Gastric Hyperemic Response During Vagally Mediated Acid Secretion by TRH Analog in Rats

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ABSTRACT

The mechanisms of gastric hyperemic response during vagally mediated acid secretion induced by YM-14673, an analog of thyrotropin-releasing hormone, were investigated in urethane-anesthetized rats. The stomach was mounted on an ex vivo chamber and perfused with saline, and gastric mucosal blood flow (GMBF) was measured using a laser Doppler flowmeter, simultaneously with acid secretion. The i.v. injection of YM-14673 (0.1 – 1 mg/kg) increased both GMBF and acid secretion in a dose-dependent manner, and these responses persisted during a 90-min test period. The increases in GMBF and acid secretion induced by YM-14673 (0.3 mg/kg) were totally abolished by either bilateral vagotomy or atropine. Sensory ablation by capsaicin also significantly attenuated GMBF response without affecting acid secretion. On the other hand, N²-nitro-L-arginine methyl ester (L-NAME), but not D-NAME, significantly attenuated the increase in GMBF in an L-arginine-sensitive manner, although acid secretion was slightly augmented; in particular, gastric hyperemic response during the first 30 min (early period) was almost totally abolished. In contrast, omeprazole significantly attenuated GMBF response only in the late period, although it completely inhibited acid secretion in response to YM-14673. Combined treatment of omeprazole and L-NAME totally abolished hyperemic responses induced by YM-14673 during the test period. YM-14673 significantly elevated the release of nitrate and nitrite into the gastric lumen, and this response was inhibited by either atropine or L-NAME. These results suggest that YM-14673 increases GMBF as well as acid secretion, mediated by vagal-cholinergic pathways, and that gastric hyperemia is further regulated by two distinct mechanisms. The response in the early period is independent of acid secretion and mediated mainly by nitric oxide, whereas that in the later period occurs in association with acid secretion and may be mediated by nitric oxide and sensory neurons.

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ABBREVIATIONS: GMBF, gastric mucosal blood flow; PG, prostaglandin; TRH, thyrotropin-releasing hormone; NO, nitric oxide; NOx, nitrate and nitrite; L(D)-NAME, N²-nitro-L-D-arginine methyl ester.

Materials and Methods

Male Sprague-Dawley rats (220–250 g, Charles River, Atsugi, Japan) kept in individual cages with mesh bottoms were deprived of food for 24 h before and during experiments. To ensure homeostasis, the animals were kept in a temperature-controlled room on a 12-h light-dark cycle. The animals received water ad libitum. The stomach was removed, mounted on an ex vivo chamber and perfused with saline, and gastric mucosal blood flow (GMBF) was measured using a laser Doppler flowmeter, simultaneously with acid secretion. The i.v. injection of YM-14673 (0.1 – 1 mg/kg) increased both GMBF and acid secretion in a dose-dependent manner, and these responses persisted during a 90-min test period. The increases in GMBF and acid secretion induced by YM-14673 (0.3 mg/kg) were totally abolished by either bilateral vagotomy or atropine. Sensory ablation by capsaicin also significantly attenuated GMBF response without affecting acid secretion. On the other hand, N²-nitro-L-arginine methyl ester (L-NAME), but not D-NAME, significantly attenuated the increase in GMBF in an L-arginine-sensitive manner, although acid secretion was slightly augmented; in particular, gastric hyperemic response during the first 30 min (early period) was almost totally abolished. In contrast, omeprazole significantly attenuated GMBF response only in the late period, although it completely inhibited acid secretion in response to YM-14673. Combined treatment of omeprazole and L-NAME totally abolished hyperemic responses induced by YM-14673 during the test period. YM-14673 significantly elevated the release of nitrate and nitrite into the gastric lumen, and this response was inhibited by either atropine or L-NAME. These results suggest that YM-14673 increases GMBF as well as acid secretion, mediated by vagal-cholinergic pathways, and that gastric hyperemia is further regulated by two distinct mechanisms. The response in the early period is independent of acid secretion and mediated mainly by nitric oxide, whereas that in the later period occurs in association with acid secretion and may be mediated by nitric oxide and sensory neurons.
food but allowed free access to tap water for 18 hr before the experiments. Studies were carried out using five rats per group.

