Clinical Investigations

Cholinergic Stimulation Improves Autonomic and Hemodynamic Profile During Dynamic Exercise in Patients With Heart Failure

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ABSTRACT

Background: Parasympathetic dysfunction is an independent risk factor for mortality in heart failure for which there is no specific pharmacologic treatment. This article aims to determine the effect of pyridostigmine, an anticholinesterase agent, on the integrated physiologic responses to dynamic exercise in heart failure.

Methods and Results: Patients with chronic heart failure (n = 23; 9 female; age = 48 ± 12 years) were submitted to 3 maximal cardiopulmonary exercise tests on treadmill in different days. The first test was used for adaptation and to determine exercise tolerance. The other tests were performed after oral administration of pyridostigmine (45 mg, 3 times/day, for 24 hours) or placebo, in random order. All patients were taking their usual medication. Pyridostigmine reduced cholinesterase activity by 30%, inhibited the chronotropic response throughout exercise, up to 60% of maximal effort (pyridostigmine = 108 ± 3 beats/min vs. placebo = 113 ± 3 beats/min; $P = .040$), and improved heart rate reserve (pyridostigmine = 73 ± 5 beats/min vs. placebo = 69 ± 5 beats/min; $P = 0.035$) and heart rate recovery in the first minute after exercise (pyridostigmine = 25 ± 2 beats/min vs. placebo = 22 ± 2 beats/min; $P = .005$), whereas peak heart rate was similar to placebo. Oxygen pulse, an indirect indicator of stroke volume, was higher under pyridostigmine during submaximal exercise.

Conclusions: Pyridostigmine was well tolerated by heart failure patients, leading to improved hemodynamic profile during dynamic exercise. (*J Cardiac Fail 2009;15:124–129*)

Key Words: Autonomic nervous system, vagal, acetylcholinesterase, oxygen pulse.

Despite the improvements in heart failure treatment, its prognosis remains poor, with an overall mortality rate of 80% in the first 8 years after diagnosis. In particular, sudden cardiac death in heart failure patients is 6 to 9 times greater than in general population.[1] Several mechanisms may contribute to sudden death in heart failure patients, such as myocardial ischemia, prolongation of action potentials, genetic predisposition, alterations in calcium homeostasis, abnormal stimulus conduction, and altered neurohumoral signaling. Most of these mechanisms are highly influenced by autonomic nervous system dysfunction.[2]

Different studies have shown that sympathetic hyperactivity[3] as well as parasympathetic dysfunction[4] are independent risk factors in heart failure. Investigations to counteract sympathovagal dysfunction have been concentrated mainly on reducing sympathetic effects, which led to the widespread use of β-blockers in heart failure.[5]

Exercise training is a known therapeutic measure that shows great potential in reducing mortality and morbidity.[6] Indeed, the improvement of sympathovagal balance is an important mechanism implicating in better prognosis for heart failure patients who exercise regularly.[7] However, heart failure patients' adherence to long-term programs involving exercise training may be as low as 50%.[8] In addition, an unknown proportion of patients submitted to exercise training may not develop beneficial autonomic adaptations.[6]

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In this context, different investigations aimed to find a pharmacologic option to counteract parasympathetic dysfunction in heart failure. Transdermal scopolamine increased heart rate variability in some patients with advanced congestive heart failure, but this favorable autonomic modulation was not reproducible in all patients.[9] Another study found similar heart rate variability improvement during the use of transdermal scopolamine in patients with mild to moderate heart failure, without impairment of exercise tolerance and without any effects on the incidence and severity of ventricular arrhythmias.[10–13] Scopolamine failed to prevent ventricular fibrillation during physical exertion in dogs with artificially induced coronary artery occlusion.[14] Higher doses of scopolamine could not be used in this context as it becomes a vagolytic agent.[15] More than 10 years have passed since the first attempts to find a pharmacologic treatment to counteract parasympathetic withdrawal in heart failure. However, so far no parasympathetic agonist is part of the usual prescription of a heart failure patient.

