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Research Article

EVALUATION OF THE INTERACTION OF PIPERINE WITH ANTIDEPRESSANT SERTRALINE AND ANALGESIC PENTAZOCINE, USING DIFFERENT ROUTES OF ADMINISTRATION IN ALBINO MICE

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ABSTRACT

Objective: To evaluate the effect of piperine on the antidepressant activity of sertraline and analgesic activity of pentazocine and to explore the effect of using oral and parenteral routes of administration on the possible interactions.

Methods: Piperine was isolated from commercially obtained *Piper nigrum* extract. Swiss albino mice of either sex were divided into 8 groups (n=6) receiving: 2% gum acacia (oral, intraperitoneally [i.p.]), standard drug (oral, i.p.), standard drug + piperine (oral, i.p.), piperine (oral, i.p.). Tail suspension test (TST) was used for antidepressant effect and Eddy's hot plate method for analgesic effect. Sertraline and pentazocine were used as standard drugs (5 mg/kg) and piperine at 10 mg/kg.

Result: In the TST, piperine alone (both routes) decreased immobility time, but the effect was statistically insignificant. Both combination groups (oral and i.p.) showed significantly better activity compared to sertraline oral and i.p. groups, respectively ($p<0.05$). Oral combination showed activity comparable to i.p. combination ($p>0.05$). Piperine did not show any analgesic activity of its own (both routes). Piperine with pentazocine orally showed significantly better activity compared to pentazocine (oral) at 1, 2, and 4 hrs, and analgesia at 0.5 hr which pentazocine oral did not. Parenteral combination was significantly better than pentazocine (i.p.) group at 2 and 4 hrs and better than oral combination at 4 hrs.

Conclusion: Piperine has potential to be used in combination with pentazocine due to its bioenhancing effect and with sertraline due to a potentiating/ additive effect which can help reduce dose and adverse effects of these drugs.

Keywords: Piperine, Pentazocine, Sertraline, Albino mice, Bio enhancement, Potentiation.

INTRODUCTION

A "bio enhancer" is an agent capable of enhancing bioavailability and bio efficacy of a particular drug with which it is combined, without any typical pharmacological activity ofown at the dose used. Bio enhancement is beneficial in reducing the drug dose required and the adverse drug effects of the drug concerned[1]. Potentiation is the phenomenon when one drug increases the effect of another drug without having a significant effect of its own [2]. The net outcome of potentiation is either the increase or prolongation of effects. Additive effect is the term used in which the combined effect of the two drugs acing by the same mechanism is equal to that expected by simple addition[2].

Piperine has been studied extensively and reported to enhance bioavailability of many drugs by promoting rapid absorption of drugs and nutrients [3] or by inhibiting several cytochrome P450 enzymes and phase II reactions [4].

Piperine is, the chief alkaloid of *Piper nigrum* or Black pepper which is a native South Indian spice. It is commonly found in the Malabar Coast of India and is well-known due to its culinary and medicinal properties. In ayurveda, it has been used for the treatment of fevers, gastric, respiratory, and urinary diseases and used externally to treat rheumatism, neuralgias, and boils[5]. It has been also been evaluated for its anticonvulsant, antioxidant, anti-mutagenic, hepatoprotective, and various endocrine activities[6]. However, its most widely studied property is perhaps bio enhancement [7].

Following are the prominent drugs for which piperine has shown bio enhancement: Oxy-phenylbutazone, phenytoin, beta-carotene, propranolol, theophylline, rifampicin, dapsone, curcumin, ciprofloxacin, cefotaxime, cyclosporine A, and metronidazole[8]. Out of these drugs, antitubercular drug rifampicin is especially worth mentioninig; when combined with piperinethere is a significant improvement in anti-tubercular activity and reduction of dose [9]. Cadila Pharmaceuticals Ltd. India has already launched risorine, world's first boosted-rifampicin containing fixed-dose combination with piperine. Risorine uses a lower dose of rifampicin (200 mg instead of 450 mg) but maintains the same efficacy [10].

