Original

A STUDY OF MUTATIONAL PHASE OF *STREPTOCOCCUS*
MUTANS: CARIES-REDUCING EFFECT OF MUTATIONAL
PHASE III OF *STR. MUTANS* IN ANIMALS

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Abstract: Mutational phase III strains reduced caries in experimental animals. The proliferation in phase I was extremely inhibited in the presence of phase III in the oral cavity of hamsters, resulting in suppressed caries induction by phase I. In rats, the phase III strain induced from *Str. mutans* isolated from a rat could inhibit the growth of *Str. mutans* indigeneous in rats, and showed a caries-reducing effect, but the phase III strain induced from *Str. mutans* isolated from a human failed to exhibit the caries-reducing effect. There seemed to be a considerable degree of specificity between *Str. mutans* and the host.

Introduction

Mutants of *Str. mutans* were isolated with a mutagenic agents, and they were designated phase I, phase II, and phase III according to their properties¹⁾. It was demonstrated that phase III strains lost their cariogenicity and suppressed the growth and plaque formation of phase I strains¹⁾.

In the study, the inhibition of dental caries in animals using phase III strains was investigated.

Materials and Methods

Two types of experimental models, hamsters and rats, were used.

1) Hamsters and experimental design.

Two kinds of experiments were employed as shown in Fig. 1. A battery of test organisms of *Str. mutans* 10449 phase I and phase III were used. For the duration of the experiment, hamsters were fed diet 2000 of Keyes and Jordan²⁾. Diet and distilled water were provided *ad libitum*.

Exp.-1. In order to depress the indigenous bacterial flora, streptomycin sulfate (200 μ g/ml) was administered prior to inoculation of the test organisms during two days. At weaning, 21 days of age, the hamsters were divided into two groups, experimental and control, and each hamster of both groups was inoculated in its pouch with 0.2ml of 24 hour culture of the phase I strain in TYC³⁾ broth once a day for five days.

The establishment of the inoculated phase I strain was examined on the day after the last inoculation and on the day the animals were killed. Oral swab samples from all hamsters of both groups were put into 10% glycerol-3% peptone solutions. Each sample was smeared on TYC agar

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Fig. 1 Experimental designs employed in hamsters

plates and the colonies grown on them were observed.

The phase III strain of 24 hour culture in TYC broth was inoculated into the hamsters of the experimental group and saline into those of control group for five consecutive days from two days after the last inoculation of the phase I strain.

Exp. 2. Two females, each with a litter of eight pups, were derived from our own breeding colony. Half of the pups of each female were at 8 days of age when molars began to erupt. Pups of the experimental group were consecutively infected with 0.03 ml of the phase III strain, and pups of the control group with 0.03 ml of saline for 14 days till weaning. The establishment of the phase III strain in the experimental group was examined as described above in Exp.-1. The phase I strain was inoculated for another five days into hamsters of both experimental and control groups (Fig. 1). The establishment of the phase I strain was examined in both groups on the day the animals were killed.

In both Exp. 1 and 2, the period of experiment was 60 days after weaning. At the end of the experiments, the hamsters were killed, and the mandibles were removed. After the soft tissue was removed from them by autoclaving for 2 minutes at 121°C and they were washed and air-dried, carious lesions were scored on the mandibular molars of the jaws by method of Keyes⁴.

2) Rats and experimental design.

Twenty-one day wistar rats, derived from our own breeding colony, were used in this experiment. They harbor R-group indigenous *Str. mutans* in their mouths at weaning. They were divided into three groups, two experimental groups and one control group. The rats in the experimental groups were inoculated with *Str. mutans* 10449 phase III or *Str. mutans* RC-20 phase III. The

