

Can sildenafil citrate ameliorate cisplatin-induced nephrotoxicity? Crosstalk between the possible mechanisms

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SUMMARY

Nephrotoxicity is considered the most important side effect which limits cisplatin therapy in various diseases. It might be due to oxidative stress, decreased renal blood flow and reduction in the glomerular filtration. Sildenafil citrate is used for treatment of erectile dysfunction, but its effect in ameliorating cisplatin nephrotoxicity was not yet clearly studied. So the present work aimed to evaluate the protective role of Sildenafil citrate against cisplatin-induced nephrotoxicity in adult male albino rats. 24 adult male albino rats were divided into 4 groups (6 rats each): Group I, control; Group II, sham control; Group III, Cisplatin-treated group, and Group IV, Sildenafil-and-Cisplatin-treated group. At the end of the experiment, the kidney of each animal was excised, trimmed and prepared for histological, histochemical and immunohistochemical study. Blood samples were obtained to evaluate the kidney functions. Kidney sections of Group III showed marked degenerative changes in the proximal convoluted tubules, vacuolations, exfoliation of the lining epithelium, cast formation and interstitial hemorrhagic exudate. There was marked elevation of serum creatinine and urea with significant increase in nitric oxide (NO) and decrease in glutathione reductase (GSH) concentrations in the kidney tissue and weak periodic acid Schiff (PAS) reaction. Treatment with Sildenafil citrate in Group IV offered marked improvement in

the renal structure, kidney function tests and other parameters. The present study concluded that Sildenafil citrate could protect the kidney against Cisplatin-induced nephrotoxicity in adult male albino rat.

Key words: Cisplatin – Sildenafil citrate – Kidney – Oxidative stress

INTRODUCTION

Nowadays, cisplatin is considered the standard anti-cancer drug used in treatment of various malignant tumors. Cisplatin toxicity in the kidney could present as acute kidney injury, hypomagnesemia, distal renal tubular acidosis, hypocalcemia, renal salt wasting, and hyperuricemia (Miller et al., 2010).

Cisplatin therapy is frequently limited by many severe side effects such as bone marrow suppression, neurotoxicity, ototoxicity, anaphylaxis, and nephrotoxicity (Shiraishi et al., 2000; Satry and Keillie, 2005).

There are several mechanisms involved in such toxicity: proximal tubular injury, oxidative stress, inflammation and vascular injury in the kidney (Ciariboli et al., 2005; Yao et al., 2007). Proximal tubular injury involves several different mechanisms, including direct toxicity to renal tubular epithelial cells and marked apoptosis (Wei et al., 2007; Jiang and Dong, 2008).

The pathological effect of cisplatin-induced nephrotoxicity includes induction of renal ischemia, reduction of glomerular filtration and increase of serum creatinine, as well as a reduction in serum

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magnesium and potassium levels. Moreover, cisplatin causes renal vasoconstriction through injury on the renal vasculature, which reduces blood flow, causing ischemic damage to the kidney and affects the glomerular filtration rate (Gi-Su et al., 2014).

Cisplatin increases the production of peroxynitrite and nitric oxide in the kidney tissues of rats (Chirino et al., 2004). Dickey et al. (2005) found that production of reactive oxygen species (ROS) in cisplatin therapy is directly related to its cytotoxicity and can be improved by free radical scavengers.

During cisplatin therapy, ROS are produced and implicated in the pathogenesis of its nephrotoxicity. Generation of ROS directly targets the lipid components of the cell membrane, causing peroxidation and denaturation of proteins, which finally leads to enzymatic inactivation (Kawai et al., 2006). Cisplatin has a low molecular weight and uncharged character, so it is present in unbound form and is freely filtered by the renal glomerulus. Most of the cisplatin is trapped in the tubules of the renal cortex (Launay et al., 2008). Its concentration in the proximal tubular cells reaches 5 times higher than its plasma concentration, which contributes to its nephrotoxicity (Kodama et al., 2014).

Renal vasoconstriction is an important factor in the pathogenesis of cisplatin-induced nephrotoxicity, causing reduction in medullary blood flow, which results in marked tubular cell injury (Winston and Safirstein, 1985). The vasoconstriction that occurs during cisplatin therapy caused more hypoxic injury and renal tubular toxicity (Schrier et al., 2004).

