Glutamine levels predict renal failure in patients treated with cisplatinum

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SUMMARY

Glutamine is an amino acid (AA) whose mission is carrying nitrogen. Abnormalities in protein and AA metabolism has been observed in patients with acute renal failure. Several clinical trials had showed abnormalities in plasma AA and its elimination in uremic environment. Moreover, renal failure disturbs hepato-splachnic circulation of glutamin, tyrosine and proline. Cisplatin is a key drug in the chemotherapy pharmacology, and the nephrotoxicity due to tubular injury, is one of its most important side effects, which sometimes is irreversible and leads to substitutive renal treatment. The goal of this work is to find predictive factors of renal failure secondary to cisplatin. Fifty four patients treated with cisplatin were studied. The plasma AA concentration and another plasmatic and urine parameters were measured in three different days after each pulse of chemotherapy. Plasma AA modifications through the pulses and reabsortion percentages of everyone were analysed too. Significant differences were observed in 13 AA reabsortion percentages and 16 plasmatic concentration. Glutamin concentration through the pulses was higher in 13 patients (24%) who presented renal failure (Plasmatic glutamin concentration higher than 1.000 mM/L at the third day after cisplatin administration was highly predictive value about getting renal failure, with significant difference from patients with o normal renal function. Others parameters analysed did not rise significant predictive values, so as it was not found relation between hyperaminoaciduria and renal function. It is concluded that cisplatin leads to renal failure in a 24% of this patients. Glutamin, concentrations higher than 1.000 mM/L at the third day after cisplatin administration have a high predictive value about getting renal failure; so, it is suggested this could be a early marker of cisplatin nephrotoxicity before the serum creatinine is elevated, in order to get an early and suitable treatment of it.

Key words: ***Amino acids. Glutamin. Renal failure. Cisplatin.***

CONCENTRACIONES DE GLUTAMINA EN PACIENTES TRATADOS CON CISPLATINO PREDICEN EL DESARROLLO DE INSUFICIENCIA RENAL

RESUMEN

La glutamina es un aminoácido encargado del transporte sanguíneo de nitrógeno. En pacientes que desarrollan insuficiencia renal se ha detectado alteración de

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los niveles plasmáticos de aminoácidos (AA) y alteración en su excreción urinaria. El cisplatino es un fármaco utilizado en quimioterapia, siendo la nefrotoxicidad uno de sus efectos secundarios más importantes. Con el objetivo de encontrar factores predictivos para el desarrollo de insuficiencia renal secundaria al cisplatino, se estudiaron 54 pacientes con diversos tumores en tratamiento con cisplatino, cuantificándose la concentración de AA y otros parámetros bioquímicos en plasma y en orina de 24 horas en tres días diferentes tras cada ciclo de quimioterapia. Se analizaron las variaciones de los AA plasmáticos a lo largo de los distintos ciclos de cisplatino y los porcentajes de reabsorción de cada uno de ellos. Se detectaron diferencias significativas a través de los ciclos en el porcentaje de reabsorción de 13 AA y en las concentraciones plasmáticas de 16. La concentración de glutamina a lo largo de los ciclos fue mayor en pacientes que desarrollaron insuficiencia renal. El valor de glutamina sérica > 1.000 mM/L analizada el tercer día tras la administración de cisplatino fue altamente predictivo de desarrollar insuficiencia renal, con una diferencia significativa respecto a los pacientes que no la desarrollan. Se concluye que concentraciones superiores a 1.000 mM/L de glutamina plasmática al tercer día tras la administración de cisplatino tienen un alto valor predictivo respecto al desarrollo de insuficiencia renal aguda.

Palabras clave: ***Aminoácidos. Glutamina. Insuficiencia renal. Cisplatino.***

INTRODUCTION

Nephrotoxicity is an adverse effect inherent to certain anti-tumoral agents.[1] Currently, cisplatinum is a key drug for chemotherapy of a vast number of solid tumors. Nephrotoxicity, which is basically produced by tubular damage, is common and one of the first impairments that may be seen within 48-72 hours after cisplatinum administration.[2]

Patients with acute renal failure present disorders in protein and amino acids metabolism.[3] Several studies have shown changes in amino acid levels and their half lives[4,5] when there is renal failure.[6,7] Besides, renal failure alters enterohepatic circulation of several amino acids, with a special impact on glutamine, proline, and tyrosine plasma levels.[8]

Glutamate and glutamine are key amino acids in nitrogen transport. Amino groups of the twenty amino acids from proteins are cleared during their degradation. If they are not reused, they are derived within the liver to ammonia synthesis. Glutamine is formed by the glutamine synthetase, which combines glutamate to ammonia.[9] Thus, glutamine is the main form of ammonia transport and its serum levels are higher than those for other amino acids.

