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Comparison of activated charcoal and sodium polystyrene sulfonate resin efficiency on reduction of amitriptyline oral absorption in rat as treatments for overdose and toxicities

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ABSTRACT

Objective(s): Comparative *in vivo* studies were carried out to determine the adsorption characteristics of amitriptyline (AMT) on activated charcoal (AC) and sodium polystyrene sulfonate (SPS). AC has been long used as gastric decontamination agent for tricyclic antidepressants and SPS has showed to be highly effective on *in-vitro* drugs adsorption.

Materials and Methods: Sprague-Dawley male rats were divided into six groups. Group I: control, group II: AMT 200 mg/kg as single dose orally, group III and IV: AC 1g/kg as single dose orally 5 and 30 min after AMT administration respectively, and group 5 and 6: SPS 1 g/kg as single dose orally 5 and 30 min after AMT administration, respectively. 60 min after oral administration of AMT (T_{max} of AMT determined in rats), C_{max} plasma levels were determined by a validated GC-Mass method.

Results: The C_{max} values for groups II to IV were determined as 1.1, 0.5, 0.6, 0.1 and 0.3 µg/ml, respectively.

Conclusion: AC and SPS could significantly reduce C_{max} of AMT when administrated either 5 or 30 min after AMT overdose ($P<0.05$). However, SPS showed to be more effective than AC in reducing C_{max} when was administrated immediately (5 min) after AMT overdose. The results suggest a more efficient alternative to AC for AMT and probably other TCA overdoses.

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Introduction

Amitriptyline (AMT) is a tricyclic antidepressants used to treat mental depression (1). As well as reducing depressive symptoms, it is also helpful to alleviate migraines, tension headaches, anxiety attacks and some schizophrenic symptoms. AMT has a narrow therapeutic index and hence its therapeutic dose is close to its toxic dose (2), so its toxicity is a common cause of death from prescription drug overdoses (3). Treatment includes aggressive supportive care, activat-ed charcoal (AC) oral administration, alkalinization therapy and management of arrhythmias, hypotension and seizures (4, 5). AC has been used for gastric decontamination over the last century. It prevents absorption of substances in the gastrointestinal tract; thereby decreasing systemic absorption of potentially toxic agents (6). Large reductions in drug absorption occur when AC is administered soon after drug ingestion (7). AC is recommended for the treatment of tricyclic antidepressants poisoning (TCA) (8, 9).

Sodium polystyrene sulfonate (SPS) is a potassium-binding resin used for the treatment of hyperkalemia (10). SPS is not absorbed from the gastrointestinal tract. As the resin passes through the gastrointestinal tract, it removes the potassium ions by exchanging it by sodium ions. In clinical practice, SPS is often mixed with cathartics such as sorbitol to prevent constipation which sometimes occurs with SPS (11, 12).

The present study was performed to see whether there is any difference in the effectiveness of AC compared with SPS and also to see whether giving the adsorbents at different times would show any significant difference in their effectiveness.

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Materials and Methods

Chemicals and reagents

Standard sample of AMT was purchased from Roozdaroo (Tehran, Iran). Papaverine was purchased from Terop Co (Switzerland). GC-grade acetonitrile was obtained from Merck Co, (Germany). AC (Asche~5%; Fe<0.3%; Particle size 75 %< 40 µ; Loss on drying~10%) and SPS were obtained from Modava Pharmaceutical Co, (Iran). All other chemicals were of analytical grade and purchased from Merck Co, (Germany) unless otherwise specified.

Study design

Thirty six male Wister rats (170-200 g) were selected from the Laboratory Animals Research Center, Shiraz University of Medical Sciences. The rats were maintained under controlled temperature, 12 hr light/12 hr dark conditions for one week before study. They were allowed to feed standard laboratory chaw and tap water *ad libitum*. The research protocol was according to the guidelines for animal care of Shiraz University of Medical Sciences. The LD_{50} of amitriptyline obtained from reference sources was confirmed by giving 200 mg/kg to 30 rats by oro-gastric administration in pilot study (13). As designed, the rats were randomly divided into 6 groups, each consisting of 6 animals. Group I received distilled water (5 ml/kg/orally) as normal control; group II received AMT 200 mg/kg/orally; group III received AC (1 g/kg 1:5 in distilled water, orally) 5 min after AMT administration; group IV received AC (1 g/kg 1:5 in distilled water, orally) 30 min after AMT administration; group V received SPS (1 g/kg 1:5 in distilled water, orally) 5 min after AMT administration; and group VI received SPS (1 g/kg 1:5 in distilled water, orally) 30 min after AMT administration. One hr after AMT administration, the animals were anaesthetized by ether and blood samples were withdrawn from their hearts. Then, the respective plasma was separated for subsequent measurement of AMT concentrations.