Cisplatin-induced vascular toxicities might be due to thrombotic microangiopathy and myocardial infarction. Vascular endothelial injury is an important component of cisplatin-induced renal injury (Bonventre and Zuk, 2004).

Sildenafil citrate is a selective phosphodiesterase-5 inhibitor, and it has multiple therapeutic actions that involve increase in intracellular cGMP levels, scavenging of free radicals and reduction in the inflammatory cytokines (Cadirci et al., 2011). Sildenafil is now used to treat not only erectile dysfunction but also pulmonary hypertension, ischemia/reperfusion injury, myocardial infarction, heart failure, cerebrovascular stroke and neurodegenerative diseases (Sandner et al., 2007).

Therefore, the present study aimed to evaluate the possible protective role of Sildenafil on Cisplatin-induced nephrotoxicity in adult male albino rats.

MATERIALS AND METHODS

Animals

The present study was carried out on 24 adult male albino (Sprague-Dawley) rats weighing 180-220 g. The rats were obtained from the Animal House, Faculty of Medicine, Cairo University. The rats were used after two weeks for proper acclimatization to the standard housing conditions ($25 \pm 2^\circ\text{C}$ temperature and 12 h light/dark cycle) and were

supplied with standard chow and tap water ad libitum. They were housed in separate clean stainless steel cages (4 rats/cage) under normal hygienic conditions maintained under standard laboratory and environmental conditions. All the animals were treated in accordance with the international guidelines for the care and use of laboratory animals.

Chemicals

Cisplatin: (cis-diamminedichloroplatinum, CDDP): It was supplied by Mylan Pharmaceutical Co., France, in the form of vials (vial 50 mg/50 mL). Each vial contains Cisplatin dissolved in normal isotonic saline. Nephrotoxicity was induced by cisplatin in a dose of 1 mg/kg daily by intraperitoneal injection (i.p.) for 7 days (Huang et al., 2001).

Sildenafil citrate (Viagra): obtained as tablet form from Galaxo Smithkline Pharmaceutical Company. Each tablet contains 50 mg Sildenafil citrate. The tablet was dissolved in 50 ml of distilled water (1 ml contains 1 mg sildenafil citrate) to obtain the recommended dose for each rat (5 mg/kg) (Abdel-Latif et al., 2013).

Experimental design

The rats were divided into four equal groups (6 rats each) as follow:

Group I (normal control): the rats received nothing.

Group II (sham control): the animals received 1 ml of isotonic saline (vehicle of cisplatin) by intraperitoneal (i.p.) injection daily for 7 days.

Group III (cisplatin- treated): each animal received a dose of 1 mg/kg daily by intraperitoneal injection (i.p.) for 7 days (Huang et al., 2001).

Group IV (sildenafil citrate and cisplatin treated): each animal received a dose of 5 mg/kg of sildenafil citrate (Abdel-Latif et al., 2013) orally via gastric tube concomitant with administration of 1 mg/kg cisplatin daily by i.p. injection for 7 days (Huang et al., 2001). The drugs were given to each animal daily for 7 days at a fixed time (9.00 am).

At the end of the experiment, the rats were sacrificed by cervical dislocation; then the abdomen was opened, 3 ml blood sample was obtained from the heart and collected in heparinized tubes for biochemical study; and the kidneys of each animal were excised, trimmed and prepared for histological and histochemical study. Kidney specimens were immediately stored at -70°C to use for histochemical study, while other specimens were stored in 10% formol saline for further histological and histomorphometric study.

The kidney sections were subjected to:

- Light microscopic study: using
 - Hematoxylin and Eosin stain (H&E): for routine histological examination
 - PAS stain reaction: for examination of the PAS reaction of the basal lamina and the brush border of the renal tubules.

Biochemical analysis: estimation of the kidney functions by measuring the level of serum urea and creatinine in the blood of the different experi-

mental groups.

Histochemical study: measuring the mean values of GSH and NO enzymes as an indication for the oxidative stress in the kidney tissue of the different experimental groups.

Histomorphometric study: using Leica image analyzer computer system to study the optical density of PAS reaction in the kidney sections of all experimental groups.

Statistical analysis: of the resulting data using the statistical package for the social science (SPSS program).