Piperine itself has been shown to have antidepressant property at dose of 10-20 mg/kg[11]. Sertraline is an antidepressant of the selective serotonin reuptake inhibitor (SSRI) class. It is primarily prescribed for major depressive disorder, as well as obsessive-compulsive disorder, panic disorder, and social anxiety disorder [12]. Sertraline causes adverse effects such as sedation, dryness of mouth, blurring of vision, urinary retention, weight gain, and erectile dysfunction.

We wanted to see the combined effect of piperine and sertraline when given by two different routes - oral and parenteral; whether there is a significant improvement in activity and explore the difference, if any, due to the route of administration.

Similarly, it has been documented that there is bio enhancement of pentazocine's analgesic activity when it is given in combination with piperine [13]. Pentazocine is a mixed opioid agonist-antagonist (analgesic) drug used to treat moderate to moderately severe pain [14]. Pentazocine has been shown to cause a headache, dizziness, drowsiness, anxiety, feelings of extreme happiness, constipation, dry mouth, blurred vision, ringing in ears, etc.

We wanted to evaluate if there is any difference in such bio enhancement if piperine is given in combination with pentazocine through oral and parenteral routes.

Such an interaction study using oral and parenteral routes, would help us commenting on the possible predominant mechanism involved in the combination effect if found significant.

MATERIALS AND METHODS

Isolation of piperine

P. nigrum extract was obtained from Amsar (P) Ltd., Indore, Madhya Pradesh, India, which contained 95% of piperine. 1 g of the extract was dissolved in 5 mL of ethyl alcohol and 10 ml of 10% w/v of alcoholic potassium hydroxide added with constant stirring. It was then filtered and allowed to stand overnight [15]. The yellow needle-shaped crystals of piperine were separated the next day. The purity of the isolated piperine was verified by checking its melting point (range 128-131°C), treating it with concentrated sulfuric acid (blood red colour obtained), by thin layer chromatography on which a single spot was obtained and ultraviolet spectrophotometry which showed absorption maxima at 343 nm characteristic for piperine[16,17].

Drugs and chemicals

Ethanol (Bengal Chemicals, India), gum acacia (Himedia Lab, India), sulfuric acid (Ranbaxy Pharma Ltd. India), potassium hydroxidepellets (Ranbaxy pharmaceuticals Ltd.), sertraline (tablet Serta 50 mg, Unichem Laboratories Ltd.), and pentazocine (Fortwin injection, 30 mg/ml - 1 ml ampoule, Ranbaxy pharmaceuticals Ltd.) were purchased from their authorized representatives.

Animals

Adult Swiss albino mice (25-30 g) of either sex were procured from the Central Animal House, Mahatma Gandhi Memorial Medical College, Indore and acclimatized for a period of 7-day at room temperature (25±2°C) and 50±15% relative humidity. They were housed in a standard cage and maintained on standard pellets and water *ab libitum*. The animals had free access to water. The study was carried out in the Department of Pharmacology, Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, India. The study protocol was approved by the Institutional Animal Ethics Committee (CPCSEA registration no. 709/2010).

Preparation of drugs for animal experiment

Piperine and other drugs, to be given orally and intraperitoneally (i.p.), were dissolved in 2% gum acacia to maintain uniformity of the solvent and respective drug solutions were prepared.

Experimental design

A sub-acute study model involving once daily administration of drugs for a period of 2-week was used for evaluating interaction with sertraline and acute study model involving single dosing was used for evaluation of combined effect on analgesia with pentazocine and piperine.

Animals were grouped in 8 groups each group comprising 6 animals each.

(N=8, n=6).
Group I: 2% gum acacia (10 ml/kg, oral)
Group II: Standard drug sertraline/pentazocine (5 mg/kg oral) [18,19]
Group III: Piperine (10 mg/kg, oral)
Group IV: Sertraline/pentazocine + piperine (5 mg/kg + 10 mg/kg, oral)
Group V: 2% gum acacia (10 ml/kg, i.p.)
Group VI: Standard drug sertraline/pentazocine (5 mg, kg i.p.)
Group VII: Piperine (10 mg/kg, i.p.)
Group VIII: Sertraline/pentazocine + piperine (5 mg/kg + 10mg/kg, i.p.)

The drugs were administered orally or intraperitoneally as per the grouping done to all animals in the eight groups at the stipulated doses. The dosing was done in the same way for a total of 14 days in sertraline group study.

