THE EFFECT OF LARGE DOSE INTRATUMORAL ADMINISTRATION OF OK-432 IN ADVANCED CANCER PATIENTS

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ABSTRACT

To clarify the effect of large dose intratumoral administration of OK-432, experimental and clinical investigations were performed. In the experimental study, 250 KE/Kg OK-432 was administered into S-180 sarcoma in ddN mice. Mice treated by OK-432 showed significant increase in delayed hypersensitivity reaction and elongation in the survival times. In the clinical study, 50 KE OK-432 was injected intratumorally during the surgery for the advanced and inoperable malignancy. PHA and PPD skin test reactions, IgG titers, and lymphocyte counts were elevated in the group received the large dose of OK-432. The treatment seemed to contribute to the prolongation of survival times during 3 months after the operation.

INTRODUCTION

OK-432, prepared from penicillin G treated *Streptococcus haemolyticus* has been reported to have both direct cytocidal and indirect host mediated anticancer activity and has been used clinically as an immunotherapeutic drug for cancer in Japan.[1)2)3)]

Although the immunological mechanisms of OK-432 has not been clarified yet, the effect on the enhancement of the delayed hypersensitivity reaction has been reported both in clinical and experimental studies.[4)] In this report, attempts have been undertaken to investigate the effect of intratumoral administration of OK-432 in experimental animals and the treatment was proved to be effective. From this experimental data, clinical application was also carried out in advanced cancer patients.

MATERIALS AND METHODS

1. Experimental studies

Animals: Six week old ddN male mice weighing 25 to 30g, supplied from breeding colonies of Nagoya University were used.

Tumor: S-180 solid sarcoma maintained in our laboratory were used.

OK-432: OK-432 was supplied from Chugai Co. Ltd.

In the experiment, 2×10^6 S-180 sarcoma cells in 0.1 ml saline solution were inoculated subcutaneously to ddN strain of mice. Fifteen days later, 250 KE/Kg OK-432 was injected intratumorally. Then, 0.32 mg/Kg (1/16 dosis of LD_{50}) Mitomycin-C (MMC) on day 17, 18, 23 and 24, and 20 KE/Kg OK-432 on day 19 to 22 and 25 to 28 were followed (large

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Received for Publication. July, 11, 1979.

OK + MO group). The other experimental groups were consisted of 250 KE/Kg of OK-432 alone group (large OK group), 0.32 mg/Kg MMC on day 17, 18, 23 and 24 and 20 KE/Kg OK-432 on day 19 to 22 and 25 to 28 treated group (MO group) and untreated control group. The effect of OK-432 was judged by the survival, tumor growth and delayed hypersensitivity reaction (DHR).

Servival: The effect of the treatment was expressed as T/C, where T was mean survival time of the treated group and C was that of the untreated control. Mice surviving beyond 60 days were calculated as 60 days survivors in the determination of the mean survival time.

Tumor growth: Mean diameter of the tumor was calculated from the sum of the longest axis and the perpendicular axis to that axis. Measurement was performed on day 15 and 29.

Delayed hypersensitivity reactions (DHR): Delayed hypersensitivity reactions on the experimental animal was examined by using picryl chloride[5)6)7)]. Mice were sensitized by 7% picryl chloride dissolved in alcohol which was applied to the skin of the clipped abdomen. Seven days later, the thickness of the ear was measured with Dial Thickness Gauge and both sides of the ear were smeared with 1% picryl chloride dissolved in olive oil. The ear was measured again at 24 hours and the difference between the two measurements was calculated in units of 10^{-3} cm. This "increase in ear thickness" has been considered to represent the delayed hypersensitivity reaction in mice[4)5)].

2. Clinical studies

Thirty four patients with advanced gastrointestinal cancer admitted to the Second Department of Surgery of Nagoya University and Chūno Hospital from July 1974 to October 1977 were chosen for the present study.

One group of the patients received OK-432 intratumorally at the time of laparotomy. After the operation, intravenous biweekly administration of 4 mg MMC was performed 8 times for 4 weeks. Then oral administration of 5FU in daily dose of 200 mg, and intramuscular administration of OK-432 in 2 KE every other day were followed (large OK + MFO group).

