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Effects of TAK-637, a tachykinin receptor antagonist, on the micturition reflex in guinea pigs

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Abstract

The effects of a new tachykinin NK_1 receptor antagonist, (*aR*,9*R*)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione (TAK-637), on the micturition reflex were compared with those of drugs used for abnormally frequent micturition or incontinence. TAK-637 showed a characteristic effect on the distension-induced rhythmic bladder contractions in guinea pigs. The systemic administration of TAK-637 decreased the number but not the amplitude of the distension-induced rhythmic bladder contractions. A similar effect was observed in animals in which the spinal cord had been severed. TAK-637 also inhibited the micturition reflex induced by topical application of capsaicin onto the surface of bladder dome. From these results, it is concluded that TAK-637 inhibits sensory transmissions from the bladder evoked by both physiological and nociceptive stimuli by blocking tachykinin NK_1 receptors, possibly at the level of the spinal cord. On the other hand, the other drugs such as oxybutynin, tolterodine, propiverine, and inaperisone showed no effects on the frequency of the distension-induced rhythmic bladder contractions but decreased the contraction amplitude. Therefore, TAK-637 may represent a new class of drugs, which would be effective for abnormally frequent micturition without causing voiding difficulties due to decreased voiding pressure.

Keywords: TAK-637; Tachykinin NK_1 receptor antagonist, non-peptide; Micturition reflex; Urinary tract, lower

1. Introduction

Substance P is widely distributed in the body of mammals and has a variety of physiological functions including actions as a neurotransmitter or neuromodulator mediated by the tachykinin NK_1 receptor. Substance P receptor antagonists have great potential in the treatment of a wide variety of disorders such as pain, emesis, asthma, arthritis, and psychological disorders (Otsuka and Yoshioka, 1993). The discovery of the first non-peptide tachykinin NK_1 receptor antagonist, *cis*-3-(2-methoxybenzylamino)-2-benzhydrylquinuclidine (CP-96,345) (Snider et al., 1991), fueled the drive for further research into non-peptide antagonists (review: Betancur et al., 1997), and their clinical utility is now of much interest in spite of the variety of supposed clinical targets. Recently, antiemetic (Kris et al., 1996) and antidepressant effects (Kramer et al., 1998) have been examined in clinical trials.

There are several lines of evidence to show that tachykinins or capsaicin-sensitive neurons play a role in the micturtion reflex in both the central and peripheral nervous system (review: Maggi, 1991, 1997). Hypothesizing that a new tachykinin NK_1 receptor antagonist, (*aR*,9*R*)-7-[3,5-bis(trifluoromethyl)benzyl]-8,9,10,11-tetrahydro-9-methyl-5-(4-methylphenyl)-7*H*-[1,4]diazocino[2,1-*g*][1,7]naphthyridine-6,13-dione (TAK-637) (Natsugari et al., 1999) might modulate the micturition reflex, we first showed that systemic administration of tachykinin NK_1 receptor antagonists raises the threshold of the micturiton reflex induced by transvesical saline filling, suggesting that TAK-637 could be used as pharmacotherapy for micturitional dysfunctions (Doi et al., 1999).

In the present study, the effects of TAK-637 on the distension-induced rhythmic bladder contractions in urethra-ligated guinea pigs as well as on the chemonocicep-

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tive vesico-vesical micturition reflex induced by topical application of capsaicin onto the urinary bladder were investigated. The distension-induced rhythmic bladder contractions are believed to be a physiological micturition-related reflex of neuronal origin (Maggi et al., 1986b). In particular, the former model gives us some information on the site and the mode of action of a test compound. Changes in the amplitude and the interval of the distension-induced rhythmic bladder contractions mean primarily that the site of action lies in the motor and in the afferent arms of the micturition reflex pathway, respectively. In the present study, we studied the effect of TAK-637 on the chemical nociceptive-induced micturition reflex. To assess clinical utility, drugs for incontinence were also studied for comparison in urethane-anesthetized guinea pigs.