Pyridostigmine bromide is a reversible anticholinesterase agent (ie, an indirect vagomimetic effect) used for the treatment of myasthenia gravis because of its action on the motor plate in skeletal muscle. Concerning the cardiovascular system, cholinergic stimulation improves autonomic and hemodynamic profile of patients with coronary artery disease during exercise. In this group of patients, pyridostigmine improved peak exercise tolerance and inhibited the chronotropic responses at submaximal exercise, increasing the intensity at which myocardial ischemia occurred.[16] In some of these patients, pyridostigmine also corrected the previously abnormal heart rate recovery after peak exercise.[16] In patients with heart failure, pyridostigmine augmented heart rate variability and decreased the density of ventricular arrhythmias. However, the systemic effect of cholinergic stimulation during exercise has not been systematically studied in heart failure. Considering that acute exercise is a natural physiologic challenge that may disclose autonomic impairment and that reduced peak oxygen uptake is an important risk marker also in patients with heart failure, it is important to investigate the effects of any potential treatment on the hemodynamic profile during exercise. Therefore the purpose of this study was to determine the effects of short-term treatment with pyridostigmine on the integrated physiologic responses during dynamic exercise in patients with heart failure.

Methods

Patients

Patients with idiopathic heart failure who were clinically stable and in sinus rhythm were invited to participate in this study. The exclusion criteria were occurrence of myocardial infarction, hospitalization, or change of medication in the last 2 months, the presence of diabetes mellitus, atrioventricular blocks, implanted pacemaker, alcoholism, chronic pulmonary obstructive disease, urinary retention, constipation, and intolerance to pyridostigmine. All participants continued taking their usual medication throughout the study period. They were instructed to avoid alcohol, beverages containing caffeine, and strenuous physical activity the day before the experiments. All patients gave written informed consent to participate in the study after full explanation of the procedures and their potential risks, and the investigation conformed to the principles outlined in the Declaration of Helsinki and had been approved by the Institutional Research Ethics Committee on Human Research.

Protocol

Each patient underwent, on 3 different days, a maximal exercise test on a treadmill (KT 10400, Inbramed, Porto Alegre, Brazil), according to an individualized ramp protocol, in which the initial and final work rates were set to achieve an estimated test duration of 8 to 12 minutes, considering the individual clinical condition and physical activity habits. The recovery phase after exercise was standardized as a 2.4 km/h walk for at least 2 minutes, during which electrocardiogram and expiratory gases were continuously recorded. The first day was used for adaptation to the equipment and to determine exercise tolerance. The second and third days of the study were separated by a 48-hour period for drug washout and followed a randomized, crossover, double-blind design. During these days, the exercise test was performed with the same protocol after 4 doses (separated by 8 hours each) of pyridostigmine (45 mg) or placebo; the last dose was administered in the laboratory 2 hours before the test. The potential effect of drug sequence was controlled by using a counterbalanced design, where patients were randomly, but evenly (50%, 50%), assigned to placebo-pyridostigmine or pyridostigmine-placebo.

The 12-lead electrocardiogram was continuously monitored throughout the exercise test and recovery (with Mason-Likar electrode placement). Arterial blood pressure was measured with a cuff sphygmomanometer before the exercise test, every minute during exercise, and during the recovery phase.

Expiratory gases and pulmonary ventilation were automatically determined (Teem 100, Aerosport, Ann Arbor, Michigan). Oxygen consumption, carbon dioxide production, and minute ventilation were registered every 20 seconds. Derived variables were calculated online (Aerograph, Aerosport, Ann Arbor, Michigan). Anaerobic threshold was identified by 2 experienced evaluators by the combination of the following methods: (1) at the point of upward inflection of the ventilation vs. time curve; (2) at the beginning of a consistent increase in the ventilatory equivalent for O_2 (minute ventilation/oxygen consumption) without a concomitant increase in the ventilatory equivalent for carbon dioxide (minute ventilation/carbon dioxide production); and (3) at the beginning of an increase in expired oxygen fraction. Ventilatory threshold was considered as the point identified by at least 2 of these three criteria. There was no case in which each of the criteria identified different thresholds.

After the exercise, in second and third days, subjects had venous blood sample withdrawn to determine serum cholinesterase. The samples (5 mL) were rested for 10 minutes to coagulate and than centrifuged at 3000 rpm for 5 minutes. The separated serum was frozen and stored until the analysis. Cholinesterase activity was determined by the reaction of the serum with acetylcholine and dithiobisnitrobenzoate and the measurement of the yielded compound, 2-nitro-5-mercaptobenzoate, by the colorimetric method using an automated system (Cobas Mira Plus, Roche, Switzerland).