The other group, no OK-432 injection was performed at the time of laparotomy, but postoperative MMC, 5FU and OK-432 treatment was similarly performed after the operation (MFO group).

Eleven patients with advanced gastric cancers and 6 advanced colorectal cancers in the large OK + MFO group, and 10 advanced gastric cancers and 7 advanced colorectal cancers in MFO group were evaluable. All patients had advanced tumors which had no indication for the curative nor palliative resections.

Survival of the patients were evaluated at least 13 months after the operation, and clinical effects were evaluated according to the criteria of Karnofsky and of Japan Society of Cancer Therapy.

Complete blood cell counts and serum immunoglobulin levels were tested every two weeks after the laparotomy. Peripheral lymphocyte was counted every week, and PHA and PPD skin reactions were tested every two weeks after the operation.

RESULTS

1. Experimental studies

Survival: As shown in Figure 1, the mean survival time was 47.5 days in large OK + MO group, 41.4 days in large OK group, 41.9 days in MO group and 36.0 days in the untreated tumor bearing control group. The treatment of intratumoral large dose OK-432 + MO showed significantly longer in mean survival time ($P < 0.05$) as compared to that of the untreated tumor bearing group.

Tumor growth: On day 15, the mean diameter of the tumor reached about 13 mm in every group. On day 29, mean tumor diameter was 14.7 mm in large OK group, 17.8 mm in large OK group, 17.2 mm in MO group and 18.8 mm in untreated tumor bearing group.

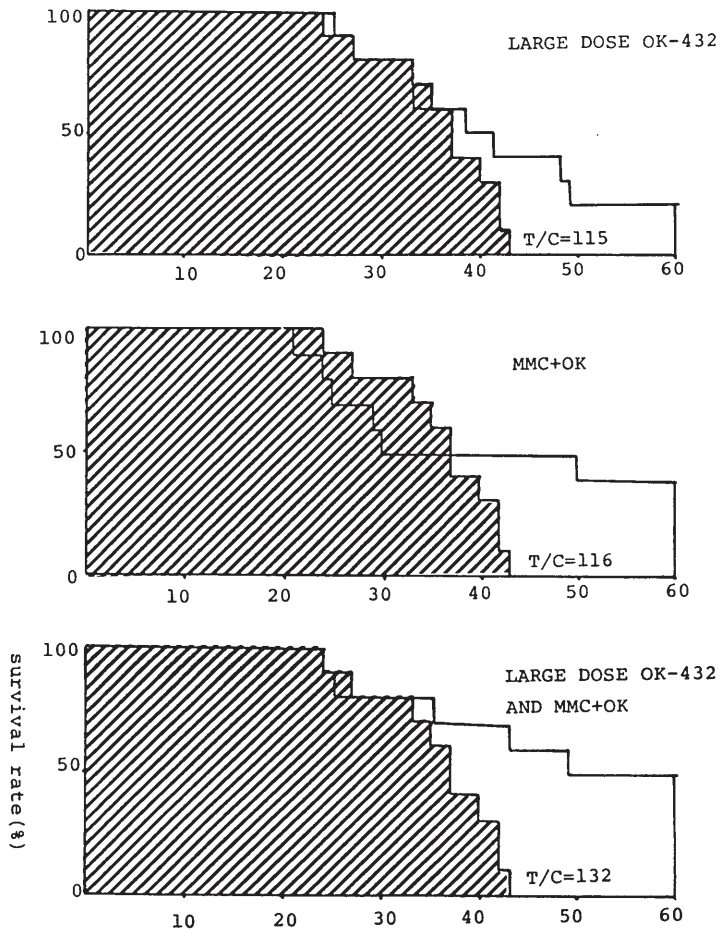

Fig. 1. Survival of ddN mice treated by large dose OK-432 and Mitomycin-C + small dose OK-432. *control animals are given in slash.

