RESEARCH ARTICLE

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IL-6 and *IL-10* Levels in Rats Blood Plasma as Immune Responses Post Radioiodine (I-131) Administration

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Abstract

Objective: The purpose of this study was to analyze the effect of oral administration of radioiodine (I-131) on the immune responses (interleukin 6 and 10) as biodosimetry markers and to support clinical trials of I-131 solution. **Methods:** The design of this study was an in vivo experimental study using twenty-seven male rats (*Rattus norvegicus* strain Sprague-Dawley) given 100 µL of I-131 solution at a dose of 260 µCi. Blood plasma was taken at 0.25, 0.5, 1, 3, 24, 48, 120, and 168 hours post oral I-131 administration, respectively. Rats without radioiodine administration as a control group. The levels of *IL-6* and *IL-10* were measured using the enzyme-linked immunosorbent assay (ELISA) method. Statistical analysis was carried out with one-way ANOVA using SPSS version 25 software. **Result:** *IL-6* level began to significantly increase at 0.25 hours post administration of I-131 (14.4 pg/mL ± 2.52 pg/mL, p=0.02). During 7 days of observation, *IL-6* levels had 2 peaks of highly significant increase at 0.5 hours (43.57 ± 5.28, p<0.001) and 120 hours (24.08 ± 2.69, p<0.001 compared to control (5.44 ± 0.95 pg/mL). *IL-10* level began to significantly increase at 0.25 hours (30.32 ± 3.22 pg/mL, p=0.03) compared to controls (20.61 ± 1.59 pg/mL). **Conclusion:** The highest increase in *IL-6* and *IL-10* levels occurred respectively in the first 0.5 hours 8 times and in the first 0.25 hours 1.47 times compared to controls. Internal irradiation with radioiodine resulted in a significant increase in immune cells in exposed blood plasma characterized by the production of the cytokines *IL-6* and *IL-10*. This appears to be a response of immune cells to reduce or stop inflammatory reactions through the release of anti-inflammatory cytokines in an effort to prevent excessive inflammatory responses that can damage cells and tissues.

Keywords: Immune responses- Interleukin 6- Interleukin 10- Radioiodine- Rats blood plasma

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Introduction

Radioiodine (I-131) is a radioactive iodine used in nuclear medicine to treat hyperthyroidism and thyroid cancer. The conditions for a radiopharmaceutical to be used for nuclear medicine therapy are that it produces alpha or beta emissions and has a half-life of about one week. Radioiodine meets this classification. Apart from producing beta and gamma emissions, I-131 also has a half-life of 8.05 days [1]. Beta particle emissions produced by I-131 have a maximum energy of 0.61 MeV with an average energy of 0.192 MeV and the ability to penetrate tissue with a thickness of 0.8 mm. Meanwhile, gamma radiation produced by I-131 has an energy of 364 KeV [2].

Exposure to I-131 can cause cell death due to its ability to penetrate the nucleus and damage the DNA [3]. A previous study demonstrated that radiation exposure increases DNA damage through micronucleus formation by 16.3 per 1 mSv equivalent dose [4]. For safety reasons, it is necessary to use I-131 at a lower dose for radiotherapy but with the same success rate as high doses as reported by Fatima et al. [5] using a dose of 30 mCi but has similar ablation success with a dose of 100 mCi in differentiated thyroid cancer (DTC) patients. The low dose of I-131 has the advantage of being safer, more comfortable, and more cost-effective for patients and hospitals [5].

The cells involved in the immune system are sensitive and susceptible to radiation and can divide quickly. Radiation exposure induces apoptosis in mature natural killer (NK) cells and T and B lymphocytes and lethal

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damage to bone marrow stem cell precursors monocytes and granulocytes. In individuals receiving high doses of radiation, for example, atomic bomb survivors, mature lymphocytes and bone marrow stem cells are severely damaged, leading to a decrease in granulocytes and natural killer cells [6]. Exposure to radiation doses not only induces DNA damage but also activates inflammatory signaling pathways. In addition, inflammation due to radiation exposure has an important role in radioresistance which can be induced in cancer cells [7, 8].