Statistical Analysis

The Kolmogorov-Smirnov method was used to determine whether continuous variables were normally distributed. The occurrence of adverse reactions was compared by the McNemar test. Variables recorded before, during, and after each test were compared by a 2-factor analysis of variance with repeated measures, where time (moment of exercise test) and drug (pyridostigmine, placebo) were the main factors. If a significant F value was obtained, analysis of variance was followed by the Student-Newman-Keuls test for pairwise post hoc comparisons. Paired *t*-test was applied to compare the magnitude of heart rate recovery after exercise. Significance was set at $P < .05$. Results are presented as mean ± SE.

Results

Twenty-three patients (9 female) with systolic dysfunction were enrolled in the study. Demographic data of the patients are presented in Table 1.

Serum cholinesterase activity was reduced by 30% after pyridostigmine (pyridostigmine: 5877.8 ± 306.12 mIU/mL) when compared with placebo (8333.5 ± 501.48 mIU/mL; $P < .001$). Some patients reported mild abdominal discomfort and diarrhea (n = 2) and excessive salivation (n = 2) after pyridostigmine ($P = .036$ vs. placebo). All symptoms disappeared after the drug was interrupted.

There was no difference of time to exhaustion between placebo and pyridostigmine. Peak values of ventilatory and hemodynamic data are presented in Table 2. Oxygen pulse was greater after pyridostigmine when compared with placebo during submaximal exercise, but not at peak exercise (Fig. 1.)

As expected, pyridostigmine reduced resting heart rate (68 ± 3 beats/min vs. 76 ± 4; $P < .001$). Concerning the chronotropic response to exercise, pyridostigmine reduced heart rate up to 60% of maximal effort, but did not change peak heart rate (Fig. 2). In addition, heart rate reserve was greater with pyridostigmine than with placebo (pyridostigmine: 73 ± 5 beats/min vs. placebo: 69 ± 5 beats/min; $P = .035$; Fig. 2), as well as heart rate recovery at the first minute after peak exercise (pyridostigmine: 25 ± 2 beats/min vs. placebo: 22 ± 2 beats/min; $P = .005$) denoting a faster heart rate recovery (pyridostigmine: 17.6 ± 1.3% vs. placebo: 15.5 ± 1.4%, $P = .015$; Fig. 2). Although there were no significant differences between conditions in resting, submaximal, or peak arterial systolic blood pressure, pulse pressure was greater under pyridostigmine when compared with placebo at 80% (pyridostigmine: 73 ± 4 mm Hg vs. placebo: 66 ± 4 mm Hg; $P = .003$) and 100% of peak exercise (Fig. 3).

Table 1. Demographic Data of Patients Included in the Study (n = 23)

Age (y)	48 ± 12
Body mass index (kg/m^2)	25.6 ± 3.6
Ejection fraction (%)	29 ± 7
Medication	
β-blockers (%)	91
Angiotensin-converting enzyme (%)	83
Angiotensin receptor inhibitor (%)	8
Digitalis (%)	78
Diuretics different from spironolactone (%)	78
Spironolactone (%)	61
Acetylsalicylic acid (%)	26
Antiarrhythmics (%)	8
Oral anticoagulants (%)	4

Table 2. Peak Exercise Data During Placebo and Pyridostigmine Administration (n = 23)

Variable	Placebo	Pyridostigmine	*P* Value
Time to exhaustion (min)	9.5 ± 0.36	9.6 ± 0.30	.597
VO_2 (mL/kg/min^{-1})	20.5 ± 1.0	20.5 ±1 .1	.962
Ventilation (L/min)	47.7 ± 2.7	48.0 ± 3.0	.816
Oxygen pulse (mL/beat)	10.1 ± 0.7	10.3 ± 0.7	.224
Heart rate (beats/min)	144 ± 4	141 ± 4	.084
Systolic blood pressure (mm Hg)	136 ± 6	141 ± 8	.064
Diastolic blood pressure (mm Hg)	62 ± 3	57 ± 4	.080
Pulse pressure (mm Hg)	74 ± 5	84 ± 6	.003