Reabsorption at the renal tubule of filtered amino acids is effective, high, and selective, and under normal conditions less than 2% of them are found in urine. Higher clearances are related to tubular damages produced by several nephrotoxic agents, including antibiotics and cytostatic agents.[10]

The present study assesses the changes in amino acids profile and other parameters in patients treated with cisplatinum with the goal of finding early predictive factors for the development of renal damage.

PATIENTS AND METHOD

Fifty-four patients with lung, head, or neck cancer, all of them out of surgical time, or with testicular tumors are prospectively studied, with a chemotherapy schedule based on cisplatinum.

Inclusion criteria

Age 18-70 years, ECOG (Eastern Cooperative Oncology Group) < 2, body weight loss < 5%, normal liver and heart functions, absence of uncontrolled arterial hypertension or infections, adequate bone marrow reserve, serum creatinine < 1.4 mg/mL with CrCl > 60 mL/min, and having not received previous chemotherapy or supplementary feeding, both enteral and intravenous.

Cisplatinum was administered with the patient hospitalized at a dose of 100 mg/m^2 as repeated cycles every 21 days with intravenous pre-hydration with 3000 cc of dextrose-saline and anti-emetics according to the protocol. Before each cisplatinum cycle administration, laboratory work-up and CrCl assessment by means of collection of 24-h urine were done.

Cisplatinum dose was reduced to 75% if there was a toxic effect according to the WHO scale, and was withdrawn if toxicity was observed. About nephrotoxicity, if serum creatinine level within 21 days of the previous cycle was higher than 1.5 mg/dL, supplementary hydration was started between for 1 to 7 days. If then creatinine was between 1.5-2 mg/dL, or CrCl was 45-60 mL/min, cisplatinum dose was reduced by 50%. If serum creatinine was higher than 2 mg/dL or CrCl was lower than 45 mL/min, the patient was withdrawn from the study.

The levels of each one of the 27 amino acids were determined at baseline (1 or 2 days before treatment start) and 72 hours after the administration of cisplatinum for each cycle.

The sample was categorized into two groups for statistical analysis. Group A: patients with renal failure and hyperaminoaciduria within the same cycle, and Group B: patients with a delay between these two events (renal function impairment the cycle following onset of hyperaminoaciduria). The aim was to assess whether the defect in amino acids tubular reabsorption could predict those patients that may develop renal failure.

Amino acids (AA) levels measured with the chromatographic Beckman analyzer, system 6300/7300, were: aspartic acid, taurine, threonine, serine, asparagine, glutamic acid, glutamine, proline, glycine, alanine, citrulline, amino butyric acid, valine, cystine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, ornithine, lysine, histidine, arginine, hydroxyproline, 3-methyl-histidine, 1-methyl-histidine. We also determined in blood: glucose, urea, creatinine, sodium, potassium, chlorine, calcium, phosphorus, uric acid, proteins, albumin, GOT, GPT, alkaline phosphatase (APH), gamma-glutamyl transpeptidase, total bilirubin, lactic dehydrogenase, magnesium, beta-2-microglobulin; and in 24-h urine: glucose, proteins, density, acidity (pH), sodium, creatinine, potassium, calcium, phosphorus, magnesium, and beta-2-microglobulin.

The following equations were used to assess tubular renal function:

1. *AA/Cr ratio*. This determination was done to minimize errors in 24-h urine collection.

2. *Reabsorption fraction (Tx)* for each amino acid, calcium, phosphorus, magnesium and beta-2-microglobulin. The calculation was done by means of the following formula: $Tx = (1- [(UX \times PCr) / (PX \times UCr)]) \times 100$ (%); UX: urine concentration of the substance; PX: plasma concentration of the substance.