Measurement of GMBF and acid secretion. The animals were anesthetized with urethane (1.25 g/kg) given i.p., and a tracheotomy was performed to ensure a patent airway. GMBF and acid secretion were measured simultaneously according to a previously published method (Kato et al., 1993). In brief, the stomach was exposed and mounted in an ex vivo chamber, and the mucosa was perfused with saline at a flow rate of 0.8 ml/min using two peristaltic pumps (Mitsumine Sci., Tokyo, Japan). The perfusate was kept in a reservoir heated to 37°C, and acid secretion was measured at pH 7.0 using a pH-stat method (Hiranuma Comtite-8, Tokyo, Japan) and by adding 0.1 N NaOH to the reservoir. GMBF was measured by a laser Doppler flowmeter (ALF-21, Advance, Tokyo, Japan) and by placing a probe gently on the surface of the corpus mucosa using a balancer (Medical Agent, Kyoto, Japan). Blood pressure was measured in the femoral artery by a pressure transducer and amplifier system (TP-200TL, AP-100F, RTA-1100A Nihon Koden). In some cases, the effect of prazosin on blood pressure changes induced by YM-14673 was examined. Prazosine (0.5 mg/kg) was administered s.c. 30 min before YM-14673 (0.3 mg/kg i.v.). The body temperature was kept around 36°C ± 1°C using a heating lamp.

Experimental protocol. At least 1 hr after both GMFB and acid secretion had stabilized, YM-14673 was administered i.v. into tail vein in doses of 0.1–1 mg/kg as a single injection. The effects of the following agents on both GMFB and acid secretary responses induced by YM-14673 (0.3 mg/kg i.v.) were examined: atropine sulfate (atropine, 3 mg/kg), omeprazole (30 mg/kg), L-NAME (10 mg/kg), D-NAME (inactive enantiomer of L-NAME, 10 mg/kg). Atropine and omeprazole were given s.c. and i.p., respectively, 30 min before injection of YM-14673, whereas L-NAME or D-NAME was given i.v. into tail vein 10 min before YM-14673 treatment. In the case of L-NAME, half of the animals were administered L-arginine (500 mg/kg) i.p. 20 min before L-NAME (Takeuchi et al., 1997). Chemical ablation of sensory neurons was performed according to a method published previously (Matsumoto et al., 1992). Briefly, the animals were injected with capsaicin s.c. once daily for 3 consecutive days (20, 30 and 50 mg/kg) 2 weeks before the experiments. All capsaicin injections were done under ether anesthesia, and the rats were pretreated i.m. with terbutaline (0.1 mg/kg) and aminophylline (10 mg/kg) to prevent respiratory impairment. The effectiveness of the treatment was tested by examining the protective wiping of the eye. Vagotomy was performed bilaterally at the neck.

Measurement of NOx release into the gastric lumen. Under urethane anesthesia, the rat stomach was mounted in an ex vivo chamber, 2 ml of saline was instilled in the chamber, and, 30 min later, the gastric contents were recovered. This procedure was repeated every 30 min, two times before and four times after i.v. injection of YM-14673 (0.3 mg/kg). Gastric output of NOx was measured in aliquots of the gastric contents by the Griess method after reduction of nitrate to nitrite with nitrate reductase (from Aspergillus; Sigma Chemical Co., St. Louis, MO). Nitrites were incubated with Griess reagent (0.1% naphthylene diamine dihydrochloride and 1% sulfanilamide in 2.5% H3PO4) for 10 min at room temperature, and the absorbance at 550 nm was measured (Green et al., 1982).

Preparation of drugs. Drugs used were urethane (Tokyo Kasei, Tokyo, Japan), capsaicin, atropine and prazosine (Wako, Osaka, Japan), L-NAME, D-NAME and L-arginine (Sigma), YM-14673 (Yamanouchi Pharmaceutical Co., Tokyo, Japan) and omeprazole (Hassel Co., Mandal, Sweden). Omeprazole was suspended in 0.5% carboxymethylcellulose (CMC) (Nacalai, Kyoto, Japan) solution, and other drugs were dissolved in saline. Each drug was prepared immediately before use and was administered i.p. or s.c. in a volume of 0.5 ml/100 g b.wt. and i.v. in a volume of 0.1 ml/100 g b.wt.

