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Sodium metabisulfite induces lipid peroxidation and apoptosis in rat gastric tissue

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Abstract

Sodium metabisulfite ($Na_2S_2O_5$) is used as an antioxidant and antimicrobial agent in a variety of drugs and functions as a preservative in many food preparations. This study was performed to elucidate the dose-dependent effects of sodium metabisulfite ingestion on rat gastric tissue apoptotic changes and lipid peroxidation. Forty male wistar rats, aged 3 months were used. They were randomly divided into four groups: control (C), the group treated with $Na_2S_2O_5$ (10 mg/kg; S1), the group treated with $Na_2S_2O_5$ (100 mg/kg; S2), the group treated with $Na_2S_2O_5$ (260 mg/kg; S3). $Na_2S_2O_5$ was given by intragastric intubation for 35 days. In the S2 and S3 groups, malondialdehyde (MDA) levels increased markedly when compared with the control group. High doses of sulfite administration elevated number of apoptotic cells both in mucosa and submucosa layers of stomach in parallel with increased MDA levels. These results suggest that sodium metabisulfite increased lipid peroxidation and thus number of apoptotic cells on gastric tissue in dose-dependent manner.

Keywords

sodium metabisulfite, lipid peroxidation, apoptosis, gastric tissue

Introduction

Humans are exposed to both endogenous and exogenous sulfites. Endogenous sulfite is generated as a consequence of the body's normal processing of sulfur-containing amino acids (Cooper, 1983; Griffith, 1987). Sulfites occur as a consequence of fermentation and also occur naturally in a number of foods and beverages (Lester, 1995). Five sulfite salts including sodium metabisulfite ($Na_2S_2O_5$), potassium metabisulfite ($K_2S_2O_5$), sodium bisulfite ($NaHSO_3$), potassium sulfite (K_2SO_3) and sodium sulfite (Na_2SO_3) are commonly used as antioxidants in food preparations (Gunnison and Jacobsen, 1987). Sulfites are usually added to food, beverages and drug preparations as an antioxidant and antimicrobial agent (Lakamp and Dobesh, 2000; Rodriguez et al., 1994). Antioxidant effects of sulfite are due to its ability to serve as a reductant (Baker et al., 2002).

Sulfite is a potentially toxic molecule that might enter the body via ingestion, inhalation or injection (Kucukatay et al., 2007). Accumulating evidence indicates that sulfite compounds cause toxic and adverse effects on mammals (Aydin et al., 2005). The amounts of ingested sulfites are expressed as sulfur dioxide equivalents (SDE). An evaluation by the Federation of American Societies for Experimental Biology (FASEB) estimates that 30–100 mg SO_2 has no observed adverse effects on humans (Nair and Elmore, 2003). The FASEB report is further supported by a Joint Expert Committee in the World Health Organization (WHO), which has established an acceptable daily intake (ADI) level of sulfites as 0.7 mg/kg body weight, expressed as sulfur dioxide

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(Nair and Elmore, 2003). With this in mind, it is important to note that the mean per capita of sulfite intake from food and beverages is estimated as 19 mg SDE per day (Gunnison and Jacobsen, 1987). This level is reported to be 163 mg SDE in the 99th percentile of the population (Gunnison and Jacobsen, 1987).

Lipid peroxidation is known to have deleterious effects on structure and functions of cell membrane (Gupta et al., 1991). A substantially high level of endogenous lipid peroxidation is an indicator of biochemical disorder in cells, tissues and organs, as well as an indicator of toxicological effect of some chemical toxins on living organisms. Lipid peroxidation is believed to be involved in several disease states, such as diabetes and neurodegenerative diseases as well as the aging process (Baynes, 1991).

Sulfur dioxide or bisulfite-sulfite toxicity may involve oxidative damage in cells, tissues and organs caused by sulfur- and oxygen-centered free radicals formed in the process of sulfite oxidation (Gordon et al., 2004; Meng, et al., 2003b; Meng, et al., 2003a).