Tx ≤ 95%: reabsorption ≤ 95% of the parameter analyzed

Tx ≤ 90%: reabsorption ≤ 90% of the parameter analyzed

Tx ≤ 85%: reabsorption ≤ 85% of the parameter analyzed

3. *Phosphorus fractional reabsorption (Tp/CrCl),* which was calculated by means of the following equation:

Tp/CrCl = PP - [(UP × PCr)/UCr]; PP: plasma phosphorus; UP: urine phosphorus.

Statistical analysis

ANOVA test was used to analyze the variations in amino acids levels and other parameters throughout the cycles, for both global patients and the groups of patients with and without renal failure. For not normally distributed parameters, the non-parametric Friedman's test was used. To compare qualitative variables the Chi-squared test was used. Statistically significant values were those with a p value < 0.05.

RESULTS

Patients characteristics are shown in Table I. A total of 308 determinations were done. Each one of plasma amino acids was determined after each chemotherapy cycle, and there were some amino acids with great level variations throughout the cycles. Although for some amino acids these changes were not significant, others did present statistically changes: alanine, arginine, asparagine, phenylalanine, leucine, lysine, methionine, ornithine, serine, threonine, glycine, glutamine, histidine, tyrosine, hydroxyproline and 3-methyl-histidine (Table II). The variation in percentage of amino acid reabsorption throughout the chemotherapy cycles was significant for 13 amino acids (Table III).

Of the 54 patients included in the study, 13 (24%) presented during some of the cycles renal function impairment with plasma creatinine elevation > 1.5 mg/dL and/or CrCl decrease < 50 mL/min. En 3/13 (23%) patients, renal toxicity was progressive and irreversible, not responding to aggressive hydration and reaching plasma creatinine levels of 2.5, 3.1 and 8.1 mg/dL, respectively, and CrCl of 32, 23 and 8 mL/min, respectively, the third case requiring inclusion into a dialysis program.

Of the 10 remaining patients (77%) that developed nephrotoxicity, all of them could be improved and recovered being able to resume the treatment although with a mean delay of 3 days (range 2-6). Three patients required reducing the cisplatinum dose. However, having developed renal function impairments in spite of returning to normal on hydration increased the possibility of developing new nephrotoxicity epi-

Table I. Characteristics of treated patients

Characteristics		Patients
Age (median (years) and range)		58 (23-70)
Gender: Male		48 (88%)
Female		6 (12%)
Type of tumor:		
head-neck		27 (50%)
Lung		24 (44.4%)
Germ line		3 (5.6%)
Type of chemotherapy:		
	CDDP+ VP-16	24 (44.4%)
	CDDP+ 5-FU	27 (50%)
	BEP	1 (1.9%)
	BEP + VIP	2 (3.7%)
Num. of cycles:		
	2 cycles	1 (1.9%)
	3 cycles	3 (5.5%)
	4 cycles	27 (50%)
	5 cycles	3 (5.5%)
	6 cycles	20 (37.1%)

CDP: Adriamycin + Cisplatinum
FU: Fluorouracil
BEP: Bleomycin + Etoposide + Cisplatinum
VIP: Etoposide + Ifosfamide + Cisplatinum

Table II. Changes in blood amino acid levels throughout cycles

Amino acids	Statistical significance	Amino acids	Statistical significance
Isoleucine	NS	Hydroxyproline	0.001
Leucine	0.005	Glycine	0.03
Lysine	0.024	Cystine	NS
Glutamic acid	NS	Taurine	NS
Alanine	0.01	Tyrosine	0.001
Methionine	0.004	Histidine	0.001
Arginine	0.0001	Amino butyric acid	NS
Asparagine	0.04	Aspartic acid	NS
Ornithine	0.037	Alfa-aba	NS
Citrulline	NS	3-methyl-histidine	0.004
Phenylalanine	0.0001	Tryptophan	NS
Proline	NS	Valine	NS
Serine	0.003	Glutamine	0.0001
Threonine	0.0001		

sodes with the following cycles, which occurred in 9/10 (90%) patients.

In 38 cycles we observed renal function impairment within the cycle itself in which samples were taken (Group A), and in 42 cycles renal dysfunction occurred after cycle administration (Group B). Of all studied amino acids, with none of them a statistically significant difference was found when cycles with hyperaminoaciduria (at each one of established cut-off points) were compared with presence of renal function impairment, at both the same cycle (Group A) and the next cycle (Group B).