Light microscopic study

Kidney specimens of each animal were fixed in 10% formal saline and processed for paraffin blocks; then sections of 5 micrometers thickness were sliced, prepared and stained with Haematoxylin and Eosin (H&E) and PAS reaction (Bancroft and Gambel, 2008) for light microscopic study. The study was carried out in the Research Center, Faculty of Agriculture, Cairo University.

Biochemical study

Blood samples were obtained from the rat hearts. Blood urea and serum creatinine were estimated with an Abbott-Aeroset auto-analyzer (Chicago, IL, USA), using the original kits done in Biochemistry department in agricultural center, Faculty of Agriculture, Cairo University. Plasma creatinine was determined according to the method of Bartles et al. (1972). The absorbance of samples was read at 495 nm against a reagent blank, and creatinine concentration was expressed in mg/dl. Plasma urea was determined according to the method of Fawcett and Scott (1960). The absorbance of samples was read at 550 nm against a reagent blank, and urea concentration was expressed in mg/dl.

Histochemical study

Measurement of Glutathione Reductase (GSH) concentration in the renal tissues

The determination of glutathione reductase (GSH) concentration was carried out according to the method described by Beutler et al. (1963). This method is based on the development of relatively stable yellow color, when 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) is added to sulfhydryl compound. Kidney homogenate 100 μ l (0.2 g/ml) and 100 μ l saline were added to 1.8 ml distilled water using a calibrated micropipette; then 3 ml of precipitating solution were added. The mixture was allowed to stand for 5 minutes at room temperature, and was then filtered. 2 ml of the filtrate were added to 8 ml phosphate buffer and 1 ml DTNB. GSH concentration was expressed as μ g protein.

Measurement of nitric oxide (NO) concentration in the renal tissues

NO measurement was done by measuring the tissue nitrite (NO₂⁻) and nitrate (NO₃⁻) and then estimated as an index of NO production. Samples were initially deproteinized with Somogyi reagent.

Total nitrite (nitrite + nitrate) was measured after conversion of nitrate to nitrite by a spectrophotometer at 545 nm. Results were expressed as nmol/g wt tissue (Cortas and Wakid, 1990).

Histo-morphometric study

Using Leica Qwin 500 LTD image analyzer computer system (software Qwin 500, Cambridge, UK), the mean optical density of PAS stained sections in different experimental groups was measured. Ten non-overlapping fields were measured for each specimen and the mean values were calculated and recorded. These measurements were done in the Research Center, Faculty of Agriculture, Cairo University.

Statistical study

The mean values and standard deviation of the data obtained from the chemical study, histochemical study and image analyzer (the optical density of PAS stained sections) were analyzed using SPSS (Statistical Package for Social Science, version 9) Chicago, USA. Comparison between the different groups was calculated using ANOVA test. The results were considered statistically significant when P value > 0.05. and P > 0.001 was considered highly significant. The data obtained was tabulated and represented graphically (Armitage and Berry, 1994).

RESULTS

Histological results

Haematoxylin and eosin (H&E) stained sections

Histological examination of H&E stained sections of the kidney of control group (group I) revealed the classic architecture of the renal cortex; the renal glomeruli, proximal and distal convoluted tubules. The renal corpuscle consisted of a glomerular capillary tuft surrounded by Bowman's capsule with a narrow Bowman's space in-between. The proximal convoluted tubules had narrow lumen, lined with simple cuboidal cells with large rounded vesicular nuclei, and acidophilic cytoplasm with intact preserved brush border. The distal convoluted tubules had wider lumen, lined with simple cuboidal cells with central rounded nuclei and acidophilic cytoplasm. The renal medulla consisting of collecting tubules showed wide regular lumen, and lined with simple cuboidal cells with central rounded nuclei and acidophilic cytoplasm (Figs. 1-A and 1-B). Kidney sections of sham control group (group II) revealed no histological differences as compared to the results of control group.