Discussion

Parasympathetic dysfunction is known to occur in heart failure patients.[17] Recent studies have shown that the pathophysiologic basis to this parasympathetic dysfunction lies on presynaptic mechanisms, such as decreased vagal nerve activity, ganglionic transmission, or altered synthesis of acethylcholine.[18] In addition, animal models of heart failure exhibit an upregulation of muscarinic receptors leading to an increase in its membrane density.[19] Different studies have focused on potential pharmacological options to

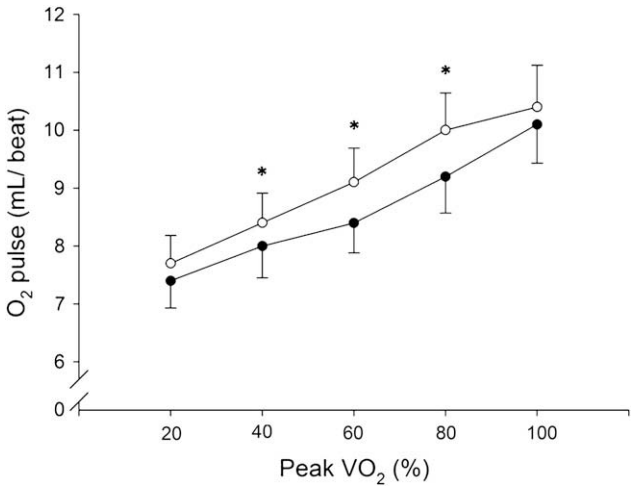

Fig. 1. Oxygen pulse during progressive dynamic exercise in patients with heart failure (n = 23) given pyridostigmine (white circles) or placebo (black circles). The data points correspond to 20%, 40%, 60%, 80%, and 100% of peak oxygen uptake during each condition. At the same percentage of peak oxygen, pyridostigmine vs placebo: *$P < .05$.

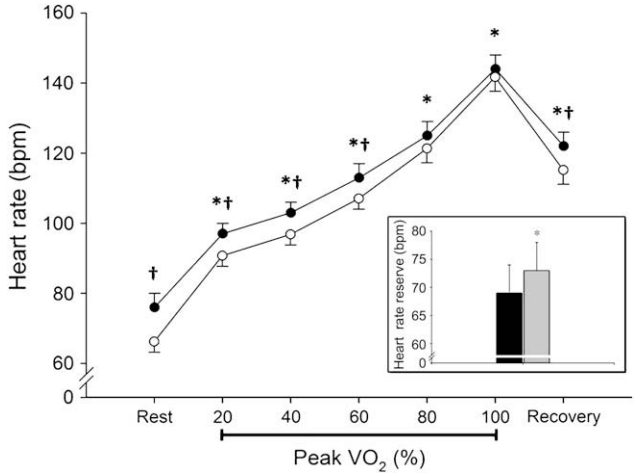

Fig. 2. Heart rate response during rest, progressive dynamic exercise and recovery in patients with heart failure (n = 23) given pyridostigmine (white circles) or placebo (black circles). The data points correspond to rest, 20%, 40%, 60%, 80%, and 100% of peak oxygen uptake, and first minute of recovery during each condition. *$P < .05$ vs. precedent moment within groups; †$P < .05$ placebo vs. pyridostigmine at the same moment. Insert: Heart rate reserve at peak effort in patients with heart failure (n = 23) given pyridostigmine (gray bar) or placebo (black bar). *$P < .05$ vs. placebo at the same moment.

counteract parasympathetic dysfunction in heart failure. Although scopolamine improved autonomic modulation in heart failure patients,[9] it failed to prevent ventricular fibrillation in dogs with artificially induced coronary artery occlusion during exercise.[14] Scopolamine crosses the blood−brain barrier and thus, even if this drug prevented ventricular fibrillation, its long-term use would probably be limited by side effects through action on the central nervous system.[15]