Table III. Changes in the percentage of reabsorption (Tx) for each amino acid throughout cycles

Amino acid	Statistical significance (p)	Amino acid	Statistical significance (p)
3-methyl histidine	NS	Isoleucine	NS
Amino butyric acid	NS	Leucine	NS
Aspartic acid	NS	Lysine	NS
Glutamic acid	0.01	Methionine	0.0002
Alanine	0.0008	Hydroxyproline	NS
Alfa-aba	NS	Ornithine	0.03
Arginine	NS	Proline	NS
Asparagine	0,01	Serine	0.0003
Cystine	0.0026	Taurine	0.01
Citrulline	NS	Tyrosine	0.02
Phenylalanine	NS	Threonine	NS
Glycine	0.0002	Tryptophan	0.04
Glutamine	0.0006	Valine	NS
Histidine	NS	1-methyl histidine	0.025

Considering the cycles in which urinary output impairment for four amino acids or more was detected, and comparing those with renal dysfunction with those without it, the following results were observed: within Group A, in cycles with renal function impairment, it was found that when the reabsorption percentage was less than 95%, the number of cycles with 4 altered amino acids was 2 out of 38 cycles vs. 2 out of 254 in those without renal dysfunction ($p = 0,02$); for $Tx \leq 90\%$, 1 cycle vs. 0 cycles ($p = 0.007$), the same for $Tx \leq 85\%$. As for Group B, the difference between cycles with renal dysfunction as compared to those without renal dysfunction was: $Tx \leq 95\%$, the number of cycles with 4 altered amino acids was 1 out of 42 cycles vs. 3 out of 208 cycles; and for $\leq$ 90% and 85%, at both cut-off point was 1 cycle vs. 0 cycles. In no case statistically significant differences were found. About other biological parameters and their possible relationship with renal function impairment, the reabsorption percentage (Tx) was analyzed for: la beta-2-microglobulin, calcium, phosphorus, magnesium, and total protein. Serum phosphorus did not show significant changes through cycles; however, there were differences the groups of patients with and without renal failure ($p = 0.004$). No other parameter showed significant differences or a predictive value for cisplatinum-induced renal failure.

Of the 27 amino acids studied, virtually almost all of them showed substantial variability in their plasma levels when comparing patients that had developed some sort of renal failure and those having not. However, only mean glutamine levels above 1000 mM/L

was significant (Figure 1). As a whole, modifications in plasma glutamine levels showed a statistical significance throughout the cycles ($p = 0.0001$), but these changes were only really relevant for those patients with nephrotoxicity ($p = 0.0002$), whereas there was no statistical significance in patients with renal failure. Statistically significant differences ($p = 0.004$) were observed in glutamine variation throughout the cycles between these two groups of patients.

There was a positive correlation between the following variables: plasma creatinine level before the cycle administration and plasma glutamine level gathered at the previous cycle ($r = 0.689$, $p < 0.0001$) (Figure 2) as well as between CrCl before cycle administration and plasma glutamine level gathered at the previous cycle ($r = -0.563$, $p < 0.0001$) (Figure 3).

In 42 out of 254 (16.5%) chemotherapy cycles administered some degree of renal failure was detected and in 41 cycles (16.1%) an increase in plasma glutamine level > 1000 mM/L was observed. In 36 cycles, renal function impairment coincided with glutamine levels increase above 1000 mM/L in the previous cycle, in such a way that the relationship between cycles with glutamine increase above those levels and the development of some degree of renal failure with the administration of the following cycle was very significant ($p < 0.000001$).

Considering the correlation between glutamine increase above 1000 mM/L and the development or renal failure, it was observed that 13 (24%) patients developed some degree of renal failure and 16 (29.6%) had glutamine increase in some cycle. The 13 patients showing nephrotoxicity had had previous glutamine increase, and only 3 patients that had this amino acid increased at any of the cycles did not further developed nephrotoxicity. In this way, the relationship between patients that have developed renal toxicity and the fact of having previously had increased glutamine > 1000 mM/L still is very significant ($p < 0.000001$).