Meanwhile, kidney sections of cisplatin-treated group (group III) displayed marked distortion of the renal architecture and obvious degenerative changes in the renal cortex: renal glomeruli, disruption of the Bowman's capsule and widening of Bowman's space. The proximal and distal cortical tubules were distorted, and dilated with marked degeneration and vacuolations of the lining epithelium and loss of the brush border (Fig. 1-C). There were interstitial hemorrhagic exudate and extrava-

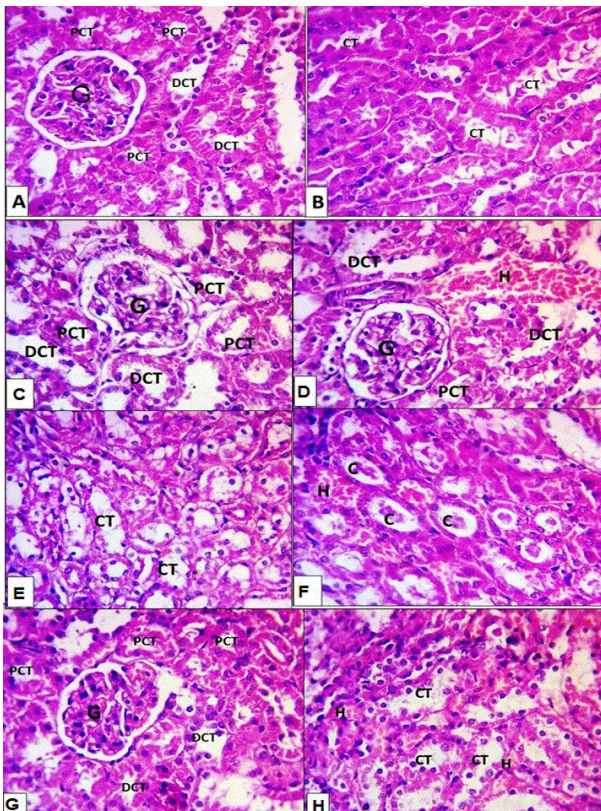


Fig 1. Haematoxylin and eosin stained sections of kidney of control group (A and B), cisplatin treated group (C, D, E and F) and sildenafil citrate treated group (G and H). x 400. G: glomerulus; PCT: proximal convoluted tubule; DCT: distal convoluted tubule; CT: collecting tubule; C: cast; H: hemorrhage

sation of the red blood cells (RBCs) in between the tubules (Fig. 1-D). The renal medulla demonstrated distortion and dilatation of the collecting tubules and exfoliation of the epithelial lining cells (Fig. 1-E) with interstitial hemorrhagic exudate and formation of hyaline cast (Fig. 1-F).

On the other hand, kidney sections of sildenafil treated group (group IV) displayed remarkable improvement and restoration of the normal renal architecture. The renal cortex showed normal structure of the renal glomeruli, the proximal and distal convoluted tubules. The proximal tubules appeared nearly normal with preserved intact brush border (Fig. 1-G). The renal medulla showed improvement of the structure of the collecting tubules with nearly normal lining epithelium and mild interstitial hemorrhagic exudate in-between (Fig. 1-H).

PAS stained sections

PAS stained sections of the kidney of control group (group I) (Fig. 2-A) and sham control group (group II) (Fig. 2-B) revealed strong positive PAS reaction in the parietal layer of Bowman's capsule, the basal membrane of the cortical tubules and in the luminal brush border of proximal convoluted tubules. Meanwhile, PAS stained sections of cisplatin-treated group (group III) revealed weak PAS reaction (Fig. 2-C). On the other hand, PAS

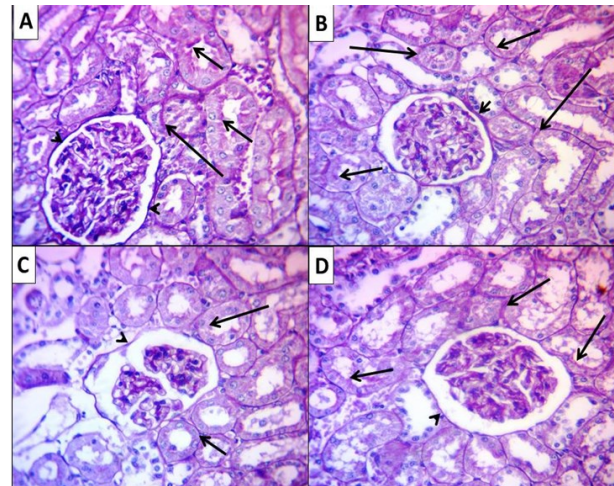


Fig 2. PAS stained sections of the kidney of control group (A), sham control group (B) showing strong positive PAS reaction in the parietal layer of Bowman's capsule (arrow heads), the basal membrane of the cortical tubules (long arrow) and in the luminal brush border of PCT (short arrows); weak PAS reaction in sections of cisplatin-treated group (C) and strong positive PAS reaction in sections of sildenafil citrate treated group (D). x 400

stained sections of sildenafil-citrate-treated group (group IV) revealed strong positive PAS reaction (Fig. 2-D).