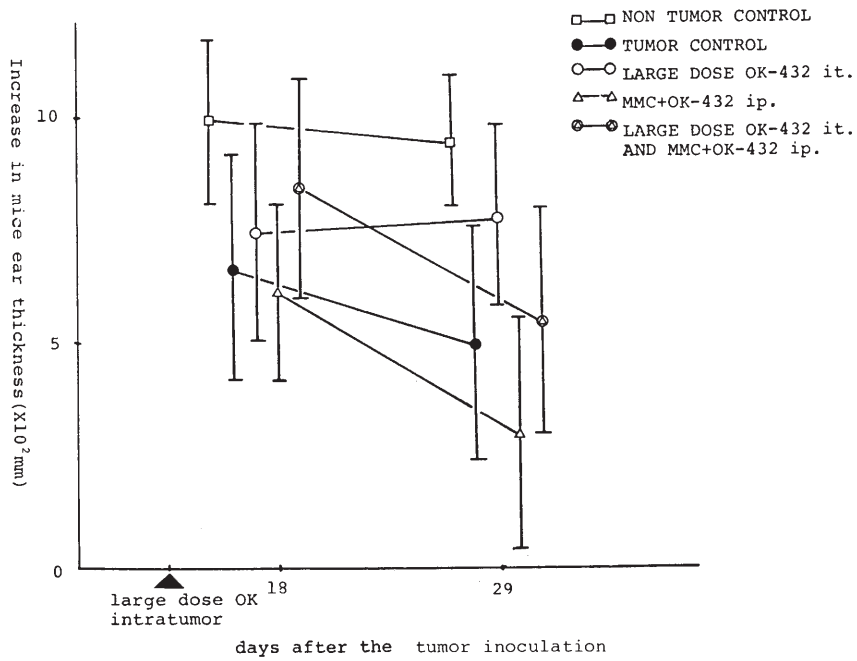

Fig. 2. Delayed hypersensitivity reactions induced by the sensitization and challenge of the picryl chloride in treated and untreated tumor bearing ddN mice.

Large OK + MO group had a significantly smaller tumor as compared to the untreated control group.

DHR: As shown in Figure 2, on day 18, DHR was almost even in each group. However, on day 29, large OK group and large OK + MO group are enhanced DHR as compared to that of the MO group.

2. Clinical studies

1) Survival and clinical effects

As shown in Figure 3, survival of the patients of large OK + MFO group predominated MFO group until three months after the operation. However, this phenomenon disappeared after four months. Fifty percent survival of the large OK + MFO group was 3.1 months in gastric carcinoma and 4.0 months in colorectal carcinoma, whereas in MFO group, it was 2.4 months in gastric carcinoma and 2.5 months in colorectal carcinoma.

Clinical effects were evaluated by the criteria adopted by Karnofsky. In large OK + MFO group, two of them were judged as 0-C and in MFO group, one of them was considered to be 0-C (Table 1).

By the criteria of the Japan Cancer Society, two patients in the large OK + MFO group patients were considered to be improved. In MFO group, one patient was judged to be improved. There were only one exacerbated case in the large OK + MFO group, whereas there were 8 cases in the MFO group.

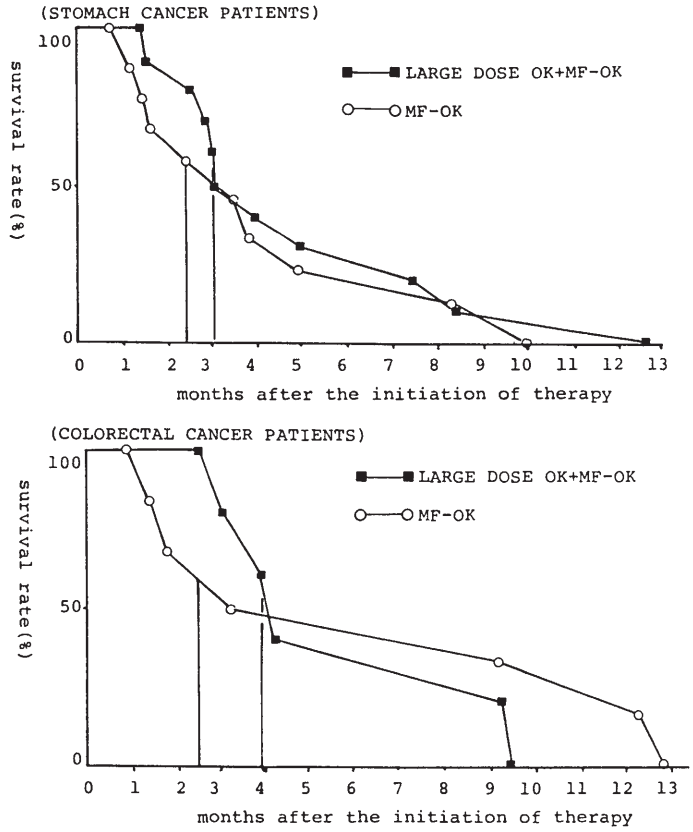

Fig. 3. Survival of the patients treated by "large OK + MFO" and "MFO" in stomach and colorectal malignancy.