Apoptosis, a physiological mechanism of eliminating damaged or aged cells, also plays a major role in gastrointestinal epithelial cell turnover (Fadeel, 2003; Obst et al., 2002; Philchenkov, 2003). In the stomach, mucosal surface epithelial cells are constantly exfoliating to the gastric lumen and completely replaced within 3–5 days under physiological conditions. Apoptosis has been reported to take place in all regions of the stomach with apoptotic cells occurring predominantly in the superficial parts of the gastric glands (Herbay and Rudi, 2000; Szabo and Tarnawski, 2000).

Recently, several studies have shown that sulfur dioxide and its derivates are the systemic toxic agents that cause DNA damage and oxidative damage in multiple organs in mice, including liver, brain, lung, spleen and stomach (Bai and Meng, 2005a, b; Meng and Bai, 2003). A variety of gastric lesions can be induced in rats by $Na_2S_2O_5$, including the formation of scattered hyperplastic glands in the fundic mucosa (Beems et al., 1982).

This study aimed to determine the dose-dependent effect of $Na_2S_2O_5$ ingestion on rat gastric tissue. Therefore, in the present study, oral sulfite treatments by gavage with doses 10, 100 and 260 mg/kg/day were established to examine lipid peroxidation and apoptotic changes in rats exposed to sulfite. Thus, thiobarbituric acid reactive substances (TBARS) were determined as an indicator of lipid peroxidation and terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) technique was used to detect apoptosis in gastric tissue.

Materials and methods

Preparation of animals

Forty male albino rats, aged 3 months, were used in this study. The animals were obtained from the Laboratory Animal Unit of the University of Akdeniz, and the study protocol was approved by the Akdeniz University Laboratory Animals Welfare and Ethics Commitee. The animals were fed a standard laboratory diet and water ad libitum. They were housed at 22°C $\pm$ 1°C and in a 12:12-hour light-dark cycle. Rats were randomly divided into four groups, each consisting of 10 animals: group 1; control (C), group 2; treated with $Na_2S_2O_5$ (10 mg/kg; S1), group 3; treated with $Na_2S_2O_5$ (100 mg/kg; S2) and group 4; treated with $Na_2S_2O_5$ (260 mg/kg; S3; Hui et al., 1989). Since the given dose of $Na_2S_2O_5$ was dissolved in sterile distilled water, the control group received 1 mL/kg/day distilled water. $Na_2S_2O_5$ (Merc, Darmstadt, Germany) and distilled water were given by intragastric intubation for 35 days (Derin et al., 2008; Hui et al., 1989).

The animals in all the groups were killed with ether anesthesia and their abdomens were opened by midline incision. The stomach was removed and opened at the lesser curvature. One part of the tissue was used for the terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) technique to detect apoptosis in gastric tissue sections. Other parts of the tissues were used for malondialdehyde (MDA) assays, in order to detect lipid peroxidation.

Measurement of lipid peroxidation

Tissue supernatants were transferred to clean tubes and used for lipid peroxidation analysis. Levels of MDA, a product of lipid peroxidation, were measured using the thiobarbituric acid (TBA) fluorometric assay (Wasowicz et al., 1993) with 1,1,3,3-tetraethoxypropane as a standard. Tissue samples (50 µL) were introduced into a tube containing 29 mM/L. 2-Thiobarbituric acid (TBA) acetic acid (8.75 M/L) samples were placed in a water bath and heated for 1 hour at 95°C–100°C. After the samples

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had been cooled, 25 μL of 5 M HCl was added and the reaction mixture was extracted by agitation for 5 min with 3.5 mL *n*-butanol. After centrifugation, the butanol phase separated and the fluorescence of the butanol extract was measured in a spectrofluorimeter (Shimadzu RF-5500, Kyoto, Japan) using wavelengths of 525 nm, for excitation, and 547 nm, for emission. The results were given as nmol/g protein.

