**72. Takeo Ueda, Shigeshi Toyoshima, Tsuneo Wachi, Mitsuharu Taniguchi
and Hiroshi Tatsumi: Researches on Chemotherapeutic Drugs against
Viruses. III. Studies on Antiviral Effect of Naphthionic Acid.**

(Pharmaceutical Institute, Keio-Gijuku University*)

As described in the foregoing paper¹⁾, naphthionic acid has been found to possess a considerable protective effect on Japanese encephalitis virus. Therefore, the acid was examined in detail as to its antiviral effect and an assumption has been made that the antiviral activity of the acid might be caused by direct interaction between the acid and the virus. This paper describes the antiviral effect and mechanism of naphthionic acid.

Virucidal Effect *in vitro* on the Virus. For tests, sodium naphthionate was employed. The Nakayama strain of *Encephalitis japonica* and young mice of 10~12g. weight were employed. Mice with symptoms of the extreme encephalitis were sacrificed, the brains harvested, emulsified with saline and diluted with saline to give desired virus dilutions. Mixtures of infective brain suspension in concentration of $10^{-6.5}$ were prepared in 0.3~1.0% solutions of the drug and inoculated intracerebrally into groups of 20 mice at intervals (0~24 hours at 23°) after exposures of the virus to the drug.

The results are given in the Table I by survival rate (%).

Table I. Virucidal Effect *in vitro* on the virus.

Concentration of the Drug. (%)	pH of solution.	Virus concentration.	Interval (hours) after exposure of the virus to the drug.					
			0	1	2	3	4	24
0.3	8.0	$10^{-6.5}$	0	0	0	0	0	71
0.5	8.0	$10^{-6.5}$	0	0	0	40	50	100
1.0	8.0	$10^{-6.5}$	0	0	75	60	80	100
Control	7.6	$10^{-6.5}$	0	0	0	0	0	0

It is shown that naphthionic acid possesses an antiviral activity *in vitro* in concentrations from 0.3% to 1.0%.

Protective Effect on the Virus. Solutions of the drug (NA) were injected intraperitoneally into groups of 20 mice in a dose of 800 mg./kg. with addition of hyaluronidase (HD) of 0.1 viscosity unit/kg., once daily for four consecutive days. Later, dilutions of the virus (LD_{50} corresponded to $10^{-8.5}$ dilution) were inoculated intracerebrally, intranasally or intravenously into the treated mice. The results are given in the following Tables by survival rate (%).

Table II. Intracerebral Infection.

Days after Intracerebral Inoculation of the Virus ($10^{-6.5}$ Dilution).

	1~4	5	6	7	8~14
Treated Group. (with 4 Doses of 100 mg./kg.).	100	100	45	10	10
Control Group.	100	85	40	0	—

* Shinano-machi, Shinjuku-ku, Tokyo. (上田武雄, 豊島 滋, 和智恒雄, 谷口三春, 辰巳 熙).

1) T. Ueda, S. Toyoshima, S. Ito and T. Kosuge: This Journal, 71, 972 (1951).

Table III. Intranasal Infection (1).
Days after Intranasal Inoculation of the Virus (10^{-1} Dilution).

	1~4	5	6	7	8	9	10~14
Treated Group with NA.	100	100	85	80	25	0	—
Treated Group with NA and HD.	100	100	65	25	25	25	25
Control Group.	100	85	80	80	0	—	—

Table IV. Intranasal Infection (2).
Days after Intranasal Inoculation of the Virus (10^{-2} Dilution).

	1~5	6	7	8	9	10~14
Treated Group with NA.	100	85	85	40	5	5
Treated Group with NA and HD.	100	90	90	50	45	45
Control Group.	100	65	65	20	20	20

Table V. Intravenous Infection (1).
Days after Intravenous Inoculation of the Virus (10^{-2} Dilution).

	1~4	5	6	7	8	9	10~14
Treated Group with NA.	100	100	55	55	25	25	25
Treated Group with NA and HD.	100	100	45	45	45	35	35
Control Group.	100	75	40	25	0	—	—

Table VI. Intravenous Infection (2).
Days after Intravenous Inoculation of the Virus (10^{-3} Dilution).

	1~4	5	6	7	8	9	10	11	12~14
Treated Group with NA.	100	100	75	65	50	50	50	40	40
Treated Group with NA and HD.	100	85	80	80	80	75	65	65	65
Control Group.	100	75	50	50	20	0	—	—	—

Curative Effect on the Virus.—Dilutions of the virus were inoculated by various routes into groups of 20 mice, and after that, solutions of the drug were injected by various routes into the infected mice with single or more doses of 1 g./kg. The results are given in the following Tables by survival rate (%).

Table VII. Intravenous Injection of NA with a Single Dose following Inoculation.
Days after Intracerebral Inoculation of the Virus ($10^{-5.5}$ Dilution).

	1~4	5	6	7	8
Treated Group with NA.	100	80	40	10	0
Treated Group with NA and HD.	100	100	45	15	0
Control Group.	100	75	35	0	—

Table VIII. Intravenous Injection of NA with a Single Dose following Inoculation.
Days after Intranasal Inoculation of the Virus (10^{-1} Dilution).

	1~5	6	7	8	9	10
Treated Group with NA.	100	100	60	25	0	—
Treated Group with NA and HD.	100	90	55	25	10	0
Control Group.	100	5	0	—	—	—

Table IX. Intravenous Injection of NA with a Single Dose following Inoculation.