Table 1. Clinical results in advanced cancer patients treated by intratumoral administration of large dose OK-432.

GROUPS	LARGE DOSE OK+MF-OK		MF-OK only	
	GASTRIC	COLORECTAL	GASTRIC	COLORECTAL
CASES	11	6	10	7
EVALUATED CASES	10	5	10	5
KARNOFSKY CRITERIA				
0-0	5	3	7	3
0-A	2	2	2	2
0-B	0	0	0	0
0-C	2	1	1	0
1-A	0	0	0	0
1-B	0	0	0	0
CRITERIA OF JAPAN SOCIETY FOR CANCER THERAPY				
EXACERBATED	1	0	5	3
UNCHANGED	7	5	5	1
IMPROVED	2	1	0	1

2) PHA and PPD skin test

Mean diameter of the PHA and PPD skin test was measured before and 2 weeks after the operation.

In large OK + MFO group, PHA skin test changed from 18.1 mm to 14.5 mm. In MFO group, the change was from 18.4 mm to 9.9 mm. The postoperative decrease in reaction was smaller in large OK + MFO group as compared to that in MFO group. (Figure 4)

In large OK + MFO group, PPD skin test reaction was 15.2 mm before operation and 14.6 mm after the operation. In MFO group, it was 17.6 mm before the operation and 12.5 mm after the operation. (Figure 5)

Both reactions showed no significant difference between the two groups when the skin test was measured 4 and 6 weeks after the operation.

3) Serum immunoglobulin

As shown in Figure 6, the change of IgG titers were measured before and two weeks after the operation. In large OK + MFO group, the change was from 1444 to 1761 mg/dl and 1317 to 1391 mg/dl in MFO group. IgA changes were from 235 to 281 mg/dl in large OK + MFO group and from 288 to 302 mg/dl in MFO group. IgM changes were from 109

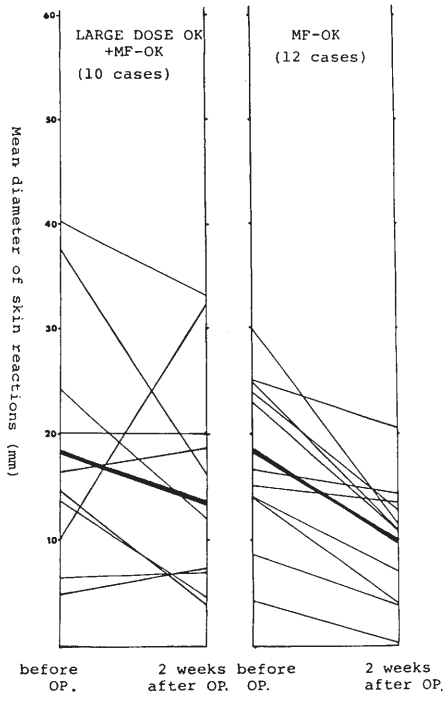

Fig. 4. PHA skin test changes in patients 2 weeks after the intratumoral administration of OK-432 in large doses.
*Heavy lines indicate the mean values.

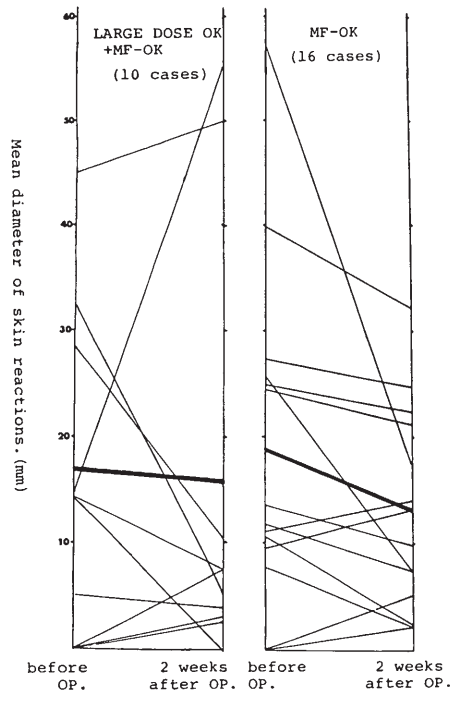

Fig. 5. PPD skin test changes in patients 2 weeks after the intratumoral administration of OK-432 in large doses.
*Heavy lines indicate the mean values.