Quantification of amitriptyline in rat plasma instrumentation

A GC-MS plus gas chromatographic mass spectroscopic system (Agilent Technologies) was used for samples analysis. The capillary column used was HP-5MS (30 m×0.25 mm ID ×0.25 µm). Chromatographic data were collected and recorded by GC-MASS real-time analysis software. Sample injection was done in splitless manner. Helium was used as the carrier gas at flow rate of 1 ml min^{-1}. The GC injector temperature was set at 270 °C. The column oven temperature was kept at 60 °C for 2 min and increased by 20 °C min^{-1} up to 300 °C. Mass spectrometry conditions were as follows: electron ionization source set at 70 Ev, MS source temperature at 230 °C and solvent cut time was 3 min. The mass spectrometer was run in full scan mode (m/z 40-500) and in SIM mode at m/z 58 and 338. The quantization of samples was done by using the SIM mode. Total run time was 20 min. The areas under the curves (AUC) of AMT and papaverine (internal standard) were calculated for plasma samples.

Preparation of standard solutions

For construction of calibration curve, the stock solution of the AMT was prepared in acetonitrile at the concentration of 1 mg/ml^{-1}, and then 1.25, 2.5, 5, 20, 37.5, 100, 200, 500 $\mu g/ml^{-1}$ solutions were prepared from stock solutions by dilution. Ten µl of each solution was added to 190 µl of plasma and final solutions were obtained at the concentrations of 0.0625, 0.125, 0.25, 1, 1.75, 5, 10 and 25 $\mu g/ml^{-1}$. The stock solution of papaverine (as internal standard) was prepared in distilled water at the concentration of 40 $\mu g/ml^{-1}$.

Sample preparation

Blood samples (1 ml) obtained from rats were stored in separate glass tubes containing EDTA and centrifuged at 4000 rpm for 10 min. Then, the supernatant (plasma) was transferred to polypropylene tubes and stored until analysis. In time of preparation, 200 µl of plasma samples was thawed at room temperature and added with10 µl internal standard working solution (0.4 $\mu g/ml^{-1}$), 2 ml phosphate buffer 1 M, pH=7 and 4 ml deionized water in a glass-stoppered 10 ml centrifuge tubes. After vortex mixing (1 min), the mixtures were centrifuged at 4000 rpm for 15 min and the upper layer was transferred to other glass-stoppered tubes and 2 ml phosphate buffer 1 M, pH=6 was added to each tube. The mixtures were vortex-mixed for 1 min and were prepared for drug extraction.

Solid-phase extraction method (SPE)

At first, UTC Clean Screen columns were prepared as follows.3 ml of hexane, methanol, distilled water and 1ml phosphate buffer 0.1 M pH= 6 were added to columns and aspirated, respectively. Then, the samples were immediately transferred to columns and 2×3 ml distilled water, 2 ml acetic acid 1M and 3 ml methanol were added and vacuumed for 2 min and aspirated. In next step, the columns were dried under vacuum for 2 min and 2 ml hexane was added to columns and aspirated. Then, 3 ml hexane/ ethylacetate (50/50 v/v) was added and aspirated in order to elute acidic and neutral drugs. Afterward, 3 ml methanol was added to columns and aspirated under vacuum. Then, 2 ml hexane was added to columns and columns were dried under vacuum for 5 min. Finally, 3 ml dichloromethan/ isopropanol/ amonia (78:20:2) (prepared daily) was added and aspirated to elute basic drugs. The collected samples

Table 1. The intra-day precision results of amitriptyline quantification by GC-MS (n=3)