Days after Intraperitoneal Inoculation of the Virus (10^{-2} Dilution).

	1~5	6	7	8	9	10	11~14
Treated Group with NA.	100	50	25	5	0	—	—
Treated Group with NA and HD.	100	75	75	75	40	25	25
Control Group.	100	75	50	30	10	10	10

Table X. Intravenous Injection of NA with 5 400 mg./kg.

Doses at Interval (0~4 days) after the Inoculation.

Days after Intravenous Inoculation of the Virus (10^{-2} Dilution).

	1~4	5	6	7	8	9	10	11	12~14
Treated Group with NA.	100	90	70	50	40	40	40	40	30
Treated Group with NA and HD.	100	100	100	60	40	40	40	40	40
Control Group.	100	65	50	50	50	30	15	15	15

Table XI. Intravenous Injection of NA with 5 1 g./kg.

Doses at Interval (0~4 days) after Inoculation.

Days after Intraperitoneal Inoculation of the Virus (10^{-2} Dilution).

	1~4	5	6	7	8	9	10	11~14
Treated Group with NA.	100	100	100	50	50	30	30	20
Treated Group with NA and HD.	100	100	100	100	100	100	80	60
Control Group.	100	90	90	75	75	60	50	30

Table XII. Intravenous Injection of NA with 5 1 g./kg.

Doses at Interval (0~4 days) after Inoculation.

Days after Intravenous Inoculation of the Virus (10^{-2} Dilution).

	1~4	5	6	7	8	9	10	11~14
Treated Group with NA.	100	90	90	80	80	80	70	55
Treated Group with NA and HD.	100	100	100	70	70	70	60	60
Control Group.	100	100	100	100	90	75	50	35

Table XIII. Intraperitoneal Injection of NA with 5 400 mg./kg.

Doses at Interval (0~4 days) after Inoculation.

Days after Intracerebral Inoculation of the Virus ($10^{-6.5}$ Dilution).

	1~4	5	6	7	8
Treated Group with NA	100	50	0	—	—
Treated Group with NA and HD.	100	100	50	15	0
Control Group.	100	85	10	0	—

Table XIV. Intracerebral Injection of NA with 5 45 mg./kg.

Doses at Interval (0~4 days) after Inoculation.

Days after Intranasal Inoculation of the Virus ($10^{-1.5}$ Dilution).

	1~5	6	7	8	9	10	11	12~14
Treated Group with NA and HD.	100	100	85	40	40	40	40	40
Control Group.	100	90	60	25	10	5	0	—

Discussion and Summary (1) Observations on the virucidal effect *in vitro* with naphthionic acid as described in the experimental part show that this drug possesses a considerable activity *in vitro* against the virus. (2) The observations on the protective effect with this acid show that this drug exerted a considerable protective effect on the virus and was activated with hyaluronidase. (3) The observations on the curative effect of the acid show that this drug possesses a little curative effect on the virus and was activated with hyaluronidase. (4) The antiviral activity of this compound might be caused by direct interaction between the compound and the virus. It might be concluded, from the results of experiments *in vivo*, that the milder the process of infection was and the more direct the route of injection, the more marked will be the effect of naphthionic acid. (5) It might, therefore, be expected that more effective drugs could be obtained among compounds related to naphthionic acid, if the substances were found which possessed more neurotropic and active (*in vitro* and *in vivo*) properties than naphthionic acid.

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73. Takeo Ueda, Shigeshi Toyoshima and Tsuneo Wachi: Researches on Chemotherapeutic Drugs against Viruses. IV. Studies on the Syntheses and Antiviral Effects of 3-Phenylazonaphthionic Acid and its Derivatives.

(Pharmaceutical Institute, Keio-Gijuku University*)

Studies on the effect of naphthionic acid against the Nakayama strain of *Encephalitis japonica* have shown that naphthionic acid possessed a marked activity *in vitro* and *in vivo* against the virus and a comparatively low toxicity. In order to find more effective substances than naphthionic acid, compounds related to the acid such as those of 3-hydroxy-4-amino-naphthalenesulfonic acid, 3,4-diamino-naphthalenesulfonic acid, 1,2-naphthoquinonesulfonic acid-(4), 1,4-naphthoquinone, Trypan red, Trypan blue, sulfanilic acid, 4-aminophenol, 4-hydroxybenzenesulfonic acid, 3-phenylazo-4-amino-naphthalenesulfonic acid (3-phenylazonaphthionic acid), etc. were examined as to their antiviral activities, and it was found that among these compounds 3-phenylazonaphthionic acid and its derivatives were of interest as to their antiviral activities²⁾.

This communication describes the syntheses and the antiviral activities *in vitro* of 3-phenylazonaphthionic acid and its derivatives.

Syntheses of 3-phenylazonaphthionic Acid and Its Derivatives.

3-Phenylazonaphthionic acid and its derivatives were synthesized by diazotized aniline and its derivatives containing ring-substituents and coupling the resulting diazonium compounds with naphthionic acid, according to the following equations:

* Shinano-machi, Shinjuku-ku, Tokyo. (上田武雄, 豊島 滋, 和智恒雄).

1) T. Ueda, S. Toyoshima and T. Wachi: This Journal, **71**, 972 (1951).