Determination of proteins

The amount of protein in the tissues was determined by using Lowry's method (Lowry et al., 1951), referring to the albumin as standard.

TUNEL labelling

Apoptosis in mucosa and submucosa of stomach tissue was detected by enzymatic labeling of DNA strand breaks using TUNEL. Cryo sections of 5-μm thickness from the stomach tissues were taken onto slides covered with poly-L-lysine and fixed with paraformaldehyde for 1 hour at room temperature. Slides were washed twice in phosphate buffer saline (PBS) for 5 min. Following the incubation of slides with the permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) for 2 min at 4°C and upon washing twice with PBS for 5 min, the labeling reaction was performed using 50 μL TUNEL reagent for each sample, except negative control, in which reagent without enzyme was added and incubated for 1 hour at 37°C. Following PBS washings, slides were incubated with converter reagent for 30 min at 37°C. After washing, color development for localization of cells containing labeled DNA strand breaks was performed by incubating the slides with Fast Red substrate solution for 10 min. TUNEL labeling was conducted using a Cell Death Detection kit (Roche; Mannheim, Germany) and performed according to the manufacturer's instructions. The apoptotic index was determined by counting a total of at least 100 nuclei subdivided in six fields chosen randomly at ×400 magnification. Slides were examined by Zeiss Axioplan Microscope and photographs were taken with Spot Advanced Software.

Statistical analysis

Analysis of variance (ANOVA) was used to compare MDA levels. Post hoc comparisons of the means were carried out using the Tukey's test. Results are expressed as mean ± standard deviation (SD). Significance levels were set at $p < 0.05$. TUNEL results were analyzed by ANOVA on ranks. Then, multiple comparisons between pairs of groups were carried out by Dunn tests. The calculated value of $p < 0.05$ was considered statistically significant.

Results

MDA levels of gastric tissue

No significant changes were observed in S1 group in MDA levels when compared to control group (control: 0.10 nmol/g protein, S1: 0.13 nmol/g protein). In the S2 group, TBARS levels were found to be increased with respect to the control group (S2: 0.15 nmol/g protein, $p < 0.05$). Similarly, MDA levels increased markedly in the S3 group when compared with the control group (S3: 0.18 nmol/g protein, $p < 0.05$; Figure 1).

TUNEL results of gastric tissue

As represented in Figure 2, the number of TUNEL-positive cells in both gastric mucosa and submucosa were significantly less in control and S1 groups. However, in the S2 and S3 groups, the number of apoptotic cells increased significantly in both mucosa and submucosa.

Figure 3 shows the apoptotic index of TUNEL-positive cells. The number of TUNEL-positive cells in the S1 group did not differ from control (control: 1.1%, S1: 3.5%). However, the percentage of apoptotic cells were significantly higher in the S2 and S3

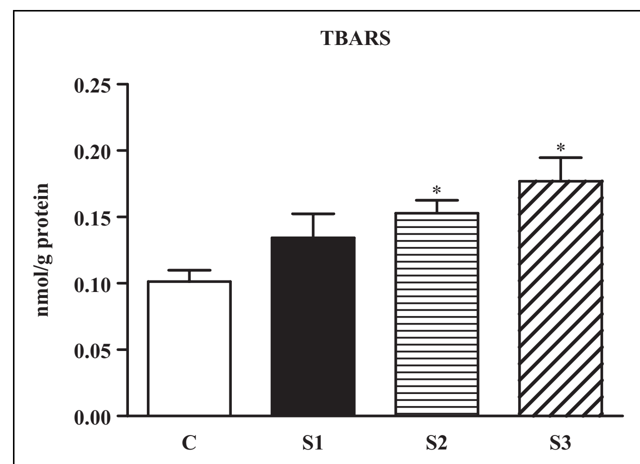

Figure 1. The effect of sodium metabisulfite on gastric tissue malondialdehyde levels. *$p < 0.05$ from control values are means ± SD.