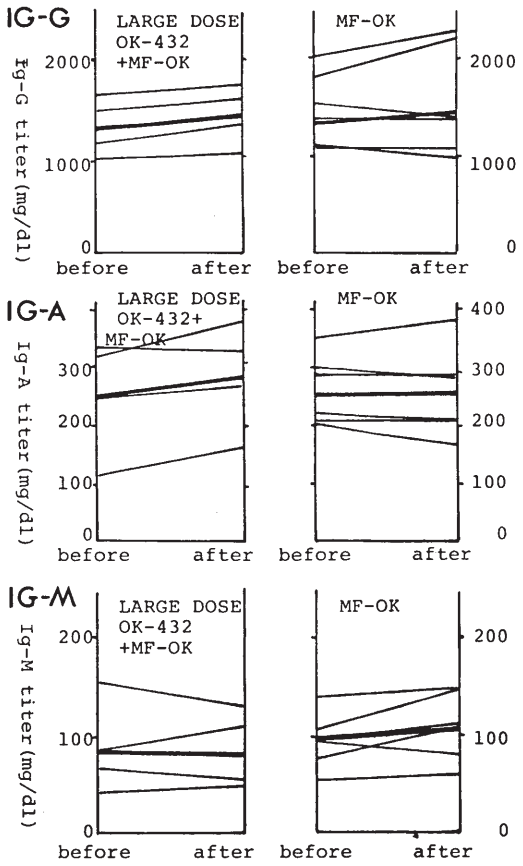

Fig. 6. Serum immunoglobulin changes in advanced cancer patients 2 weeks after the intratumoral administration of OK-432 in large doses.
*Heavy lines indicate the mean values.

to 114 mg/dl in large dose group, and 132 to 150 mg/dl in MFO group. IgA and IgM had no appreciable difference between the two groups, but in large OK + MFO group, IgG showed slight increase in two weeks after the operation.

4) Complete blood count and peripheral lymphocyte count

As in Figure 7, WBC and RBC showed no significant changes during the treatment. Platelet counts were higher in large OK + MFO group than in the MFO group. Hemoglobins were higher in MFO group than in the large OK + MFO group.

Lymphocyte counts were increased in large dose group one week after the operation, but after two weeks, no significant difference was observed between the two groups. (Figure 8)