DISCUSSION

Cisplatinum induces both acute and chronic nephrotoxicity by accumulation, and this was the reason why its use was restricted when the first trials were carried out. The mechanism by which this drug damages tubular cells is not well understood. Recent investigations suggest that enzymes of the type cysteine S

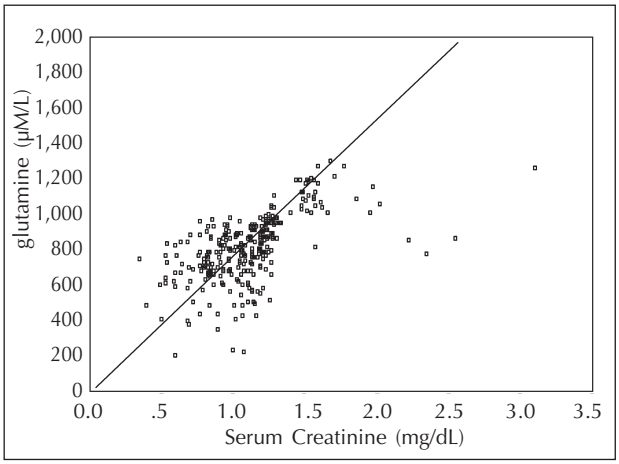

Fig. 2.—Correlation between glutamine and serum creatinine at the cycle following cisplatinum administration.

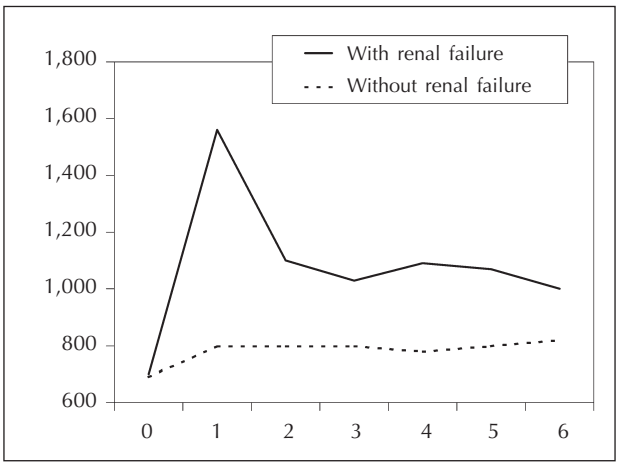

Fig. 1.—Glutamine levels at the different cisplatinum cycles in patients by presence or absence of renal failure.

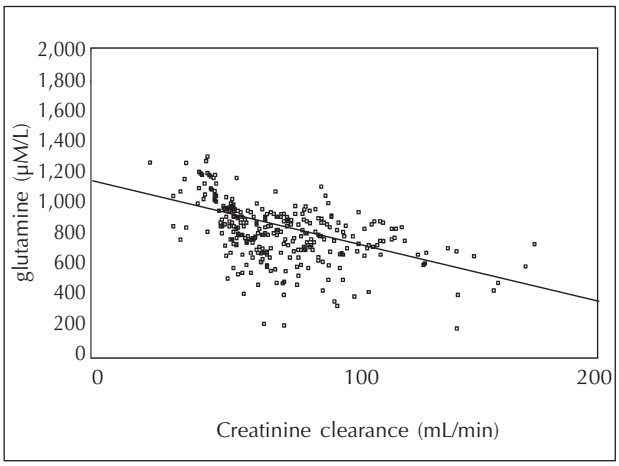

Fig. 3.—Correlation between glutamine and creatinine clearance en el cycle at the cycle following cisplatinum administration.

are implicated in cisplatinum-induced renal toxicity.[11] There are very few studies analyzing the variations in plasma amino acid profiles at the initial phase of renal failure. In other studies the results have been controversial, likely due to the low number of studied cases and the heterogeneity of included patients: pathology, age, degree of renal dysfunction, and different treatments at the time of the analysis.[12,13]

Overhydration and diuretics use are the most frequently used means to prevent cisplatinum nephrotoxicity. Conventional hydration regimens providing at least a urinary volume of 3 liters for 24 hours, as the one used in this study, usually protect against nephrotoxicity, which is detected in 5-10% of the cases. The selection of solutions in which to dilute CDDP is also important. Hypertonic saline solution (3-4.5%), used here, has been shown to have a protective effect, possibly because the high concentration of chlorine may reduce the binding of cisplatinum to sulphydryl groups from proteins.[14]

Twenty-four percent of the patients had renal failure at some cycle and this is somewhat higher than other reported series. However, we should highlight that only in 3 cases (5.5%) toxicity was irreversible, this event being the one registered in toxicity studies using cisplatinum as anti-tumor agent and which is in agreement with the percentage observed in our series.