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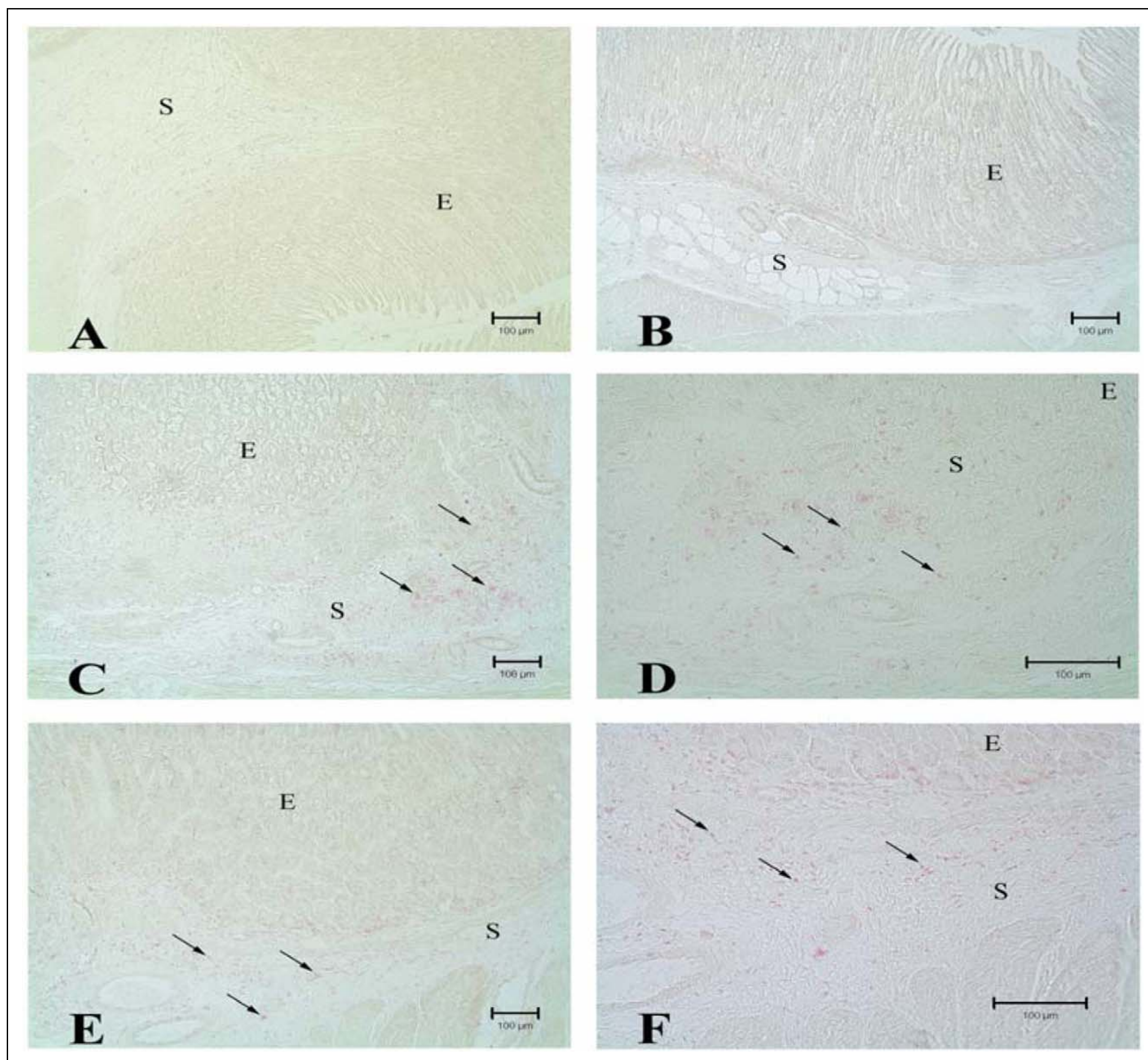