Initial injected concentrations (µg/ml)	Obtained concentrations (µg/ml)			Average (µg/ml)	%RSD (%CV)	% Accuracy
0.0625	0.064	0.066	0.068	0.066	3.03	105.6
0.5	0.41	0.043	0.42	0.42	2.8	86
1.5	1.43	1.47	1.41	1.43	2.13	95.3

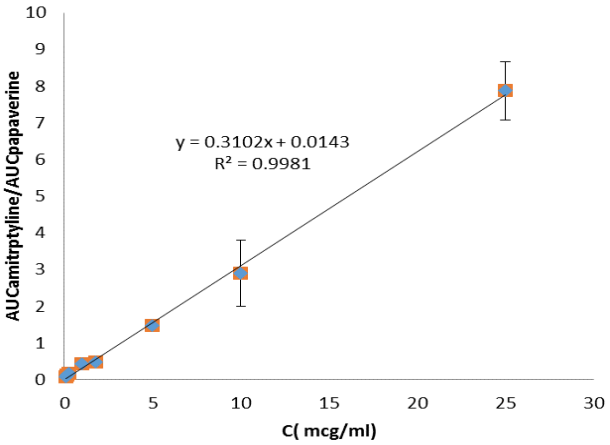


Figure 1. The calibration curve of amitriptyline obtained by GC-MASS Each point is the mean of three injections. Values are the Mean±SD (n= 3)

were dried in 50 °C under nitrogen and the residual was dissolved in 50 µl acetonitrile and 1 µl was injected to GC-MS.

Method validation

To prepare the calibration curve, different concentrations of AMT in range of 0.0625 to 25 µg/ml^{-1}and a constant amount of papaverine (to reach an identical concentration of 0.4 µg/ml^{-1} in all samples) were added to rat plasma and after sample preparation and extraction, were injected to GC-MS. The AUC ratios of AMT to papaverine were calculated and plotted versus AMT concentrations. The calibration curve was described by the linear equation: $y= mx+c$ Where y is peak area, x is the concentration, m is the slope and c is the intercept. LOD and LOQ were determined as the concentration causing a peak height of three times of noise and the least concentration lying on regression line, respectively.

Statistics

In order to investigate the difference between the group means, One-way ANOVA was performed following by Danett *post hoc* test using SPSS ver18 software.

Results

Sample preparation and solid phase extraction

AMT is a basic drug (pka 9.4) and is often presented as water soluble hydrochloric salt (AMT. HCl) in dosage forms in market. However, in plasma pH 7.4, it partially converts to deionized form which is highly lipophilic (log P 4.9) and has a high plasma protein binding (>90%) (13). Therefore, a preparation method should be used to separate it from plasma proteins and provide neat and concentrate samples for injection to analysis instrument. In this research, we have used a multi-step protocol for this purpose. Papaverine was used as internal standard because it is a lipopholic basic compound and is highly bound to plasma proteins (90%) (14) and has a similar behaviour to AMT in this regard. For solid extraction of AMT, subsequent eluting with polar and nonpolar solvents could elute acidic and neutral compounds and plasma proteins efficiently. The recovery efficiency was determined and the final sample was nearly clean and free of interventing compounds.