2. Materials and methods

2.1. Experimental animals

Male Hartley guinea pigs (250–300 g body weight) were used. The animals were anesthetized with an intraperitoneal injection of urethane (1.2 g/kg) and the urinary bladder was exposed through an incision in the abdomen, and the urethra was ligated. Two 23-gauge needles connected to a polyethylene tube (PE-90) were inserted into the bladder dome: one for recording the intravesical pressure and the other for injection of saline into the bladder. Warmed saline-soaked cotton wool swabs were put on the exposed bladder dome to keep it moist and warmed saline was dropped on it if necessary.

Spinal animals were prepared by severing the spinal cord at the Th13-L1 segmental level under ether anesthesia. After a Th12-L2 laminectomy, the spinal transections were performed using aseptic surgical techniques, with visual confirmation of complete transection. Sterile sponge (Yamanouchi) was placed between the cut ends of the spinal cord and local anesthetic (Xylocaine jelly) was applied topically. The overlying muscle and skin were closed with suture. Post-operatively, 10,000 units of penicilline G potassium was intramuscularly injected once a day to prevent infection. All the animals were kept in individual cages with easy access to food and water. Sawdust was put on the floor of the cage to prevent pressure sores. The room temperature and humidity were kept in the range of 23 ± 2°C and 55 ± 10%, respectively. The animals were used in the experiments 2 days later. No special care was taken to help them with voiding because they could void by themselves for the 2 days of recovery.

2.2. Experimental protocol

Distension-induced isovolumetric bladder contractions were caused by stepwise injections (0.2-ml portions) of warmed physiological saline (39°C) into the bladder. Immediately, after stable rhythmic contractions had been confirmed by monitoring intravesical pressure, drugs were administered intravenously. The effects of drugs were evaluated from both the shutdown time (the time of complete cessation of the bladder contractions after the drug administration) with a cut-off time of 30 min and the maximum intravesical pressures. TAK-637 was dissolved in dimethylsulfoxide (DMSO) and intravenously injected in a volume of 0.05 ml/100 g and the other drugs were dissolved in physiological saline and intravenously injected in a volume of 0.1 ml/100 g.

Capsaicin-induced bladder contractions were caused by topical application of capsaicin (0.8 μg/10 μl in a 10% ethanol–saline solution). In each animal, physiological saline was infused into the bladder stepwise (0.2-ml portions) until isovolumetric bladder contractions appeared, and then fluid was withdrawn from the bladder in amounts such that the volume was just below the threshold for isovolumetric contractions. Topical capsaicin was applied on the outer surface of the bladder dome by means of a Gilson Pipetmann®. TAK-637 was dissolved in DMSO and intravenously injected in a volume of 0.05 ml/100 g.

2.3. Statistics

As the vehicle of TAK-637 (DMSO) affected the shutdown time of the distention-induced isovolumetric bladder contractions in some animals, the shutdown times were compared between the drug-treated groups and vehicle-treated group using a non-parametric Dunnett's test or Student's *t*-test. The intravesical pressure of distention-induced isovolumetric bladder contractions was not sensitive to vehicle administration, and their statistical comparison was made between the predrug and postdrug value with a paired *t*-test.

The statistical analysis of the effect on the capsaicin-induced bladder contractions was performed with a non-parametric Dunnett's test.

A probability of $P < 0.05$ was accepted as significant. The study was approved by Takeda's Experimental Animal Care and Use Committee.

2.4. Chemicals

TAK-637, (±)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine · 2HCl ((±)-CP-99),994 · 2HCl), (±)-CP-99,994), inaperisone hydrochloride (inaperisone), and tolterodine hydrogen tartrate (tolterodine) were synthesized in Takeda's Pharmaceutical Research Laboratories. Oxybutynin hydrochloride (oxybutynin: Kodama) and propiverine hydrochloride (propiverine: Taiho) were extracted from the respective commercially available tablets in Takeda's Pharmaceutical Research Laboratories. Urethane (Aldrich.), atropine (Sigma), capsaicin (Sigma), hexamethonium bromide (Sigma), tetrodotoxin (Wako), xylocaine jelly (Fujisawa), and penicillin G potassium (Crystalline Penicillin-G Potassium: Asahi Kasei) were purchased.