Figure 2. Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) assay in control and experimental groups. Arrows indicate the positive signals of apoptosis. Representative pictures have been chosen in order to illustrate gradually increasing levels of apoptosis. (A) Control tissue section with minimal apoptosis. (B) S1 group tissue section showing similar levels of apoptotic cells to controls. (C) S2 group tissue section showing increased number of apoptotic cells both in mucosa and submucosa. (D) Higher magnification image of S2 with arrows showing the positive signals of apoptotic cells. (E) S3 group tissue section showing increased apoptosis in mucosa and submucosa layers of stomach. (F) Higher magnification image of S3 with arrows showing the positive signals of apoptotic cells. E, epithelium, S: submucosa

groups when compared to control (S2: 17.2%, S3: 18.7%, $p < 0.05$).

Discussion

The human body is exposed to sulfite (SO_3^{2-}) through the inhalation of SO_2, which is mainly derived from industrial emissions (Jameton et al., 2002), and the ingestion of SO_3^{2-} (or HSO_3^{2-}) used as a preservative in food (Meng and Zhang, 1992; Shapiro, 1977).

The reported data showed that $Na_2S_2O_5$ significantly elevated lipid peroxidation and apoptosis on gastric tissue when given at doses of 100 and 260 mg/kg/day. The dose of 260 mg/kg/day sulfie was selected from a previous study (Derin et al., 2008; Hui et al., 1989) designed to represent human exposure to

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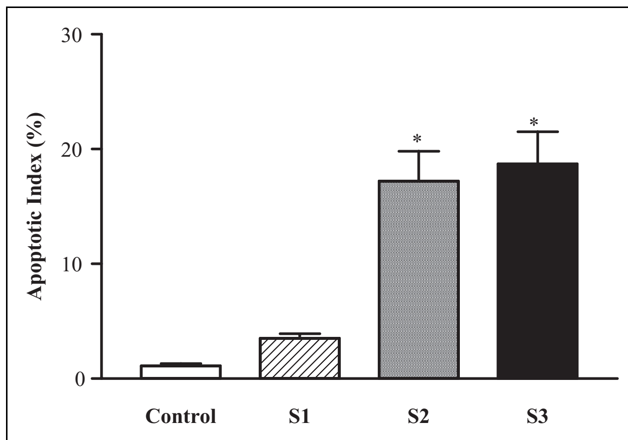

Figure 3. Graph shows the apoptotic index of terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling (TUNEL) positive cells in control and experimental groups. The percentage of apoptotic cells were significantly higher in S2 and S3 groups when compared to control. $*p < 0.05$.

high levels of sulfites, which occur through consumption of certain foods and drugs that contain high levels of sulfite. In particular, there are several amino acid preparations utilized in total parenteral nutrition (TPN) solutions that contain large amounts of sulfites (Lakamp and Dobesh., 2000). Sulfite toxicity is also considered possible with peritoneal dialysis fluids, some of which contain $Na_2S_2O_5$ in concentrations of 0.005%–0.012% (MacPherson, 2001).

Earlier studies have shown that it is possible to consume 180–200 mg/ kg body weight/sulfite from foods and beverages in a single day or meal (Gunnison and Jacobsen, 1987; Taylor et al., 1986). Considering that many foods such as sausage, dried fruit, beer and wine contain SO_3^{2-}, the daily level of normal SO_3^{2-} intake (0.7 mg/SO_2/kg) determined by WHO (FAO/WHO, 1994) can easily be exceeded. Sulfite exposure can be greater than 163 mg/day with consumption of food and drink rich in sulfites and with air pollution (Derin et al., 2006; Lester, 1995). In fact, it is seen that this limit is exceeded in a number of studies in which the daily SO_3^{2-} intake is over the determined levels of 0.7 mg/SO_2/kg (Joint FAO/WHO Expert Committee). Thus, the utilized doses of $Na_2S_2O_5$ in our study are consistent with that of the earlier research.