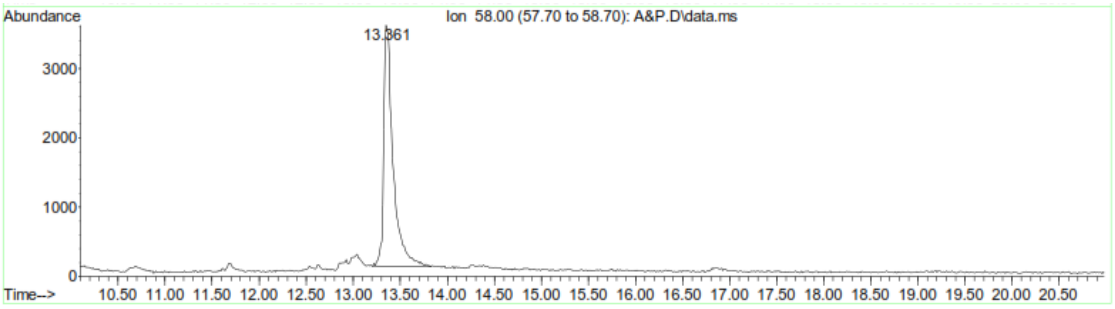
AMT quantification by GC-MS

As noted above, AMT is a very lipophilic drug and has a broad distribution in body which produces very low concentrations in plasma. This phenomenon makes the plasma drug quantifications difficult and requires the analysts to develop more sensitive methods in this regard. Although many researchers reported the common HPLC methods with UV detectors for analysis of AMT, but in the most of them, a specific preparation or derivatization technique has been used for concentrating the drug in sample and increasing the sensitivity of detection, respectively (15, 16). A HPLC method was also validated by our team to analyse the plasma samples (data not presented in the paper) but was not sufficiently sensitive to quantify the AMT in plasma. GC-Mass is a very sensitive technique commonly used for determination of very low concentrations of drug in biological fluids. Some researchers have utilized this technique for AMT quantification in plasma successfully (17). In current study, the validated method was linear in range of .0.0625 µg/ml^{-1} to 25 µg/ml^{-1} WithR^2=0.9981 (Figure 1) and had LOD and LOQ values as 0.01 and 0.0625, respectively. An accuracy of 96% was obtained for medium concentration of 0.75 µg/ml^{-1}. Figure 2 A and B show the chromatograms of AMT and papaverine, respectively and Tables 1 and 2 summarize the method intraday and interday precision and accuracy. As seen, the method is precise (%CV less than 5%) and accurate (% accuracy error less than 20%) in the range of study.

Determination of pharmacokinetic parameters of AMT in rats

The plasma concentration-time profile was plotted and T_{max} and C_{max} were graphically determined which were 60 min and 1.1 µg/ml^{-1}, respectively

A)



B)

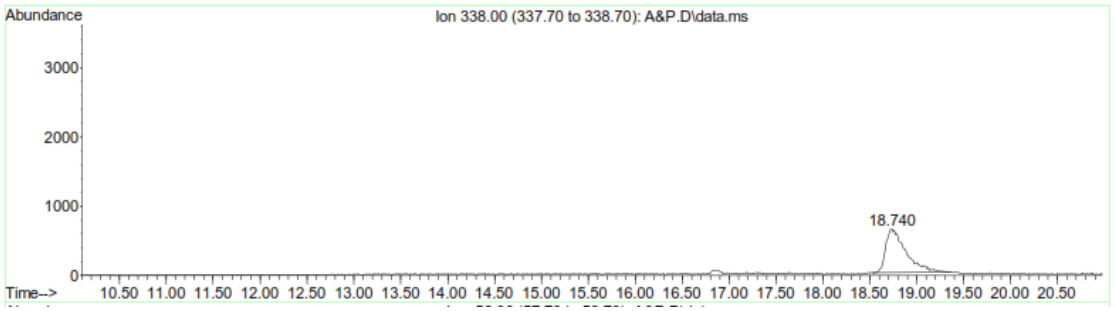


Figure 2. GC-MS chromatograms obtained from (A) amitriptyline and (B) papaverine

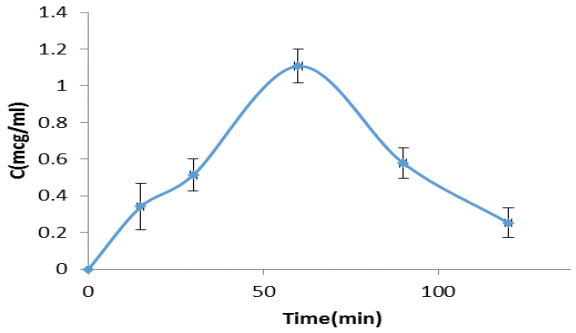


Figure 3. The plasma concentration-time profile of amitriptyline in rats after oral ingestion of drug (n=3)

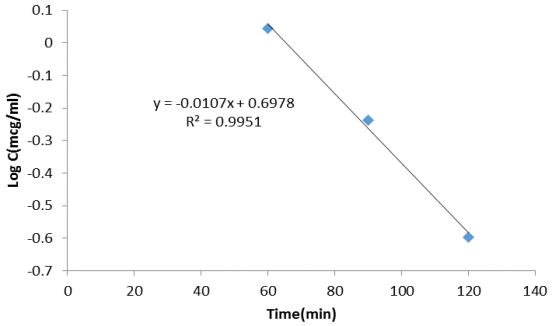


Figure 4. The logarithmic plot of terminal concentrations in elimination phase of plasma profile of amitriptyline after oral ingestion. Elimination constant and half-life were calculated from regression line equation

(Figure 3). The results were in good agreement with other studies done in rats (16). A semi-log plotting of terminal concentrations versus time showed an elimination constant (k) of 0.023 min and a consequent half-life of 30.04 min were calculated (Figure 4).