Procedure and experiment

Tail suspension test (TST) to demonstrate antidepressant activity

For evaluation of antidepressant activity of sertraline in combination with piperine, a sub-acute study model involving once daily administration of drugs for a period of 14-day was used. TST was employed to evaluate the effect of antidepressant activity [20].

Principle of TST

The tail suspension test is based on the observation that a mouse suspended by the tail shows alternate periods of agitation and immobility. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn reflects depressive disorders in humans[21]. The cumulative immobility time is a measure of the animal's degree of helplessness ("depression"). Treatment with antidepressant drugs reduces the immobility time.

Procedure

TST was performed according to the method described by Streu *et al.* [22] with slight modifications. The eight groups of mice were administered the different drugs/combinations for 14 days. TST was performed on day 0 (baseline) and 14th day (end of study; 2 hrs after administration of the drug). The mice were suspended by their tail at a height of 58 cm above the table top, from an aluminum wire strung between two stands. The movements of the mice were observed and video recorded for a period of 10 minutes. Periods, where the mice lay motionless or passive, were considered as the immobile phase.

Eddy's Hot plate model to demonstrate analgesic activity

Principle

Painful reactions can be produced in experimental animals by applying noxious stimuli such as thermal - using radiant heat as a source of pain, chemical - using irritants such as acetic acid and bradykinin and physical pressure - using tail compression.

In the Eddy's hot plate model, the animals are placed on the Eddy's hot plate which consists of an electrically heated surface. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics (like pentazocine), whereas peripheral analgesics do not generally affect these responses [23].

Procedure

Animals were weighed, dosed according to grouping, and placed on the hot plate maintained at temperature of 55-56°C. Responses such as jumping, withdrawal, and licking of the paws were seen. The time period (latency period), from when the animals were placed and until the responses occurred, were recorded using a stopwatch. To avoid tissue damage of the animals 10 seconds was kept as a cut off time [24,25].

The time obtained in all the untreated groups of animals was considered the basal/normal reaction time. Increase in the basal reaction time was the index of analgesia. All the animals were screened initially at least three times in this way and the animals showing a large range of variation in the basal reaction time were excluded from the study. After selecting the animals, the drugs were administered to all animals as per grouping. The reaction times of the animals were then noted at 0.5, 1, 2 and 4 hrs interval after drug administration.

Optimum care was taken of the animals during the both studies, and antiseptic measures were followed.

Statistical analysis

Data were expressed as mean ± standard error of mean, and statistical analysis was carried out by paired t-test, and one-way ANOVA followed

by multiple Tukey's comparison test using SPSS software version 20.0. $p<0.05$ was considered to be statistically significant.

RESULTS

Antidepressant activity (Table 1)

Piperine per se (Group III and Group VII) showed some decrease in immobility time, but not statistically significant, by both oral and parenteral route (i.p.) as compared to respective control and standard groups ($p>0.05$). Both sertraline alone groups (oral and i.p.) showed statistically significant decrease in immobility period compared to oral and i.p. control (Group I and V) on day 14 ($p<0.05$).

Both sertraline and piperine combination Groups IV and VIII (oral and i.p.) showed a significant decrease in immobility period as compared to the respective control Groups I and V and also the respective sertraline alone Groups II and VI (oral and i.p.) on day 14 ($p<0.05$). Sertraline and piperine combination given intraperitoneally showed better activity as compared to sertraline and piperine combination given orally, but there was no statistical difference ($p>0.05$); effect of the oral combination was comparable to the i.p. combination. Importantly, the oral combination Group IV showed activity which was significantly better than the sertraline alone i.p. Group VI.

Analgesic activity (Table 2)

Piperine per se did not show any analgesic activity by oral or parenteral route (i.p.) (Group III and Group VII) compared to the control and standard groups ($p>0.05$). Pentazocine alone Groups II and VI (oral, i.p.) showed significantly better activity compared to control Groups I and V (oral, i.p.) ($p<0.05$).

Pentazocine given in combination with piperine orally showed a significant increase in reaction time as compared to the pentazocine alone (oral) group at 1, 2, and 4 hrs ($p<0.05$). Pentazocine in combination with piperine (oral) showed a significant increase in reaction time at 0.5 hr as compared to control group ($p<0.05$), whereas pentazocine alone (p.o.) did not ($p>0.05$); faster onset of action with the oral combination.