Recently, several studies have found that sulfite and its derivatives enhance lipid peroxidation in rats (Aydin et al., 2005; Derin et al., 2006; Derin et al., 2008; Elmas et al., 2005; Yargiçoğlu et al., 1999). The concentration of TBARS has been shown to be a good indicator of endogenous lipid peroxidation. The results of this study showed that $Na_2S_2O_5$ administration significantly elevated TBARS levels in a dose-dependent manner at the concentrations of 100 mg/kg and 260 mg/kg. Mechanisms of sulfite-induced lipid peroxidation are thought to initially involve sulfite (SO_3^{2}) oxidation into a sulfite radical ($SO_3^{-\cdot}$; Elmas et al., 2005). The sulfite radical can subsequently react with molecular oxygen to form sulfite peroxyl radical and sulfate radical, which in turn react with lipids (Zaloga and Marik, 2003). Sulfite radicals can also directly react with lipids, resulting in the formation of a lipid alkyl radical. Molecular oxygen adds onto the alkyl radical forming a lipid peroxyl radical (Baker et al., 2002). The breakdown of lipid peroxides results in the formation of aldehydic cleavage products, including MDA (Dahle et al., 1962).

Recently, it has been accepted that oxidative stress is an apoptosis inducer (Chandra et al., 2000). The production of reactive oxygen species, in particular, has been associated with programmed cell death in many conditions such as stroke, inflammation, ischemia, lung edema and neuro-degeneration (Buttke and Sandstrom, 1994; Kannan and Jain, 2000; Tan et al., 1998). Bai and Meng (2005a, b) demonstrated that both the mRNA levels and the number of p53 and bax increased after SO_2 inhalation, while bcl-2 mRNA levels decreased in the lungs and liver, which indicate that expression of p53, bax and bcl-2 affected by SO_2 might result from the transcriptional and posttranscriptional regulations.

Normal gastric mucosal integrity is maintained when the rate of cell loss by cell apoptosis is matched by the rate of new cell production by cell proliferation. As hyperproliferation that is not balanced by cell loss (apoptosis) may contribute to malignant transformation in the long term due to defective apoptosis, it is thought to play an important role in gastric carcinogenesis, as it occurs in colorectal cancer (Bedi et al., 1995; Moss et al., 1996; Thompson, 1995). It was reported that sulfite and its derivatives caused DNA damage to multiple organs in mice and rats (Bai and Meng, 2005a, b; Bedi, et al., 1995; Meng, 2003; Shapiro, 1977). In our study, high doses of sulfite administration elevated the number of apoptotic cells both in mucosa and submucosa layers of the stomach, in parallel with increased TBARS levels in a dose-dependent manner.

Meng et al. have reported that SO(2) exposure can cause oxidative damage to the stomach and intestines of mice, and SO(2) is a toxic agent not only to the respiratory system but also to the stomach and

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intestines of mammals (Meng et al., 2003c). Another study demonstrated that sodium bisulfite and sodium sulfite at various doses (125, 250 or 500 mg/kg body weight) significantly increase DNA damage on stomach and different organs (Meng et al., 2004). Our previous study also showed that cold-restraint stress caused apoptosis in gastric mucosa secondary to a decrease in antioxidant capacity and an increase in oxidant activity (Ercan et al., 2007). Recent studies showed that indomethacin and helicobacter pylori induce mitochondrial pathology that ultimately activates the mitochondrial pathway of apoptosis in gastric mucosa to develop gastropathy (Calvino-Fernandez et al., 2008; Maity et al., 2009).

In summary, the results reported in this paper indicate that intragastric administration of $Na_2S_2O_5$ can cause oxidative stres and thus increase lipid peroxidation on gastric tissue. Our findings demonstrated that sulfite also increased number of apoptotic cells, in both mucosa and submucosa layers of stomach, in a dose-dependent manner. Therefore, it may be important to be careful while using this agent in food and beverages as a preservative.

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