Effect of AC and SPS on AMT plasma concentration in rats

The AMT plasma concentrations at 60 min as maximum plasma level of AMT (C_{max}) were determined and compared among groups. The C_{max} values for groups II to IV were 1.1, 0.5, 0.6, 0.1 and 0.3 µg/ml, respectively. Figure 5 and 6 compare the effect of AC and SPS on AMT plasma concentrations 5 and 30 min after AMT administration. The results showed that when SPS and AC were administrated 5 min after AMT, the C_{max} values were significantly lower for SPS (P<0.05) though both of them were effective on C_{max} compared to AMT alone (P<0.05).

Table 2. The inter-day precision results of amitriptyline quantification by GC-MS (n=3)

Initial injected concentrations (µg/ml)	Obtained concentrations (µg/ml)		Average (µg/ml)	%RSD (%CV)	% Accuracy	
0.0625	0.062	0.066	0.068	0.065	4.68	104.0
0.5	0.41	0.43	0.40	0.41	3.70	82.0
1.5	1.50	1.47	1.41	1.46	3.14	97.3

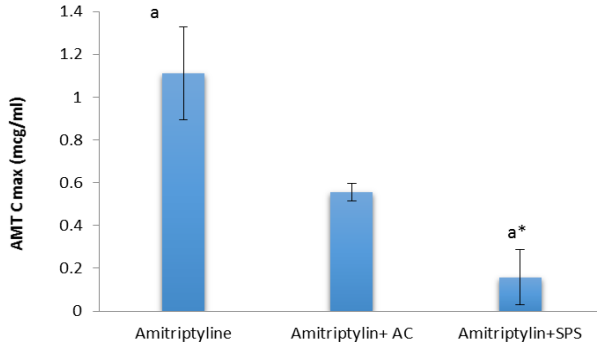


Figure 5. Effect of activated charcoal (AC) and sodium polystyrene sulfonate (SPS) on plasma C_{max} of amitriptyline (AMT), 5 min after oral administration of amitriptyline
Values are the Mean±SD (n= 6)
*P<0.05 compared with amitriptyline alone, a: P<0.05 compared with amitriptyline+AC

However, when SPS and AC were administrated 30 min after AMT, the C_{max} values were not significantly different (P>0.05) though both were effective on C_{max} compared to AMT alone (P<0.05).

Discussion

The Initial treatment of an acute overdose includes gastric decontamination of the patient (18). AC has the capacity to adsorb a wide range of substances and organisms (19). AC is an orally non-absorbable compound and can bind to many drugs such as amitriptyline, clomipramine and doxepin, prohibiting them from oral absorption. In the present study, SPS, a cation-exchange resin, showed an effective adsorption of AMT, presumably because of its cationic properties.

The previous *in-vitro* studies indicated that the molecular structure of drugs is an important factor in the adsorption mechanism by adsorbents (20). Higher adsorption is usually seen for aromatic compounds compared to aliphatic ones of similar molecular size and in branched-chain molecules compared to straight-chain ones. Tricyclic antidepressants (TCA), having an aromatic tricyclic structure and a branched-chain structure have been described to be well adsorbed to AC *in vitro* (20). Scheme 1 shows the chemical structures of AMT for a more sensible discussion regarding the adsorption mechanisms.