Biochemical analysis

The kidney functions tests of group III showed marked increase in serum urea (48.6 ± 3.2 mg/dl) and creatinine (4.2 ± 0.14 mg/dl), which were statistically significant when compared to the results of the control group (28.4 ± 1.4 and 0.82 ± 0.16 mg/dl, respectively). Meanwhile, treatment with Sildenafil citrate in Group IV revealed marked reduction in the serum levels of urea (32.8 ± 2.5 mg/dl) and creatinine (1.12 ± 0.62 mg/dl), which were statistically insignificant when compared to the results of the control group. (Table 1).

Histochemical results

The mean GSH enzyme concentration in the kidney tissues of group III was markedly decreased (0.10 ± 0.23 μ g/g) if compared to the group I (0.24 ± 2.12 μ g/g), which was statistically high significant. There was a marked increase in the renal NO concentration (0.86 ± 0.04 nmol/g) as compared to group I (0.18 ± 0.62 nmol/g), which was statistically high significant. Treatment with sildenafil citrate in Group IV showed obvious improvement in the form of increase in GSH concentration (0.19 ± 0.16 μ g/g) and reduction in NO concentration (0.23 ± 0.08 nmol/g) in the renal tissue, which was statistically insignificant when compared to group I (Table 2).

Histomorphometric results

The kidney section of group III showed marked decrease in the optical density of PAS reaction (0.22 ± 0.02), which was statistically significant when compared to the control group (0.46 ± 0.12).

Meanwhile, group IV showed increase in the optical density of PAS reaction (0.38 ± 0.8), which was statistically significant when compared to the control group and significant when compared with the same finding in group III (Table 3).

DISCUSSION

In the present study, cisplatin-treated group (group III) displayed marked pathological changes in the kidney mainly affecting the proximal tubules of the renal cortex in comparison to the histological picture of the control group. There were marked distortion of the renal architecture, disruption of the Bowman's capsule and widening of Bowman's space. The proximal and distal cortical tubules were distorted, dilated with marked degeneration and vacuolations of the lining epithelium and loss of the brush border. There were interstitial hemorrhagic exudate and extravasation of the RBCs in between the tubules. The renal medulla demonstrated distortion and dilatation of the collecting tubules and exfoliation of the epithelial lining cells, with marked interstitial hemorrhagic exudate and formation of intratubular hyaline cast.

These findings were in accordance with Aman et al. (2008), who mentioned that cisplatin administration caused a conspicuous alteration in the histological structure of many PCT of the subcap-

sular nephrons in the form of epithelial swelling with an intracytoplasmic vacuolations and narrowing of their lumina; others exhibited a shorter tubular hyperchromatic epithelium with loss of striated edge and discontinuity of tubular wall. They added that many tubules have no cellular lining and lost their nuclei, while others were lined with flattened elongated cells. Most of the tubular epithelial cells were fragmented and sloughed into their lumina and some tubules contained hyaline casts.

Moreover, these findings were agreed with Ozkok and Edelstein (2014). The pathophysiology of cisplatin-induced acute kidney ischemia (AKI) involves proximal tubular injury, oxidative stress, inflammation, and vascular injury in the kidney. There is predominantly acute tubular necrosis and also apoptosis in the proximal tubules. The present study revealed that affection was mainly in the proximal convoluted tubule.

These findings were supported by Kodama et al. (2014), who mentioned that concentration of cisplatin in the proximal tubular cells is 5 times higher than the serum concentration, and thus such an accumulation of cisplatin in kidney contributes to its nephrotoxicity.