Statistics. Data are presented as the mean ± S.E. from five rats per group. Statistical analyses were performed using a two-tailed Dunnett’s multiple comparison test, and values of P < .05 were regarded as significant.

**Results**

Effects of YM-14673 on GMFB and Acid Secretion

Under urethane anesthesia, acid secretion was maintained in a range of about 3 to 5 µEq/10 min with stable GMFB values (an arbitrary unit; 100 mV) during a test period. The i.v. injection of YM-14673 (0.1 ~ 1 mg/kg) caused an increase in acid secretion and GMFB in a dose-dependent manner (fig. 1). At 0.3 mg/kg, YM-14673 increased acid secretion from 5 ± 0.7 µEq/10 min to 20.0 ± 2.7 µEq/10 min, and GMFB to 149.2 ± 33.6% of basal values, at 60 min after treatment, and these responses persisted for over 90 min. The magnitude of these responses was lower than that induced by 1 mg/kg of YM-14673 but much higher than that induced by 0.1 mg/kg of this agent. The following experiments were performed using YM-14673 at a dose of 0.3 mg/kg, which stimulated acid secretion submaximally.

![Fig. 1. Effect of YM-14673 (0.1–3 mg/kg) on GMFB and acid secretion in the anesthetized rats. The agent and vehicle were given i.v. into tail vein as a bolus injection. GMFB is expressed as % increase from basal values, acid secretion as µEq/10 min. Data are presented as the mean ± S.E. of values determined every 10 min from five rats per group.](image)
Effects of Various Treatments on Acid Secretion and GMBF Responses Induced by YM-14673

Effects of atropine, sensory ablation and vagotomy. The i.v. injection of YM-14673 at a dose of 0.3 mg/kg produced a substantial increase in GMBF and in acid secretion (fig. 2). The increase in acid secretion induced by YM-14673 was totally inhibited by bilateral vagotomy and atropine (3 mg/kg s.c.), the inhibition being 102.1% and 92.1%, respectively, at 60 min after injection of YM-14673. Likewise, these treatments almost totally attenuated gastric hyperemia in response to YM-14673, the inhibition being 104.9% and 87.3%, respectively. On the other hand, chemical ablation of sensory neurons by capsaicin had no effect on acid secretory response but significantly suppressed the increase in GMBF during acid stimulation caused by YM-14673; the inhibitory effect was more prominent in the later period, i.e., from 30 min after YM-14673 injection.

Effects of L-NAME. Pretreatment of the animals with L-NAME (10 mg/kg i.v.) significantly suppressed the increase in GMBF during acid secretion (fig. 3). The inhibitory effect of L-NAME on the GMBF response induced by YM-14673 was more marked in the early period (the first 30 min after YM-14673) and gradually weakened in the later period; the inhibition was 77.1% and 40.2%, respectively, at 30 min and 60 min after injection of YM-14673 (fig. 4). Acid secretion induced by YM-14673 was enhanced by L-NAME; the acid output obtained for the second 30-min period was 67.4 ± 8.4 μEq/30 min, which is significantly greater than that (42.5 ± 6.6 μEq/30 min) observed in control rats. These effects of L-NAME on GMBF and acid secretory responses induced by YM-14673 were significantly antagonized by co-administration of L-arginine (500 mg/kg i.p.), although L-arginine alone at this dose did not have any effect on basal acid secretion and GMBF (not shown). On the other hand, D-NAME (10 mg/kg i.v.) had no effect on the increase of either GMBF or acid secretion in response to YM-14673.

Effect of omeprazole. Gastric acid secretion induced by YM-14673 was completely inhibited by prior administration of omeprazole (60 mg/kg s.c.), resulting in fluctuations at the

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Fig. 2. Effects of vagotomy, atropine and sensory deafferentation (CAP) on the increase in GMBF and acid secretion induced by i.v. injection of YM-14673 (0.3 mg/kg) in the anesthetized rats. Atropine (3 mg/kg) was administered s.c. 30 min before YM-14673 injection. Vagotomy was performed acutely at the cervical portion 1 hr before YM-14673 injection, whereas CAP entailed three consecutive injections of capsaicin 2 weeks before the experiment. GMBF is expressed as % increase from basal values, acid secretion as μEq/10 min. Data are presented as the mean ± S.E. of values determined every 10 min from five rats per group. *Significant difference from control at P < .05.