Pro-inflammatory cytokines are key components of the immune system that can be rapidly activated following tissue irradiation. They combine with the effects of ionizing radiation where both produce free radicals including reactive oxygen and nitrogen species (ROS/RNS). The molecules formed after irradiation signal the production of ROS and cytokines. As a result, the feedback control system will gradually shape the response to tissue damage due to radiation exposure. The pro-inflammatory phase continues until antioxidants and anti-inflammatory cytokines act to restore homeostatic conditions. The balance between pro-inflammatory and anti-inflammatory forces can fluctuate for a long time after radiation exposure, creating a surge of cytokines as the body tries to deal with persistent pathogenesis [9].

Interleukin-6 (*IL-6*) and interleukin-10 (*IL-10*) are two types of cytokines that play an important role in the immune system. *IL-6* is a pro-inflammatory cytokine whose role is to stimulate acute phase protein synthesis, hematopoiesis, immune reactions, and neutrophil production in the bone marrow [10]. *IL-6* is secreted by macrophages in response to specific microbial molecules, referred to as pathogen-associated molecular patterns (PAMPs). These PAMPs bind to an important group of innate immune system detection molecules, called pattern recognition receptors (PRRs), including Toll-like receptors (TLRs). It is present on the cell surface and intracellular compartments and induces intracellular signaling cascades that give rise to the production of inflammatory cytokines [11]. Elevated levels of *IL-6* suppress radiation-induced cell death, and blockade of *IL-6* signaling by tumor cells to radiation. The radioresistance effect of *IL-6* is associated with reduced DNA damage after radiation. *IL-6* was also reported to activate the Nrf2 antioxidant pathway, leading to a significant reduction in oxidative stress by upregulating Mn-SOD [12].

Interleukin-10 is an anti-inflammatory cytokine that is mostly secreted by monocytes and has pleiotrophic effects on the immune system and inflammation. The main role of *IL-10* is to inhibit or eliminate the inflammatory response, control the development and differentiation of B cells, NK cells, TH cells, CD8 T cells, mastocytes, granulocytes, dendritic cells, keratinocytes, and endothelial cells, and is immunosuppressive against myeloid cells [13]. Previous research reported that there was a decrease in *IL-10* expression immediately after cells received radiation exposure [14]. *IL-10* is also able to improve endothelial cell activation induced by radiation so that it can be used to treat radiation damage [15].

Investigation and information related to the biological effects of I-131 on cells have not been widely carried out, while its use in the fields of medicine and radiotherapy is increasingly widespread. In addition, the immune response in rats after exposure to internal radiation by I-131 has not been widely studied. This is the novelty that this paper seeks to achieve. The purpose of this study was to analyze the effect of oral administration of I-131 on the immune responses (interleukin 6 and 10) as biodosimetry markers and to support clinical trials of I-131 solution.

Materials and Methods

Ethical approval

The ethical approval certificate for this present study was issued by Ethical Commission of Animal Care and Use, National Research and Innovation Agency of Indonesia (BRIN), on February 21, 2023 with certificate number 032/KE.02/SK/02/2023.

Radioiodine (I-131)

I-131 was made using the dry distillation method based on a study by Pratama et al. [16]. Fifty grams of TeO_2 target material was exposed to radiation at the G.A. Siwabessy multipurpose reactor's facility. TeO_2 was moved from hot cell No. 4 (dry-hot cell) to a distillation tube (vycore) following the irradiation procedure. Firstly, the furnace is turned on and the temperature is gradually raised to 800°C to begin the distillation process. With vacuum conditions in the distillation system reaching -2 to -3 incHg, the distillation process takes five hours to complete. By adjusting the vacuum level and the glass wool's color change in the activated charcoal column, the distillation process is monitored. After five hours, the distillation process was finished, and the temperature was lowered to 250°C. It takes about four hours to complete. Three times, 5 mL of 0.05N NaOH solution was poured into the activated charcoal column to complete the elution process.