3. Results

3.1. Effects on distension-induced rhythmic bladder contractions

Distension of the urinary bladder by infusion of saline (3–4.5 ml) induced high amplitude (> 15 mm Hg) rhythmic contractions as shown in Fig. 1. The contractions were completely eliminated by dropping tetrodotoxin (20 μg/0.1ml) onto the surface of the bladder dome, and the contractile pressure was reduced by the intravenous injection of atropine (0.3 mg/kg, i.v.). From these results, it is quite clear that in guinea pigs, the distension-induced rhythmic bladder contractions are neurogenic in nature and regulated partly via cholinergic parasympathetic nerves.

The results observed with the various drugs are shown in Table 1. Intravenous injection of TAK-637 as well as (±)-CP-99,994 completely suppressed the bladder contractions. The effects on the frequency of the distension-induced rhythmic bladder contractions were quantitatively evaluated in terms of shutdown time. TAK-637 dose dependently increased the shutdown times with a minimum effective dose of 1.0 mg/kg, i.v. When the contractions reappeared, the contractile amplitude was unaffected. The structurally unique tachykinin NK_1 receptor antagonist, (±)-CP-99,994, showed the same effects: increase in the shutdown time and no effect on the amplitude.

Animals in which the spinal cord had been severed were used to clarify the site of action of TAK-637. The distension-induced rhythmic bladder contractions in the spinal guinea pigs were almost the same as those in the intact animals for both frequency and amplitude, indicating that the spinal cord plays a crucial role in the distension-induced rhythmic bladder contractions in guinea pigs. TAK-637 at a dose of 1.0 mg/kg, i.v. significantly increased the bladder shutdown time in spinal animals exactly as in the intact animals (Table 1). In contrast to the effect in the intact animals, however, TAK-637 significantly decreased the contractile amplitude in the animals in which the spinal cord had been severed.

All the drugs other than the tachykinin NK_1 receptor antagonists, such as oxybutynin, tolterodine, propiverine, and inaperisone decreased the amplitude of the distension-induced rhythmic bladder contractions and did not change the shutdown time (Table 1), supporting the notion that their sites of action are in the motor component of the micturition reflex pathway including the detrusor muscle (Fredericks et al., 1975; Nilvebrant et al., 1997; Haruno, 1992; Morikawa et al., 1992).