Also, Winston and Safirstein (1985) postulated that renal vasoconstriction, caused by endothelial dysfunction and impaired vascular autoregulation, is an important component of the pathophysiology of cisplatin-induced nephrotoxicity. The cisplatin-treated group showed marked alteration in the kidney functions and increased in the serum urea and creatinine. These findings were in consistent with Helmy et al. (2014), who recorded significant increases in blood urea nitrogen and serum creatinine concentrations following acute cisplatin administration, resulted in cisplatin injection.

Oxidative stress might be the causative mechanism of cisplatin nephrotoxicity. In the present study there was marked depletion of GSH enzyme in the kidney, with marked increase in NO enzyme. These findings were in accordance with Badar et al. (2005), who found that cisplatin had negative inhibitory effects on antioxidant enzymes, and therefore significantly decreased the renal activities of superoxide dismutase, glutathione peroxidase, and catalase. Moreover, these findings were supported by the findings of Kawai et al. (2006), who mentioned that, following treatment with cisplatin, ROS are implicated in the pathogenesis of acute renal injury. They added that ROS directly target the lipid components of the cell membrane, causing peroxidation and denaturation of proteins.

These findings could be explained by Wainford et al. (2008), who suggested that cisplatin is conjugated with reduced glutathione (GSH) in the liver and reaches the kidney as a cisplatin-GSH conjugate, which is cleaved to a nephrotoxic metabolite mainly by the action of gamma-glutamyl transpeptidase (GGT), an enzyme primarily located in the brush border of the proximal convoluted tubule of the kidney. The metabolite formed is a highly reactive thiol/platinum compound that interacts with

Table 1. Mean \pm SD serum levels of urea and creatinine of the different experimental groups

Groups	Urea (mg/dl)	Creatinine (mg/dl)
Group I	28.4 \pm 1.4	0.82 \pm 0.16
Group II	29.2 \pm 1.1	0.78 \pm 0.22
Group III	↑ 48.6 \pm 3.2*	↑ 4.2 \pm 0.14*
Group IV	↑ 32.8 \pm 2.5	↑ 1.12 \pm 0.62

Table 2. Mean \pm SD of level of GSH and NO in the renal tissues of the different experimental groups.

Groups	GSH (μ g/g)	NO (nmol/g)
Group I	0.24 \pm 2.12	0.18 \pm 0.62
Group II	0.25 \pm 0.16	0.19 \pm 0.03
Group III	↓ 0.10 \pm 0.23**	↑ 0.86 \pm 0.04**
Group IV	0.19 \pm 0.16	0.23 \pm 0.08

Table 3. Mean \pm SD of the optical density of PAS reaction of the different experimental groups.

Groups	Mean \pm SD
Group I	0.46 \pm 0.12
Group II	0.44 \pm 0.24
Group III	↓ 0.22 \pm 0.02*
Group IV	0.38 \pm 0.8

* P > 0.05 statistically significant

macromolecules leading eventually to renal cell death.

Sildenafil treated group revealed obvious improvement in the histological picture of the kidney with remarkable restoration of the renal architecture of the renal glomeruli and cortical tubules. These findings were in agreement with Morsy et al. (2014), who mentioned that concomitant administration of sildenafil with gentamicin restored the histopathological insult induced by gentamicin in the rat kidney, as it showed regular epithelial cells lining the tubules with normal morphology of renal cortex.

There was normalization of the kidney functions parameters (serum urea and creatinine). These findings were matched with that of Choi et al. (2009), who recorded that blood urea nitrogen (BUN) and serum creatinine levels were lower in sildenafil-treated rats.

Moreover, Zhao et al. (2011) reported that sildenafil effectively inhibited lipopolysaccharide-induced production of NO both in N9 cells and primary rat microglial cells. The decrease in NO level may be due to decrease in iNOS level, although eNOS level is increased, as the amount of NO generated by eNOS is small, while large quantities of NO are synthesized by iNOS (Raj and Baylis, 1995).

On the other hand, Cadirci et al. (2011) showed that sildenafil decreases malondialdehyde in the kidney tissues. This inhibitory effect of sildenafil on lipid peroxidation may be a result of its suppressing effect on iNOS expression (Goligorsky et al., 2002).

In conclusion, the present work elucidates that sildenafil citrate could protect the kidney against cisplatin-induced nephrotoxicity in adult male albino rat. This nephron-protective effect might be due to its antioxidant and reno-vascular effect.

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