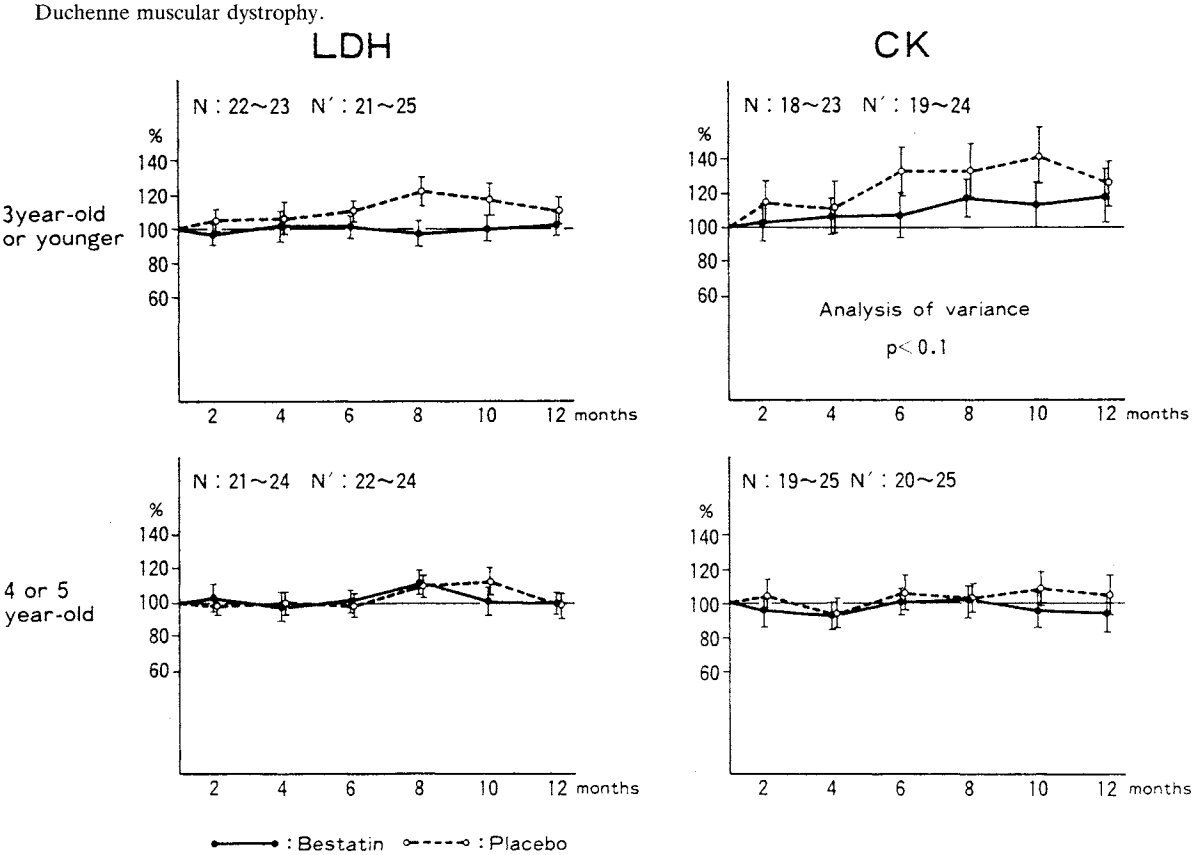


Fig. 4. Change in serum lactic dehydrogenase and creatine kinase in patients with Duchenne muscular dystrophy before and after treatment.

inhibitor drugs on progressive muscular dystrophy could not be proven. However, these trials may show the difference of the effects between animals and human beings and various factors which may be related to the clinical effects as well.

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