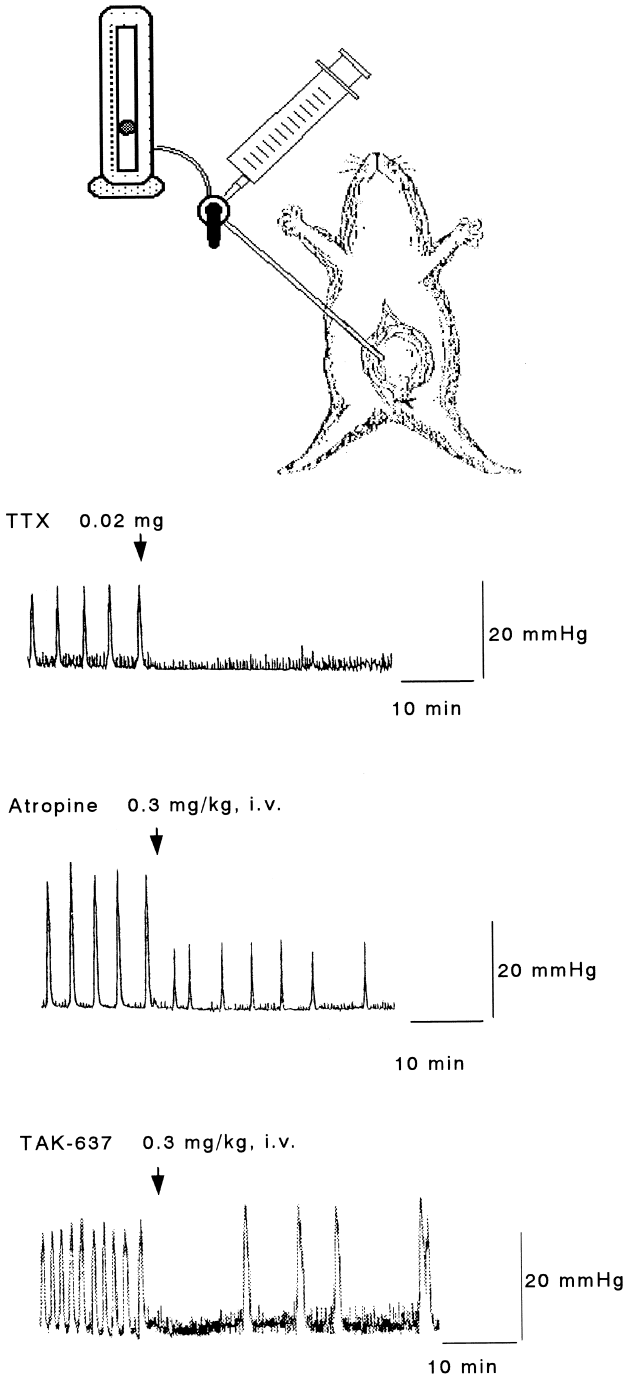

Fig. 1. The effects of various drugs on distension-induced bladder contractions. Isovolumetric bladder contractions were measured as shown in the scheme. The topical application of tetrodotoxin to the bladder dome completely blocked the isovolumetric contractions (the upper chart). Intravenous injection of atropine clearly decreased the amplitude of the bladder pressure (the middle chart). TAK-637 decreased the frequency of the rhythmic contractions without changing the amplitude of the pressure (the lower chart).

3.2. Effects on capsaicin-induced bladder contractions

This experiment was performed to determine if the chemical nociceptive-induced micturition reflex can be blocked by TAK-637 because it is well known that the micturition reflex can be elicited by nociceptive stimuli as well as by the distension-induced stimulation of the bladder (Maggi et al., 1984). It should be mentioned that topical capsaicin did not cause a tonic increase in basal bladder pressure in any animal because the dose of cap-

Table 1
The effects of various drugs on isovolumetric bladder contractions
Pre and Post indicate the mean intravesical pressure before and after drug administration, respectively. Data are expressed as the means ± S.E.M. The difference in the number of animals in parenthesis for bladder shutdown and intravesical pressure is the number of animals in which the shutdown time exceeded the cut-off time (30 min).

	Dose (mg/kg, i.v.)	Bladder shutdown (min)	Intravesical pressure (mm Hg)	
			Pre	Post
DMSO		8.5 ± 2.6 (10)	29.8 ± 1.9	29.8 ± 1.9 (9)
TAK-637	0.1	11.9 ± 3.4 (10)	22.3 ± 2.8	23.2 ± 2.8 (8)
	0.3	16.3 ± 4.1 (10)	21.3 ± 1.3	21.3 ± 0.8 (6)
	1	22.4 ± 2.8[a] (10)	22.2 ± 2.8	21.6 ± 2.3 (6)
DMSO		10.3 ± 3.3 (7)	31.4 ± 1.8	30.7 ± 1.9 (6)
TAK-637 (Spinal)	1	23.3 ± 3.3[b] (7)	28.4 ± 4.5	20.8 ± 6.0[c] (3)
Saline		5.1 ± 1.0 (10)	26.3 ± 2.0	26.6 ± 2.3 (10)
(±)-CP-99,994	0.3	7.5 ± 1.3 (10)	25.1 ± 2.2	24.3 ± 2.2 (10)
	1	15.5 ± 3.4[a] (10)	18.8 ± 1.3	20.2 ± 1.6 (7)
	3	24.4 ± 2.4[d] (10)	19.3 ± 1.1	21.0 ± 1.6 (4)
Oxybutynin	0.1	5.5 ± 1.2 (10)	21.9 ± 1.6	19.5 ± 1.4 (10)
	0.3	5.1 ± 0.9 (10)	23.3 ± 1.8	18.6 ± 1.3[e] (10)
	1	5.0 ± 1.0 (10)	21.6 ± 1.6	17.5 ± 0.9[e] (10)
	3	9.4 ± 2.8 (10)	25.4 ± 2.1	18.9 ± 2.3[e] (10)
Saline		4.0 ± 0.7 (10)	22.5 ± 1.7	22.4 ± 1.8 (10)
Tolterodine	0.1	4.7 ± 1.2 (10)	28.4 ± 3.1	28.0 ± 3.0 (10)
	0.3	2.5 ± 0.4 (10)	24.3 ± 1.6	22.0 ± 1.7[c] (10)
	1	3.2 ± 0.6 (10)	24.4 ± 2.5	15.5 ± 1.7[e] (10)
Propiverine	3	4.2 ± 0.9 (10)	22.5 ± 2.1	21.7 ± 2.5 (10)
	10	3.6 ± 1.2 (10)	25.0 ± 1.9	23.3 ± 1.9[e] (10)
Saline		2.8 ± 0.5 (10)	21.0 ± 1.9	21.0 ± 2.2 (10)
Inaperisone	0.3	2.9 ± 0.3 (10)	21.4 ± 1.6	21.0 ± 1.8 (10)
	1.0	2.2 ± 0.2 (10)	20.4 ± 1.1	19.0 ± 1.3[c] (10)
	3.0	3.3 ± 0.7 (10)	23.2 ± 1.1	19.6 ± 1.1[e] (10)