inoculations were repeated once a day for 8 days⁵¹. The control group received no treatment. For inoculation, the organisms were cultured in TYC broth (300 ml) for 24 hours at 37°C, centrifuged, and then washed and suspended in 10 ml of sterilized saline. 0.2 ml of the cell suspension were directly applied into the rat's mouth with sterilized cotton rolls. During the experiment, rats were fed cariogenic diet 6 PMV⁶⁰. Diet and distilled water were provided ad libitum. The experimental period was 30 days after weaning (at 21 days old), and at the end of the experiment, the rats were killed and the jaws were removed. Individual jaws were stained with 0.5 % basic fuchsin solution⁷², sectioned with a thin-sectioning machine* into about 200 μ thickness, and the fissures of the molars were scored for early-lesion caries⁸⁹ with a dissection microscope. At weaning on the 10th day and at the end of the experiment, oral swabbings were taken from all rats for bacteriological survey. The number of colonies of total streptococci on Mitis Salivarius agar** and wild *Str. mutans* on TYC agar were determined. On the 10th and the end of experiment, the establishment of inoculated phase III strain was investigated.

Results

The caries-reducing-effect of phase III strains of *Str. mutans* was investigated in hamsters and rats.

1) Hamsters and (Table 1)

In Exp. 1, all hamsters were infected with *Str. mutans* 10449 phase I for 5 days after weaning. All hamsters had this strain in their mouths at weaning. In the experimental group, hamsters were received following inoculation with *Str. mutans* 10449 phase III. When killed, although all hamsters of the control group harbored phase I strains, none of the experimental group harbored them in their mouths. The caries scores of the experimental group were much lower than those of the control group, as seen in Table 1.

Table 1 Caries scores on the mandibular molar surfaces of hamsters infected with *Str. mutans* 10449 phase I and phase III

Group	No. of animals	Weight gain (g)	Caries scores according to Keyes			
			Total	1st molar	2nd molar	3rd molar
Experimental	8	93.4	71.67	3.68	16.54	51.45
Control	8	62.1	247.51	76.23	78.13	93.15

Exp. 1. *Str. mutans* 10449 phase III was inoculated after the establishment of *Str. mutans* 10449 phase I.

Group	No. of animals	Weight gain (g)	Caries scores according to Keyes			
			Total	1st molar	2nd molar	3rd molar
Experimental	8	63.5	50.72	3.92	9.90	36.90
Control	8	62.8	177.34	34.20	59.11	84.03

Exp. 2. *Str. mutans* 10449 phase III was inoculated before the inoculation of *Str. mutans* 10449 phase I.

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In Exp. 2, the phase III strain was confirmed in the mouth of all hamsters at weaning. After weaning, phase I strain was given for the next 5 days to both groups. The first procedure in the experimental group, inoculation of the phase III strain, suppressed the phase I strain. That is, the phase I strain was not found at all in the mouths of hamsters of the experimental group when killed, although all hamster in the control group had the phase I strain. The caries scores were also much lower in the experimental group than in the control group, in which the phase I strain remained till the end of the experiment (Table 1).

Table 2 Comparison of caries-reducing effect of *Str. mutans* 10449 phase III and *Str. mutans* RC-20 phase III in rats

Group	No. of rats	Inoculated organism	Weight gain (g)	Prevalence (%)	Caries scores (No. of carious fissures per head)
1	20	10449 phase III	30.1	95	5.05
2	19	RC-20 phase III	30.0	79	2.75
3	20	Control	33.7	100	4.80

Table 3 Comparison of the number of wild *Str. mutans* and total streptococci in the mouths of rats

	Group 1 Colony count			Group 2 Colony count			Group 3 Colony count		
Experimental Day	wild* <i>S. mutans</i>	total* Strept	wild <i>S. mutans</i> total Strept	wild <i>S. mutans</i>	total Strept	wild <i>S. mutans</i> total Strept	wild <i>S. mutans</i>	total Strept	wild <i>S. mutans</i> total Strept
At weaning	4.80	7.03	0.01	4.52	6.72	0.01	4.63	6.46	0.01
10	5.62	6.61	0.10	4.74	6.67	0.01	5.41	6.85	0.04
At sacrifice	6.39	7.07	0.21	6.22	6.99	0.17	6.04	6.76	0.19

* Colony counts of wild *Str. mutans* and total streptococci are given in logarithm.