Fig. 3. Effects of L-NAME, of L-NAME plus L-arginine and D-NAME on the increase in GMBF and acid secretion induced by i.v. injection of YM-14673 (0.3 mg/kg) in the anesthetized rats. L-NAME (10 mg/kg) or D-NAME (10 mg/kg) was administered i.v. 10 min before YM-14673 injection, whereas L-arginine (500 mg/kg) was administered i.p. 20 min before L-NAME. GMBF is expressed as % increase from basal values, acid secretion as μEq/10 min. Data are presented as the mean ± S.E. of values determined every 10 min from five rats per group. *Significant difference from control at P < .05.
base-line levels before and after injection of YM-14673 (fig. 5). Although omeprazole also caused a significant inhibition of the gastric hyperemic response induced by YM-14673, this inhibition was not evident in the first 50 min; rather, it was apparent in the later period, i.e., from 60 min after YM-14673 treatment. On the other hand, the combined treatment with omeprazole plus L-NAME almost completely attenuated the GMBF response and the acid secretion after injection of YM-14673, the inhibition of the GMBF response being 89.3% and 95.5%, respectively, at 30 and 60 min after YM-14673 injection from five rats per group. *Significant difference from control at P < .05.

Changes in Luminal NOx Levels Induced by YM-14673

Under basal conditions, the stomach released a substantial amount of NOx in the lumen, the value being 13.5 ± 0.1 nmol/30 min (fig. 6). The luminal release of NOx was significantly enhanced by i.v. injection of YM-14673 (0.3 mg/kg) and reached 54.8 ± 4.0 nmol/30 min at 60 min after YM-14673 injection. This increase in NOx release in the lumen was completely inhibited by bilateral vagotomy and pretreatment with either atropine (3 mg/kg i.v.) or L-NAME (10 mg/kg i.v.); the values in the latter two cases were 17.4 ± 1.7 and 15.6 ± 0.6 nmol/30 min, respectively, at 60 min after injection of YM-14673.

Changes in Systemic Blood Pressure Induced by YM-14673

Mean systemic blood pressure was 75 ± 90 mmHg in control rats under basal conditions. The i.v. injection of YM-14673 (0.3 mg/kg) significantly elevated blood pressure from 80.0 ± 2.2 mmHg to 92.0 ± 2.2 mmHg. This pressor response was inhibited by prazosine $\alpha_1$-receptor antagonist, the value observed at 15 min after administration of YM-14673 being 79.7 ± 2.6 mmHg, although this agent had no effect the GMBF response to YM-14673 (not shown). On the other hand, the increase in blood pressure caused by YM-14673 was not affected by bilateral vagotomy, sensory deafferentation, atropine, omeprazole or D-NAME; the values observed at 15 min after YM-14673 injection were 94.4 ± 5.3 mmHg, 90.0 ± 4.8 mmHg, 88.8 ± 6.6 mmHg, 87.2 ± 2.3 mmHg and 91.6 ± 3.4 mmHg, respectively, which are not significantly different from those observed in control rats given YM-14673 alone. A marked elevation of blood pressure was also observed after administration of L-NAME (10 mg/kg i.v.: 47.3 ± 4.1% of basal values), but additional treatment with YM-14673 did not further elevate systemic blood pressure. The hypertensive response caused by L-NAME was significantly antagonized by co-administration of l-arginine (500 mg/kg i.p.), the inhibition being 70.8%.
glycine or NaHCO₃ to buffer or neutralize H⁺, respectively. However, Thiefin et al. (1989) showed that gastric hyperemia induced by central injection of TRH was not secondary to stimulation of acid secretion, because this hyperemic response was observed even in the absence of acid secretion under pretreatment with omeprazole. In the present study, omeprazole had no effect on the increase in GMBF during the first 50 min, although acid secretion was completely inhibited, and the hyperemic response induced by YM-14673 significantly attenuated, in the later period, from 50 min after YM-14673 treatment. Thus different mechanisms may be involved in gastric hyperemic response during acid secretion, depending on whether the secretion is induced peripherally or centrally mediated by vagus nerves, because gastric hyperemic response induced by the latter was not abolished even in omeprazole-treated animals. The discrepancy between our results and those of Thiefin et al. (1989) may be due to different experimental conditions, such as the method of GMBF or the drugs used. The GMBF in the present study was continuously determined by laser Doppler flowmeter, whereas in their study it was measured intermittently (every 30 min) by hydrogen gas clearance. Furthermore, YM-14673 showed a long-lasting stimulatory effect on acid secretion, when compared with RX-77368 and TRH itself. It may be assumed that the mechanisms underlying gastric hyperemia induced by YM-14673 involved different factors from those reported for the hyperemic response observed with other TRH analogs. Indeed, the gastric hyperemic response induced by either central injection of TRH or electrical vagal stimulation did tend to be reduced by omeprazole (Thiefin et al., 1989; 1990).