Pentazocine given in combination with piperine intraperitoneally showed a significantly greater increase in reaction time as compared to pentazocine alone (i.p.) group at 2 and 4 hrs ($p<0.05$). Pentazocineand piperine intraperitoneal combination showed a significantly greater increase in reaction time as compared to oral combination at 4 hrs ($p<0.05$); more sustained bio enhancement signifying longer duration of action. However, this effect was not significantly greater at 0.5, 1, and 2 hrs.

DISCUSSION

From the results shown above for the two activities showing the interaction of piperine in combination with sertraline and pentazocine, using oral and i.p. routes of administration, it is clearly evident that both drugs have significantly enhanced effects in combination with piperine. The improved antidepressant activity of sertraline is possibly due to a potentiating/additive effect between the two drugs (discussed below), bolstered with contribution from piperine's ability to increase its absorption or inhibit its metabolism. The significantly increased analgesic activity of pentazocineis mainly due to the bio enhancement caused by piperine. Moreover, on evaluating the effect of using oral and parenteral (i.p.) routes of administration, it is seen that the oral combinations show effects in the two activities, which are comparable to the standard drug given by parenteral route, which is a very significant finding.

Combined effect of piperine and sertraline

The study of the interaction between the antidepressant drug sertraline and piperine shows that both the oral and intraperitoneal combination groups of sertraline given with piperine showed better antidepressant activity than the control and standard groups (oral and i.p., respectively); thus, indicating a significantly enhanced combined effect. In additional, it was also seen that the effects of the oral combination were, in fact, better than sertraline given alone i.p. In other words, the combination of piperine with sertraline by oral route makes it comparable to parenterally given sertraline.

Piperine has been shown to have the antidepressant effect of its own at doses of 10-20 mg/kg after 2 weeks of administration [11]. In our sub-acute study, piperine at dose of 10 mg/kg showed a decrease in immobility time which was not comparable to the standard drug sertraline (by both routes). However, when used in combination with sertraline, the results were significantly better than sertraline alone, as

Table 1: Evaluation of antidepressant effect using TST

Drug treatment	Dose/route	Period of immobility (in seconds) in 10 minutes
Group I: Control 2% gum acacia	10 ml/kg, oral	508.67±19.83
Group II: Sertraline	5 mg/kg, oral	394.50±17.29
Group III: Piperine	10 mg/kg, oral	480.50±21.54
Group IV: Sertraline+piperine	5 mg/kg+10 mg/kg, oral	244.67±16.40*#
Group V: 2% gum acacia	10 ml/kg, i.p.	494.50±23.17
Group VI: Sertraline	5 mg/kg i.p.	315.00±10.65*
Group VII: Piperine	10 mg/kg i.p.	485±16.40
Group VIII: Sertraline+piperine	5 mg/kg+10 mg/kg, i.p	204.83±11.81*#

TST: Tail suspension test

Table 2: Evaluation of analgesic effect using Eddy's hot plate method

Reaction time in seconds						
Drug treatment	**Dose/route**	**0 hr**	**0.5 hr**	**1 hr**	**2 hr**	**4 hr**
Control 2% gum acacia	10 ml/kg, oral	3.00±0.27	2.80±0.35	3.25±0.38	2.87±0.34	3.23±0.30
Pentazocine	5 mg/kg, oral	2.95±0.19	3.22±0.17	4.93±0.32	5.38±0.53	3.98±0.09
Piperine	10 mg/kg, oral	2.50±0.27	2.60±0.35	3.24±0.38	2.80±0.34	3.22±0.30
Pentazocine+piperine	5+10 mg/kg, oral	2.58±0.14	4.70*±0.33	6.52*±0.27	7.42*±0.28	5.43*±0.18
Control 2% gum acacia	10 ml/kg, i.p.	3.13±0.31	2.93±0.24	3.19±0.20	2.60±0.33	3.48±0.37
Pentazocine	5 mg/kg, i.p.	2.72±0.20	3.43±0.22	6.33*±0.28	6.57±0.54	4.82±0.26
Piperine	10 mg/kg, i.p.	2.40±0.27	2.20±0.35	3.35±0.38	2.25±0.34	3.30±0.30
Pentazocine+piperine	5+10 mg/kg, i.p.	2.73±0.20	3.90±0.32	7.20*±0.45	8.33*#±0.54	6.60*#α±0.34