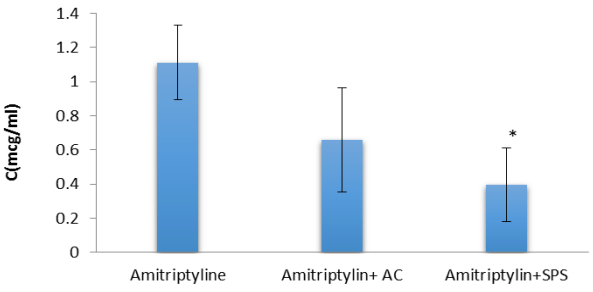
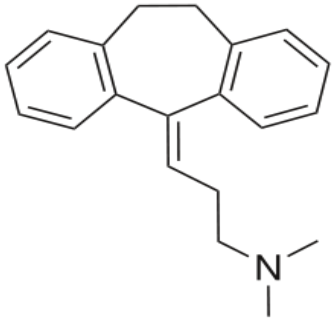


Figure 6. Effect of activated charcoal (AC) and sodium polystyrene sulfonate (SPS) on C_{max} of amitriptyline, 30 min after oral administration of amitriptyline Values are the Mean±SD (n=6). *P<0.05 compared with amitriptyline alone



Scheme 1. Chemical structure of Amitriptyline

SPS is a strongly acidic ion-exchange resin and is used to treat hyperkalemia (21). SPS has been promising in animal and healthy human volunteers for reducing Li absorption and promoting its elimination (22). The previous studies showed that calcium polystyrene sulfonate, a cation-exchange resin, could adsorb imipramine, clomipramine, mianserin, trazodone, and ciprofloxacin based on their cationic properties (23). The chemical behavior of the resin is similar to that of a strong acid (P_{ka} - 2.1) (24). This resin is highly ionized in both of acid ($R\text{-}SO_3H$) and salt (RSO_3Na) forms of sulfonic acid group ($-SO_3H$)and its Na^+ and H^+ are readily available for exchange over the entire pH range. Consequently, the exchange capacity of strong acid resins is independent of the solution pH (21).

In our study, SPS showed to effectively adsorb AMT. This fact can be attributed to the protonation at amine groups of these drugs and adsorption onto SPS surface after exchanging with sodium. The chemical behavior of this resin is similar to that of a strong acid. This resin are highly ionized in both the acid (R-SO3H) and salt (RSO3Na) form of the sulfonic acid group (-SO3H). The hydrogen and sodium forms of strong acid resins are highly dissociated, and the exchangeable Na^+ and H^+ are readily available for exchange over the entire pH range. Consequently, the exchange capacity of strong acid resins is independent of the solution pH. The anionic resins having sulfonic, phosphonic or carboxylic acid exchange groups have approximate pKa values of <1-6 . Therefore, at pH 7.2, SPS is in salt (RSO_3Na) form of the sulfonic acid group and at pH 1.2,conversion of salt to acid form is done (21).

The higher effect of SPS compared to AC when administrated immediately after AMT can be possibly due to different kinetics of drug adsorption to these agent. This observation shows that SPS has a more tendency to AMT and higher rate of binding compared to AC. It is noteworthy that the *in-vitro* results previously published by our group approve this observation (25).

Conclusion

AMT is one of most common antidepressants ever used for depression and other CNS disorders.

However, because of narrow therapeutic index, the toxicity may occur in accidentally or intentionally overdoses. For these situations, rapid gastric decontamination is the most helpful way to prohibit drug from absorption and subsequent toxic levels in plasma. In present study, AC and SPS could significantly reduce C_{max} of AMT when administrated either 5 or 30 min after AMT overdose. Also, the results showed that SPS was more effective than AC in reducing C_{max} when was administrated immediately (5 min) after AMT overdose. Although, there was no significant difference when they were administrated later (30 min).

We conclude that it can be related to pH dependency of SPS and also gastric transit time of rats. Previous studies (21) showed that capacity of SPS is depend on the solution pH and also we reported that the SPS affinity for AMT is grater in lower pH (25). The fasting state of rats could influence gastrointestinal transit time, also gastric transit time in rat is different from human. Previous research (26) has shown that 30 min after oral administration of drugs in fasted rat, 60% drugs transit from stomach to small intestine. Therefore, when we use SPS and AC 30 min after AMT, the greater amount of AMT is not in stomach and efficacy of SPS at this time in intestine pH is not different with AC.

The results suggest that SPS could be a more efficient alternative to AC for treatment of AMT and probably other TCA overdoses when administrated immediately after overdose.

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Conflict of interest

The authors report no declarations of interest.

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