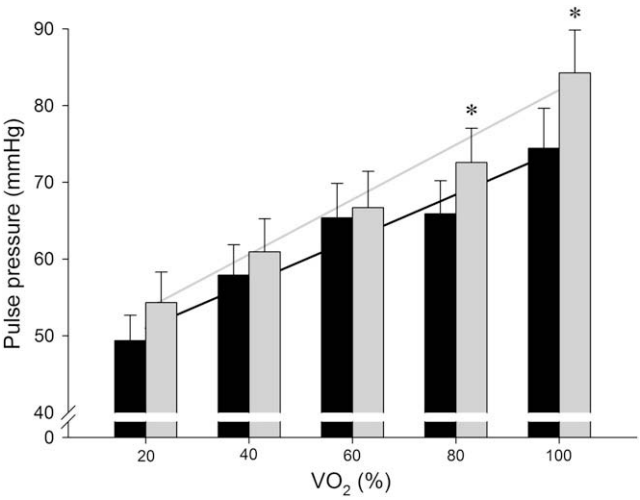

Fig. 3. Pulse pressure response during exercise in patients with heart failure (n = 23) given placebo (black bars) or pyridostigmine (gray bars). Lines indicate linear regression of pulse pressure under placebo (black line) or pyridostigmine (gray line) effect. *$P < .05$ vs. placebo at the same moment.

Pirenzepine is an M_1 selective antagonist devoid of central action, capable of paradoxically augmenting parasympathetic tone when administered at low doses. Hayano et al found that, although pirenzepine showed a vagomimetic effect in some heart failure patients, it did not significantly alter the low frequency/high frequency ratio in these patients.[20] In addition, muscarinic agonists are known to slow atrioventricular conduction[21]; thus, the use of higher doses of pirenzepine would probably not be possible.

None of the pharmacologic alternatives to counteract parasympathetic dysfunction in heart failure is usually prescribed in clinical setting. Despite the interesting results showed with different pharmacologic options, pyridostigmine seems to have several advantages. The vagomimetic effect of pirenzepine and scopolamine are paradoxical (ie, they are vagolytic agents that cause activation of vagal activity only when administered at low doses). The parasympathetic action of pyridostigmine is dose dependent and can be individually adjusted. Additionally, pyridostigmine is administered orally and does not cross the blood−brain barrier under normal conditions.[22]

Pyridostigmine increases the concentration of acetylcholine in the synaptic clefts through reversible inhibition of cholinesterase activity, enhancing parasympathetic effect. The dose of pyridostigmine administered in the present study was several times lower than the one usually prescribed in myasthenia gravis. Nevertheless, it reduced serum cholinesterase activity by 30% and caused significant hemodynamic effects with minor and limited side effects in heart failure patients.

The hemodynamic effects of pyridostigmine were previously investigated at rest and during exercise. Pyridostigmine reduced resting and exercise heart rate in healthy[23,24] and coronary artery disease patients.[16] When administered in 8-hour intervals, pyridostigmine (30 mg) caused sustained bradycardia and augmented heart rate variability in patients with heart failure.[25]

Our group has previously shown that pyridostigmine increased exercise tolerance in coronary artery disease patients.[16] In the present study, although pyridostigmine was not capable of increasing exercise tolerance in heart failure patients, it inhibited the chronotropic responses to exercise up to 60% of peak exercise. There is still controversy about the prognostic value of the rapidity of heart rate increase during exercise.[26] In patients with coronary artery disease, Falcone et al concluded that a marked heart rate increase at the onset of exercise could be useful as an independent predictor of adverse cardiac events, including death.[27] On the other hand, Leeper et al found that a rapid initial heart rate increase during exercise was associated with improved survival in a population of patients referred for exercise testing, but not in coronary artery disease patients.[28] The divergence between these 2 studies may have been caused by methodologic issues such as type of ergometer, exercise protocol, and exercise body position. The prognostic value of heart rate increase at the onset of exercise in patients with heart failure has not been previously studied. Because

heart rate response at the onset of exercise is mediated by the withdrawal of parasympathetic tone, the blunted heart rate response during submaximal exercise seems to indicate an enhancement of parasympathetic function in heart failure patients under pyridostigmine.

The effects of pyridostigmine on heart rate response to exercise can also be expressed by an increased reserve (ie, peak-resting). Although no previous study has investigated the prognostic power of this variable in cardiac failure, Myers et al[29] have recently published a follow-up of almost 2000 men who underwent maximal exercise testing for clinical reasons, and found heart rate reserve to be a strong predictor of cardiovascular death. In this scenario, pyridostigmine effect on heart rate reserve is potentially beneficial to heart failure patients.