Detecting factors that may predict high risk for renal toxicity secondary to cisplatinum, in spite of a low percentage of severe events, is highly interesting because of its clinical relevance regarding prognosis and health costs.

We did not find significant variations of plasma amino acid profile in relation to the presence or absence of renal failure, except for glutamine. This finding differs from other studies in which there really were variations in some amino acids in the presence of this complication. This is the case fro branched amino acids valine, leucine and isoleucine[15,16] serum citrulline, aromatic amino acids, specially tyrosine[17], and other amino acids which increases were detected sporadically and variably from one study to another.[18,19] Experimental studies on cisplatinum-induced nephrotoxicity conclude that quantification of amino acids tubular renal transport is a good marker of nephrotoxicity, especially in young animals.[20]

When focusing this study towards the search of biochemical changes that would predict high risk for cisplatinum-induced nephrotoxicity we performed amino acids determinations the third day after the administration of the drug, before detecting the raise in plasma creatinine, when possibly the changes in the amino acid profile would be at the highest level.

This study shows significant differences in glutamine levels when comparing the evolution curves of plasma levels throughout the cycles between patients developing some degree of renal failure and those not having it. When a cycle-to-cycle analysis was done, there was a clear trend for glutamine raise within the same cycle in which renal failure was observed, although the increase was found to be greater in the previous sample, as if the increase in this amino acid could predict an increase in plasma creatinine before having to administer the following cycle. These data were corroborated when a positive correlation was found between glutamine levels after cisplatinum and creatinine and CrCl before the administration of the following cycle.

When analyzing glutamine levels throughout the cycles in nephrotoxic patients, mean values in each of the cycles were above 1000 mM/L; thus this value was selected as the cut-off point to develop a posteriori renal toxicity; this calculation was done per cycle and per patient. In both cases, the results were similar and the relationship was highly significant, yielding very acceptable sensitivity, specificity, and positive predictive values of the test.

There are no studies with a similar design against which our results may be compared. However, some authors have detected glutamine increases and the decrease in its plasma clearance in renal failure, especially at initial phases. It has even been shown that dipeptides including glutamine, such as alanine-glutamine or glycine-glutamine, also show a decreased clearance in renal failure.[21] Other studies also support, from a pathophysiologic point of view, these results by demonstrating that in renal failure there is a decrease in glutamine uptake by the liver in order to form ammonia and then urea.[8] By the action of the two fractions of the glutaminase (liver and renal glutaminase) glutamine yields glutamate and ammonia, which then is converted into urea (in the kidney this latter step does not take place since ammonia is directly excreted in the urine as measurable acidity). Both substrates (as well as conditions leading to hyperammoniemia as in renal failure) inhibit the action of glutaminase by feed-back mechanisms.[22,23] To corroborate this, there are experimental studies in rats which kidneys were submitted to ischemia which led to inhibition of the glutaminase enzymatic system in order to prevent ammonia accumulation, which also has toxic effects in the kidney.[24] However, this inhibition is not permanent for the time renal failure remains but it occurs at very early stages and the enzymatic activity goes back to normality within few days, so that the effect on glutamine will only be observed early on.[25] This is in agreement with the results obtained in the present study in which the glutamine in-

crease was more evident that may correspond to acute tubular damage and preceding the development of evident renal failure. By contrast, when renal failure was established, glutamine levels decreased.

This study justifies the setting of new and more accurate techniques able to predict the population at risk for developing renal failure or anticipating its diagnosis at early phases.[26]

To conclude, glutamine levels higher than 1000 mM/L within 72 hours of cisplatinum treatment constitute a nephrotoxicity indicator, even more if similar changes have occurred at previous cycles. Early adjustments in cisplatinum administered dose and higher monitoring of post-cisplatinum hydration may help to prevent certain degrees of renal failure that in some cases may be irreversible.

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