It should be noted that in the present study, the gastric hyperemic response induced by YM-14673 consisted of two periods, judging from the effect of omeprazole; the hyperemic response in the early period was independent of acid secretion, whereas that in the later period was totally dependent on acid secretion and completely inhibited by omeprazole. Tanaka et al. (1993) reported that the increase in GMBF induced by RX-77368 was completely inhibited by l-NAME in an l-arginine-sensitive manner without any effects on acid secretion, which suggests the involvement of endogenous NO in this response. The present study, however, showed that gastric hyperemia induced by YM-14673 was almost totally attenuated by l-NAME in the early period, whereas that in the later period was completely inhibited only when omeprazole was given together with l-NAME. These results suggest that the increase in GMBF induced by YM-14673 is mediated mainly by NO in the early period, whereas in the later period depends on the process of acid secretion, in addition to NO. It has been reported that the increase in GMBF induced by pentagastrin was totally dependent on acid secretion, the process being partly mediated by NO (Kato et al., 1997a; Pique et al., 1991). These findings may suggest that endogenous NO is involved in gastric hyperemia during acid secretion, irrespective of whether the secretion is induced peripherally or centrally through vagus nerves.

We found in this study that YM-14673 increased the release of NOx, a stable metabolite of NO, into the gastric lumen, a result consistent with the observation by Saperas et al. (1995). Such releases of NOx into the gastric lumen were completely abolished by either l-NAME or atropine as well as by vagotomy, which suggests that this NOx release in re-

![GMBF Response During Acid Secretion](https://example.com/gmbf-graph.png)

**Discussion**

The present study confirmed, using YM-14673, a TRH analog, that gastric hyperemia during vagally mediated acid secretion was totally dependent on vagal nerves and that the mechanisms involved the vagal-cholinergic pathway. It further showed that this hyperemic response was mediated by distinct mechanisms, depending on the period. Gastric hyperemia in the early period, the first 30 min after YM-14673 injection, may be mediated mainly by NO, whereas that in the late period is closely associated with acid secretion and is possibly mediated partly by either NO or sensory neurons.

YM-14673, a potent analog of TRH, has various effects on the GI tract, cardiovascular system, and CNS (Horita et al., 1986; Fujiwara and Ida, 1989; Shimizu et al., 1989). In contrast to TRH, the effect of YM-14673 can be observed irrespective of whether the agent is given by peripheral or central administration (Fujiwara and Ida, 1989; Shimizu et al., 1989). Indeed, i.v. administration of YM-14673 produced a marked increase in GMBF and acid secretion in rats. It has been reported that central injection of TRH and RX-77368 produced a vagally mediated increase in acid secretion (Tache et al., 1980; 1985) and GMBF (Okuma et al., 1987; Raybould et al., 1990) in rats. We also reported previously that the HCO₃⁻ stimulatory effect of YM-14673 in the gastroduodenal mucosa was completely attenuated by bilateral vagotomy (Takeuchi et al., 1990; 1991). Likewise, the present study showed that the gastric hyperemic response induced by YM-14673 was totally abolished by vagotomy, which suggests that vagal nerves play an important role in acid secretion, when compared with RX-77368 and TRH.