Heart rate recovery after exercise is a known marker of parasympathetic activity. An attenuated heart rate recovery after exercise indicates greater mortality risk in healthy subjects[30] and patients with coronary heart disease with preserved left ventricular function.[31] Bilsel et al[32] showed that attenuated heart rate recovery was a reliable index of the severity of exercise intolerance in heart failure patients. Arena et al[33] concluded that heart rate recovery was an independent prognostic marker of patients with heart failure. More recently, similar results were found in heart failure patients under β-blocker treatment.[34] Pyridostigmine has corrected the heart rate recovery of 2 patients with coronary artery disease whose heart rate recovery after exercise was abnormal.[16] Androne et al[35] expanded these findings showing that a single 30-mg dose of pyridostigmine increased heart rate recovery at 1 minute after exercise in heart failure (ischemic or not). This effect was corroborated by our study, in which patients with idiopathic heart failure were evaluated after a 24-hour administration of the same drug. The improved heart rate recovery in the present study may indicate a protective role of pyridostigmine when used in patients with idiopathic heart failure.

Although not altering resting blood pressure, pyridostigmine caused higher pulse pressure values during maximal effort. Although an elevated pulse pressure is related to increased cardiovascular risk in some conditions, this seems not to be true in all heart failure patients. In patients with ischemic left ventricular systolic dysfunction, an increased pulse pressure predicts total and cardiovascular mortality.[36] On the other hand, a low pulse pressure indicates worst prognosis in patients with advanced chronic heart failure[37] and in patients with non-ischemic heart failure.[38] The different value of pulse pressure as a prognosis indicator in the various populations is explained by the complex interactions between arterial stiffness and cardiac output. Pulse pressure amplification during exercise is related to age and hypercholesterolemia, confirming the relationship between arterial stiffness and greater pulse pressure.[39] Although arterial stiffness was not measured in the present study, there is no reason to believe that pyridostigmine, a cholinergic agent, would cause any change in arterial stiffness. Consequently, it seems that the greater pulse pressure found in nonischemic heart failure during maximal effort is an indicator of enhanced systolic volume and cardiac function. Indeed, oxygen pulse, an indirect measure of systolic volume during exercise, was greater during pyridostigmine use at 60% and 80% of peak exercise consumption. This is consistent with a previous study, where pyridostigmine resulted in greater oxygen pulse during exercise when administered to patients with coronary artery disease.[16] In the present study, this effect occurred regardless of oxygen uptake, suggesting that the mechanism was the relative bradycardia during exercise. Thus, it seems that pyridostigmine, by causing a longer diastolic time, improved diastolic filling and, consequently, systolic volume, as estimated by the augmented oxygen pulse. Oxygen pulse is an indicator of risk of death, not only at peak exercise, but also at submaximal workloads.[40] Thus the increased oxygen pulse in heart failure patients during pyridostigmine use may indicate a protective effect of this drug.

The mechanism by which parasympathetic activation influences left ventricular contractility and, consequently, systolic volume is unclear. Although Takahashi et al[41] have shown in rats that acetylcholine decreases left ventricle contractility, this effect was not observed by direct vagal stimulation. It seems that the ultimate effect of cholinergic stimulation on ventricle contractility depends on the myocardial vagal innervation density, which varies among species. Further studies are needed to clarify the contractile response to specific parasympathetic stimulation in normal and diseased human hearts. The increased oxygen pulse (ie, stroke volume) during exercise with pyridostigmine observed in the present study may have been a hemodynamic consequence of the negative chronotropic effect of the drug leading to increased diastolic period, ventricular filling, and performance via Frank-Starling mechanism. Regardless of the mechanism involved, pyridostigmine was safely used in heart failure patients and, indeed, may have enhanced their systolic function during submaximal exercise. Considering that systolic dysfunction plays an important role in the impaired exercise capacity of heart failure patients, our results could be extrapolated to daily life into an improved capacity to exercise at submaximal levels after pyridostigmine administration.

Pyridostigmine caused an overall vagomimetic effect leading to increased oxygen pulse in submaximal levels of exercise, enhanced pulse pressure at maximal effort, and improved heart rate response during and after dynamic exercise. Because these are indirect indicators of improved mortality and morbidity rates, further studies including a longer follow-up and a greater number of patients are warranted.

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