2) Rats (Table 2).

As shown in Table 2, the score of caries of the second group rats infected with the phase III mutant of R-group *Str. mutans* RC-20 was significantly smaller ($p < 0.01$) than the scores of the third group and the control rats. But the caries score of the first group rats infected with the phase III mutant of Human-group *Str. mutans* 10449 was the same of that of the control rats.

The proliferation of the indigenous *Str. mutans* was less in the second group rats than in the first group and control rats two days after the last inoculation of test organisms, on the 10th day of the experiment, as shown in Table 3. But on the last day of the experiment, recoverable number of indigenous *Str. mutans* were almost the same among the three groups. No distinct

Table 4 Percentage of rats in which phase III strains were recoverable

Group	At weaning	10th Day	At sacrifice
1	—	40.4 (8/20)	20.0 (4/20)
2	—	63.2 (12/19)	21.1 (4/19)

differences were observed between the establishment of *Str. mutans* RC-20 phase III and 10449 phase III. On the 10th day of experiment, RC-20 phase III strain seemed to adapt better than 10449 phase III strain in the mouth of rats, but on the last day of the experi-

ment, the establishment rates of both phase III strains were decreased to about 20 per cent (Table 4).

Discussion

This study suggested that caries prevention might be possible ecologically using the mutational phase III of *Str. mutans*, because in both hamsters and rats, the mutational phase III greatly decreased the caries score.

Although hamsters infected with Human-group *Str. mutans* 10449 phase III were protected from caries lesions, rats were protected only when they were infected with RC-20 phase III, but 10449 phase III did not exhibit any caries-reducing effect at all. This suggests that there is a considerable degree of specificity between *Str. mutans* and the host.

Str. mutans appeared in the mouth of rats at 19 or 20 days of age. Sato⁹⁾ demonstrated that *Str. mutans* strains derived from rats were clearly different from strains derived from humans serologically, and that *Str. mutans* strains were divided into two main groups, Human-group and Rat-group, on the basis of their origin. Moreover, Sato et al.¹⁰⁾ reported that the Rat-group strain, *Str. mutans* RC-20 phase I, showed better affinity and produced more caries in rats than the Human-group strain, *Str. mutans* 10449 phase I.

In this respect we have recently focused much attention on the biochemical differences between the pilli-like substances, the binding materials, of the cell surfaces of *Str. mutans* RC-20 and 10449. The adhesive interactions between *Str. mutans* and tooth surface may be of great ecological significance, and need to be clarified.

Plaque formation and proliferation in phase I strain were extremely inhibited in the presence of phase III in vitro¹⁾. In this research, phase III strains of *Str. mutans* showed the ability to inhibit caries production by wild type strains of *Str. mutans* in the rat model and by phase I strains of *Str. mutans* in the hamster model. These findings lend support to the prospect of use of phase III strains in caries prevention.

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概要 : *Streptococcus mutans* の Mutational phase に関する研究 : *Str. mutans* mutational phase III の動物実験におけるう蝕抑制効果について, 鶴水 隆*, 福田芳生*, 佐藤 誠*, 尾崎文子**。
Str. mutans mutational phase III株は, 動物実験において, う蝕抑制効果のあることが認められた。ハムスターを用いた実験では, 口腔内に mutational phase I と phase IIIを投与すると, phase I の増殖が阻害され, その結果 phase I によるう蝕誘発が抑制された。ラットを用いた実験では, ラット由来の *Str. mutans* より生じた phase III 株は, ラットに常在する *Str. mutans* の増殖を阻害する傾向が認められ, う蝕誘発に対する抑制効果のあることが明らかにされた。しかし, 人由来の *Str. mutans* より生じた phase III 株には, う蝕抑制効果が認められなかった。したがって, *Str. mutans* とその宿主の間には, 特異性の存在することが示唆された。