[a] Vehicle group vs. drug-treated group; $P < 0.05$ (non-parametric Dunnett's test).
[b] Vehicle group vs. drug-treated group; $P < 0.05$ (Student's *t*-test).
[c] Predrug vs. Postdrug; $P < 0.05$ (paired *t*-test).
[d] Vehicle group vs. drug-treated group; $P < 0.01$ (non-parametric Dunnett's test).
[e] Predrug vs. Postdrug; $P < 0.01$ (paired *t*-test).

saicin (0.8 μg/10 μl) was chosen so as to be just above the threshold of phasic contractile responses. The capsaicin-induced bladder contractions were completely eliminated by intravenous hexamethonium (10 mg/kg, $n = 6$). From these results, it is confirmed that the responses are of reflex origin as reported and the possibility of ''the local efferent function'' can be excluded (Maggi et al., 1984).

TAK-637 dose dependently inhibited the capsaicin-induced response with a minimum effective dose of 0.3 mg/kg, i.v. (Table 2). The numbers of animals showing complete loss of the contractions were 2/10, 3/10, and 8/10 at doses of 0.03, 0.1, and 0.3 mg/kg, i.v., respectively. Thus, the effect of TAK-637 was near to all or none.

Table 2
The effects of TAK-637 on capsaicin-induced bladder contractions
Data are expressed as the means ± S.EM. for 10 animals. Statistical analysis was performed using the non-parametric Dunnett's test.

	Maximum intravesical pressure (mm Hg)
DMSO	32.1 ± 2.2
TAK-637	
0.03 mg/kg, i.v.	25.0 ± 4.7
0.1 mg/kg, i.v.	19.9 ± 5.1
0.3 mg/kg, i.v.	2.4 ± 1.8[a]

[a] $P < 0.01$.

4. Discussion

TAK-637 is an orally active tachykinin NK_1 receptor antagonist; its receptor affinity is 0.45 nM ($= IC_{50}$) in human IM-9 cells, and its in vivo activity is 33 μg/kg, p.o. ($= ID_{50}$) in capsaicin-induced trachea extravasation in guinea pigs. It also has a high selectivity for the tachykinin NK_1 receptor over tachykinin NK_2 and NK_3 receptors (Natsugari et al., 1999). In our previous study, we found that the new tachykinin NK_1 receptor antagonists, TAK-637 and (±)-CP-99,994, increased the volume threshold (volume to which the bladder can be filled with saline before voiding is induced) in the guinea pig bladder, and their potential for clinical use was discussed. The present study addressed TAK-637's mode and sites of action in the model based on the distension-induced rhythmic bladder contractions in guinea pigs. Two variables were taken into

consideration: the frequency of the distension-induced rhythmic bladder contractions in terms of shutdown time and the contractile pressure of the distension-induced rhythmic bladder contractions. The frequency of the distension-induced rhythmic bladder contractions is thought to be regulated by the micturition center in the central nervous system (Maggi et al., 1986b). The amplitude of the contractile pressure can be regarded as almost equivalent to the network of the detrusor muscle because the urethra was ligated so as not to let off any of the vesical pressure. Therefore, the measurement of these two variables gives useful information about the modes of action of drugs acting on bladder functions.