One-way ANOVA followed by multiple Tukey's comparison test, values are mean±SEM, n=6 in each group, df: 5, 30, *$p<0.05$ compared to pentazocine (p.o.), #$p<0.05$ compared to pentazocine (i.p.), α$p<0.05$ compared to pentazocine and piperine (p.o.), SEM: Standard error mean, i.p.: Intraperitoneally

discussed above. This could be explained due to a possible synergistic/additive effect between the two drugs as well as contribution from the bio enhancing mechanisms of piperine.

In the study by Li *et al*., piperine was shown to enhance the serotonin level in the hypothalamus and hippocampus without any influence on the dopamine and nor adrenaline levels. It also showed a minor monoamine oxidase inhibitory activity. Sertraline is a drug belonging to the class of SSRIs; essentially, working by the same mechanism in depression-enhancement of serotonin levels. Piperine, at sub-threshold doses has also been shown to potentiate the antidepressant-like effect of trans-resveratrol with the involvement of monoaminergic system, again indicating the involvement of serotonergic system [26].

Moreover, sertraline is a drug which has an oral bioavailability of around 44% [27] and undergoes hepatic metabolism by multiple cytochrome P 450 isoforms such as CYP2D6, 2C8, 3A4 [28]. Hence, piperine could also probably enhance the oral absorption of the drug as well as inhibit its metabolism [4]. As the bioavailability with the intraperitoneal administration is almost 100%, the combination given by this route showed more improvement in activity compared to the oral combination. However, the fact that activity of oral combination was not significantly inferior to the parenteral combination indicates that increase in absorption of sertraline (bioavailability-44%) may possibly play a role in the enhanced effect. There could also be inhibition of enzymes involved in the biotransformation of sertraline (CYP2D6, 2C8, 3A4) by piperine, contributing further, pharmacokinetically, to the pharmacodynamics potentiation leading to significantly better activity of both oral and parenteral groups compared to respective standard groups.

Combined effect of piperine and pentazocine

In the study of analgesic activity, pentazocine given in combination with piperine, by both oral and intraperitoneal routes, gave significant better analgesic activity as compared to control and pentazocine given alone (oral and i.p., respectively), but at different time points. Piperine per se did not show any analgesic activity of its own. Hence, this was a direct evidence of the bio enhancing effect of piperine.

With the oral combination group, the improved analgesia, compared to the oral standard group, was evident as early as 1 hr, and remained for the entire duration of study, i.e. up to 4 hrs. The oral combination also showed significant analgesia even at 0.5 hr when the oral pentazocine alone group did not. This indicates that the oral combination group had a faster onset of action compared to the oral pentazocine group.

With the intraperitoneal combination group, the significant improvement in analgesia compared to the i.p. pentazocine occurred first at 2 hrs and persisted at the 4 hrs mark as well. The faster onset of action of the combination with respect standard was not evident through i.p. route. This is quite understandable considering the fact that parenteral route of drug administration by itself has a faster onset of action. A very important observation was that at the end of the study duration (at 4 hrs), the i.p. the combination was significantly better than the oral combination suggesting a more effective prolongation of the duration of action of pentazocine in the i.p. combination than the oral combination.

On analyzing these findings, we can say that the bio enhancing activity of piperine appeared early in the oral combination group, and it remained significantly greater for a longer period of time in the intraperitoneal combination group. Pentazocine is a drug which reaches peak plasma concentration at about 1-3 hrs and has a mean plasma half-life of about 2 hrs. However, blood levels show considerable variation both within and between subjects due to extensive but variable pre-systemic (hepatic) elimination. Hence, the oral bioavailability ranges from 11% to 32% [29]. There is reported the role of hydroxylation, glucuronidation and CYP 2E1, 1A2 [30] - all liable to inhibition by piperine.