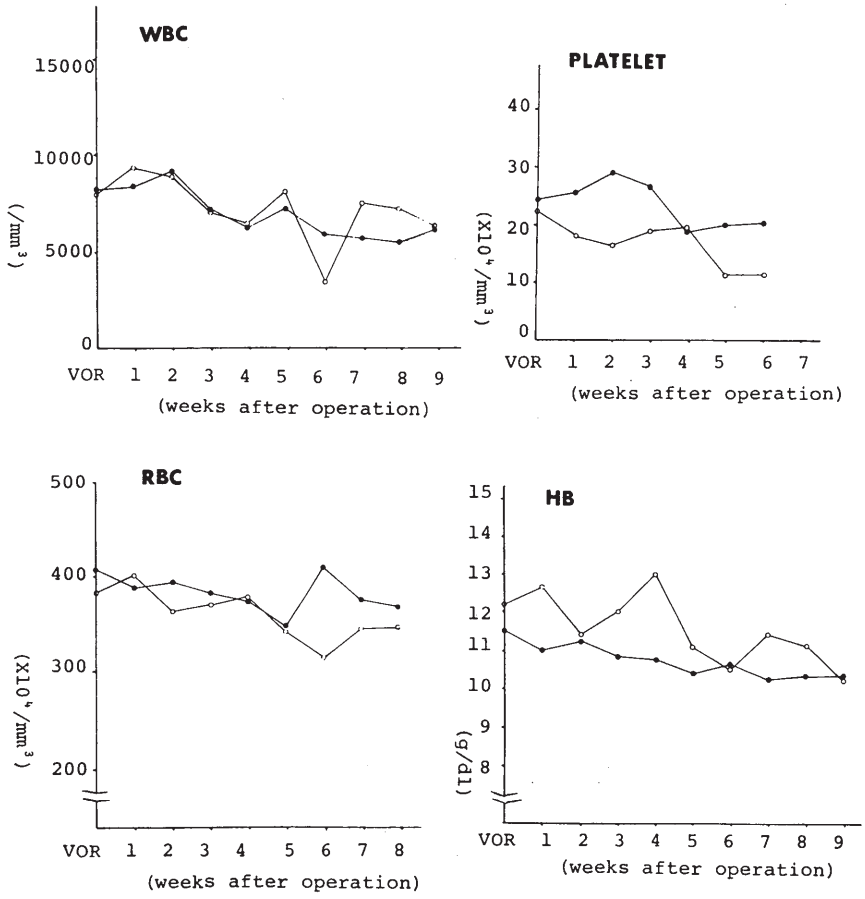

Fig. 7. Complete blood count changes in patients 2 weeks after the intratumoral administration of OK-432 in large doses. Black dots indicate the "large OK + MFO" group, and blank dots indicate the "MFO" group.

5) Side effects

In large dose group, 5 of 17 patients showed fever up to 38.0°C until the third postoperative day. But, no other significant side effects, such as shock, renal disturbance or liver damage were encountered.

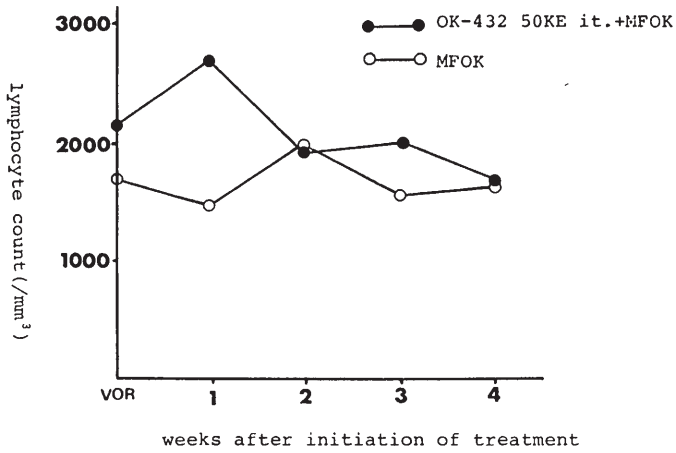

Fig. 8. Peripheral lymphocyte count changes in patients 2 weeks after the intratumoral administration of OK-432 in large doses.

DISCUSSION

Several reports have been published about the intratumoral administration of OK-432 in recent years.[8)9)10)11)] The purposes of the treatment are 1) direct cytocidal effect, 2) enhancement of the tumor specific antigenicity through degeneration of the damaged tumor and 3) elevation of the non-specific host defence mechanism by large dose administration of OK-432.[12)]

In present report, we have mainly dealt with the third item. In the experimental studies, delayed hypersensitivity reactions were increased in large dose groups compared to the untreated control groups and MO group. Also, survival of the group treated by large dose OK-432 showed significant prolongation.

In the clinical studies, PHA and PPD skin test reactions and IgG increased in large dose group as compared to those in MFO group two weeks after the operation. Peripheral lymphocyte counts increased after one week. Survival was improved in large dose group until three months after the operation.

From the experimental and clinical evaluation, it seems likely that large dose intratumoral OK-432 administration contributed to the recovery of the nonspecific immunity depressed by the operation. The postoperative immune deficiency that might have lead to the further exacerbation of the tumor might be restored by the large dose of OK-432 administration, and therapeutic effects were enhanced in large dose OK-432 treated group than in the ordinary treated group.

We propose that intratumoral 50 KE OK-432 is beneficial for the treatment of advanced inoperable cancer patients in two points of view. First, appreciable clinical therapeutic effect was obtained, and second, side effects such as shock, persisting high fever, bone marrow toxicity was not observed in the administration of either OK-432 or chemotherapeutic agents. Safe and effective treatment is vital to survival, and investigations should be continued to find out the best schedules of therapy.

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