TAK-637 and the structurally unique tachykinin NK_1 receptor antagonist, (±)-CP-99,994, dose dependently increased the shutdown time with no effect on the amplitude of the distension-induced rhythmic bladder contractions, and similar effects were also observed in the animals in which the spinal cord had been severed. These profiles coincide well with those observed following the intrathecal injection of tachykinin NK_1 receptor antagonists (Lecci et al., 1993a; Maggi et al, 1993). Therefore, it is strongly suggested that the tachykinin NK_1 receptor antagonistic effect of TAK-637 is responsible for the effects seen upon systemic administration in the present study.

In the animals in which the spinal cord had been severed, TAK-637 decreased the amplitude of the distension-induced rhythmic bladder contractions, and this seems to have affected the motor arm of the micturition reflex. The normal ''switching on/off'' mechanism of the micturition reflex functions only if there is coordination between the sacral and pontine micturition centers (De Groat, 1990; Blok and Holstege, 1998). In the animals in which the spinal cord had been severed, this coordination was impossible. Therefore, the micturtion reflex could not work in a switching manner. Thereby, the decrease in contractile pressure can be ascribed to the decrease in the afferent input caused by TAK-637.

Much evidence has accumulated that capsaicin-sensitive primary afferents play a crucial role in conveying sensory information from the bladder (Sharkey et al., 1983; Holzer-Petsche and Lembeck, 1984; Maggi et al., 1986a). It should be noted that most of the capsaicin-sensitive afferent neurons are tachykinin- and calcitonin gene-related peptide-containing afferents and that substance P is an endogenous ligand with a preference for tachykinin NK_1 receptors (review: Otsuka and Yoshioka, 1993; Maggi, 1991). Therefore, a tachykinin NK_1 receptor antagonist could be expected to have the same effects as capsaicin-induced desensitization. Indeed, this was confirmed by the intrathecal (i.t.) administration of tachykinin NK_1 receptor antagonists. The i.t. administration of tachykinin NK_1 receptor antagonists (Lecci et al., 1993a; Maggi et al., 1993) and the systemic administration of TAK-637 in the present study suppressed the distension-induced rhythmic bladder contractions without affecting the amplitude of the distension-induced rhythmic bladder contractions, supporting the notion that tachykinins, via spinal tachykinin NK_1 receptors, function as sensory transmitters for bladder distension. In addition, tachykinin NK_1 receptors are also implicated in nociceptive sensory transmission from the bladder because TAK-637 inhibited the capsaicin-induced micturition reflex in the present study.

Increasing bladder capacity or volume threshold is believed to be of clinical benefit in patients with abnormally frequent micturition or incontinence. We have reported that TAK-637 and drugs for detrusor overactivity, such as oxybutynin, increased the volume threshold in guinea pigs, and we also considered the possible clinical utility of these agents in the treatment of detrussor overactivity (Doi et al., 1999). We have now clearly demonstrated that the mechanisms of TAK-637 action are different from those of drugs for detrusor overactivity. TAK-637 has no effect on the efferent pathway and is suggested to inhibit the afferent system via tachykinin NK_1 receptors, possibly at the spinal level, leading to the increase in the volume threshold because neither peripheral NK_1 nor NK_2 receptors are suggested to play a role in bladder contractions of reflex origin (Lecci et al., 1993b).

Antispasmolytics such as oxybutynin are limited in their efficacy for inhibiting bladder overactivitiy and it is now well recognized that the sensory pathway is important for the etiology of detrusor overactivity (Klein, 1988). Intravesical capsaicin or resiniferatoxin, which is an ultrapotent capsaicin analogue (Winter et al., 1990), is effective for the treatment of human overactive detrusor (Fowler et al., 1994; Wiart et al., 1998; Lazzeri et al., 1997). In addition, increases in the substance P-containing fibers are reported in unstable human bladder (Smet et al., 1997) and interstitial cystitis (Pang et al., 1995). Taken together, these clinical findings support the idea that alteration of the sensory system might offer a pharmacologically unique mode of therapy for unstable detrusor, which would not cause the voiding difficulties that may occur with treatment involving spasmolytics. TAK-637 is expected to be the first in a new class of drugs for lower urinary tract dysfunction.

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