In their study, the gastric hyperemia induced by RX-77368 was completely inhibited by L-NAME in an L-arginine-sensitive manner without any effects on acid secretion, which suggests the involvement of endogenous NO in this response. The present study, however, showed that gastric hyperemia induced by YM-14673 was almost totally attenuated by L-NAME in the early period, whereas that in the later period was completely inhibited only when omeprazole was given together with L-NAME. These results suggest that the increase in GMBF induced by YM-14673 is mediated mainly by NO in the early period, whereas in the later period depends on the process of acid secretion, in addition to NO. It has been reported that the increase in GMBF induced by pentagastrin was totally dependent on acid secretion, the process being partly mediated by NO (Kato et al., 1997a; Pique et al., 1991). These findings may suggest that endogenous NO is involved in gastric hyperemia during acid secretion, irrespective of whether the secretion is induced peripherally or centrally through vagus nerves.

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response to YM-14673 is dependent on NO production and occurs via vagal-cholinergic pathways. On the other hand, it has been reported that capsaicin-sensitive sensory neurons and CGRP play a role in gastric hyperemic responses induced by intracisternal injection of TRH analog (Raybould et al., 1990; Kiraly et al., 1994). The present study also found that such GMBF responses induced by YM-14673 were significantly reduced, more evidently in the later period, by chemical deafferentation of sensory neurons. We recently showed that the increase in GMBF during acid secretion induced by pentagastrin is totally dependent on luminal H+ and is mediated by NO and prostaglandins as well as sensory neurons (Kato et al., 1997a). Tepperman and Whittle (1992) reported that endogenous NO and sensory neuropeptides interact with each other in the regulation of gastric microrcirculation and the modulation of mucosal integrity. It has been also proposed that intracisternal injection of TRH or its analog induces a vagal cholinergic-mediated release of PGs (Yoneda and Tache, 1993). PGs are known to stimulate sensory neurons, resulting in the release of sensory neuropeptides such as CGRP (Lundberg et al., 1992), which increases GMBF in an effect partly mediated by NO (Holzer et al., 1993). It is assumed that a vagal-dependent increase in GMBF during acid secretion induced by YM-14673, similar to pentagastrin, may be mediated by a complex mechanism involving PGs and CGRP in addition to NO. Tanaka et al. (1997) recently reported that the increase in GMBF induced by TRH analog was not affected by indomethacin, although the mucosal defensive mechanisms in relation to the central vagal activation by TRH are mediated by both PG-dependent and PG-independent pathways. Further studies are certainly needed to clarify the role of PGs in the GMBF response during acid secretion induced by TRH.

In the present study, we also observed that the acid secretory response to YM-14673 was slightly increased by pretreatment with l-NAME or sensory deafferentation. The results with l-NAME seem to be consistent with previous observations using NO synthase inhibitors, which showed an inhibitory role of NO in the regulation of acid secretion (Martinez-Cuesta et al., 1992; Takeuchi et al., 1995; Kato et al., 1997b). Indeed, NO has been shown to be capable of inhibiting acid secretion in isolated parietal cells (Brown et al., 1993). Because the levels of NOx in the lumen were increased after the administration of YM-14673, it is possible that l-NAME enhanced the acid secretion by removing an inhibitory effect of NO. On the other hand, the effects of capsaicin and sensory neurons on acid secretion remain controversial. Esplugues et al. (1990) reported that chemical deafferentation did not significantly modify the acid secretory response to peripheral secretagogues, whereas others showed a considerable reduction in acid secretion in sensory deafferented rats (Alfoldi et al., 1986; Evangelista et al., 1989; Raybould and Tache, 1989). We also reported that sensory deafferentation did not significantly affect either basal or histamine-induced acid secretion (Takeuchi et al., 1994). This discrepancy persists in the present study, but this may be due to different experimental conditions. In any case, it seems that sensory neurons play a minor role in modulation of acid secretion induced peripherally or centrally via vagus nerves. In conclusion, the present study showed that YM-14673, a peripherally active TRH analog, produced an increase in GMBF and acid secretion via a mechanism totally dependent on the vagal-cholinergic neurons. We also showed that this GMBF response during vagally mediated acid secretion involved distinct mechanisms, depending on the time period. The hyperemic response is mediated mainly by NO in the early period, the first 30 min after YM-14673 injection, whereas that in the late period is closely associated with acid secretion and is mediated, at least partly, by both NO and sensory neurons.

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