So, it appears that when pentazocine is given in combination orally, piperine counteracts its initial loss of bioavailability by probably inhibiting the first pass metabolism as well as increasing its absorption; leading to its faster onset of significant analgesic activity compared to oral pentazocine given alone.

With intraperitoneal administration, the presystemic metabolism or incomplete gastrointestinal absorption is already eliminated; this is reflected in the i.p. the combination not showing significant improvement initially as compared to i.p. pentazocine alone. But, later when the drug concentrations begin to fall, piperine may allow longer maintenance of pentazocine's action through its inhibition of metabolism and probably excretion. Moreover, this effect is more evident in the intraperitoneal combination as almost 100% of the drug is already available for action (parenteral administration).

Bio enhancement with piperine

A "bio enhancer" is defined as an agent of herbal origin or any phytomolecule, which is capable of enhancing bioavailability and bio efficacy of a particular drug or nutrient with which it is combined, without any typical pharmacological activity of its own at the dose used. The term bioavailability enhancer was first coined by the Indian Scientists at the Regional Research laboratory, Jammu (RRL, now known as Indian Institute of Integrative Medicine) who discovered and scientifically validated piperine as the world's first bioavailability enhancer in 1979[1,31].

This bio enhancement is the reason behind the improved analgesia seen with pentazocine, and also possibly contributing to the enhanced activity of sertraline (not technically bio enhancement) due to the synergistic/additive effect between sertraline and piperine toward serotonin enhancement. Various studies have indicated some generalized mechanisms behind this effect such as:

Increased absorption

A low oral bioavailability is seen with many drugs due to restricted gastrointestinal absorption. Piperine can enhance this by:

i. Increasing solubility - Increased micelle formation, essential for the absorption of lipid soluble drugs, by increasing secretion or synthesis of bile acids and inhibition of bile acid metabolism [32].
ii. Increasing blood supply - Trikatu, containing piperine has been shown to enhance absorption of drugs from the digestive tract, by enhancing gastrointestinal blood flow [3].
iii. Increasing the permeability of intestinal epithelial cells - Piperine stimulates gamma-glutamyl transpeptidase (γ GT) activity and increases amino acid uptake by epithelial cells, and thereby increasing the uptake of drugs by GI epithelium[33]
iv. Increase microvilli length - as shown by ultra-structural studies[34].

Inhibiting metabolism

Piperine is reported to inhibit many different cytochrome P-450 isoforms as well as UDP-glucuronyl transferase and hepatic arylhydrocarbon hydroxylase. It also inhibits glucuronidation in isolated intestinal cells [35,36].

There is also inhibition of mixed function oxidases as well as ertain hydroxylation, demethylation, deethylation, and deamination processes [37]. Recently, it has been shown to be a relatively selective inhibitor of CYP1A2, CYP1A1, CYP2D6, CYP3A4, and CYP2C8 [38].

Reduced efflux

Piperine inhibits p-glycoprotein, one of the major efflux pumps, and thereby could increase the duration of stay of the active drug at the target cell [39].

The inference derived from the above two interaction studies collaborates well with the proposed and proven phenomena regarding piperine's bio enhancement. Most drugs co-administered with

piperine are probably more bioavailable as a result of both of the mechanisms, i.e., increased absorption from the gut and the slowdown of biotransformation, inactivation, and elimination from the system. The latter mechanism is perhaps most important in sustaining the elevated blood levels of the drug, and making it more bioavailable to the tissue. Although a rapid absorption into the blood stream may account for increased blood levels of the drug, leading to a faster onset of action.

CONCLUSION

The study preliminarily demonstrates that piperine significantly increases the antidepressant activity of sertraline HCL by both oral and parenteral routes which can be attributed to a potentiating/additive interaction between the two with a possible contribution from bio enhancing mechanisms of piperine. There is a definite bio enhancement seen with pentazocine when combined with piperine by both routes of administration; oral combination showing a faster onset of action and the parenteral combination showing a more persistent prolongation of duration of action.

Further studies need to be conducted to further explore this potential of piperine to be used in combination with sertraline in the treatment of major depressive disorder and with pentazocine for treatment of moderate to severe pain; the combination offering an option of use of reduced doses of these drugs, thereby possibly a reduction in their troublesome adverse effects.

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