Animals

The design of this study was an in vivo experimental study using twenty-seven male rats (*Rattus norvegicus* strain Sprague-Dawley), aged 8-10 weeks, and weighing 180-200 grams. The sample size was determined using the Federer [17] formula (n-1) (t-1) ≥15, with 9 treatment groups so that 3 rats were used per group. These animals were obtained from the "Taufik Rat and Mice" Veterinary Laboratory Service, Bogor, Indonesia, and had received a health examination from a veterinarian with a certificate number 101/TRM/SK/VIII/2023. All rats were acclimatized under optimal conditions of temperature (21-24°C) and lighting (12 hours dark/light cycle) at the Center of Animal Laboratory, National Research and Innovation Agency (BRIN), Indonesia.

Blood plasma collection

The animals were grouped into 8 treatment groups based on the time of plasma collection (0.25, 0.5, 1, 3, 24, 48, 120, and 168 hours) and a group without administration of I-131 as control, each consisting of 3 mice. First, Na-I131 solution was administered orally at a dose of 260 μCi per rat using a cannula. After a certain period of sacrificed

time (0.25, 0.5, 1, 3, 24, 48, 120, and 168 hours), the rats were anesthetized using ketamine intraperitoneally at the same time. Rats in the control group were anesthetized on the last day of the experiment. Blood samples of 2-3 mL were taken immediately after the rat sacrificed. The whole blood taken is collected in a heparin tube to prevent blood lysis. The process of separating plasma from whole blood is carried out by centrifugation at a speed of 3000 rpm for 15 minutes. The plasma obtained was then collected in a 1.5 mL microtube and stored in a deep freezer at -80°C.

Measurement of IL-6 and IL-10 cytokines

Measurement of *IL-6* and *IL-10* in mouse blood plasma was carried out using the enzyme-linked immunosorbent assay (ELISA) method with a protocol according to the rat ELISA kit (ABclonal Biotechnology, Cat No: RK00020 for *IL-6* and Cat No: RK00050 for *IL-10*). Microplates were coated with antibodies specific for *IL-6* or *IL-10*. Each well on the plate was washed 3 times using wash buffer. Next, the standard solution that had been prepared and the samples were pipetted into each well as much as 100 µL and incubated at 37°C for 2 hours and then washed 3 times. A biotin-conjugated specific antibody was added to the well and incubated at 37°C for 1 hour then washed 3 times. Streptavidin-HRP (100 uL) was added to the well and continued with incubation at 37°C for 30 minutes and then washed 3 times. TMB substrate (100 uL) was added to well and incubated at 37°C for 20 minutes. After that, stop solution (50 uL) was added. The absorbance value was determined using a microplate reader (PowerWave XS2, Biotek) at wavelengths 450 and 570 nm. The absorbance value used was the absorbance value at wavelength 450 nm minus the absorbance value at wavelength 570 nm.

Statistical analysis

The absorbance values obtained were plotted on a standard curve that had been created so that *IL-6* and *IL-10* levels (pg/mL) could be determined in each treatment group. The data obtained are presented as mean ± SD. Statistical analysis was carried out using SPSS software version 25. The data normality test was carried out using the Shapiro-Wilk test. The mean levels of *IL-6* or *IL-10* in each treatment group were compared to the control and the level of significance was tested using One Way Analysis of Variance (ANOVA). Determination of significantly different groups was carried out using Tukey's advanced test. P-value <0.05 was considered statistically significant.

Results

In this study, *IL-6* and *IL-10* concentrations were measured in the blood plasma of rats given I-131 orally. Table 1 shows the concentration of each cytokine during the sacrifice time period. In the first 0.25 hours after administration of I-131, the *IL-6* concentration in rat blood plasma increased significantly by 2.65 times compared to controls (14.40 ± 2.52 vs 5.44 ± 0.95 pg/mL, p=0.02), followed by 15 minutes later there was a very significant increase of 3 times (43.57 ± 5.28 pg/mL, p<0.001). A very significant decrease in *IL-6* concentration began to occur in the first hour to 3 times lower than 30 minutes earlier (14.38 ± 2.39 pg/mL, p<0.001). In the 3 hours, 24 hours, and 48 hours periods there were insignificant fluctuations. However, the increase in *IL-6* concentration again occurred very significantly after 120 hours, 2.5 times compared to the 48 hours (24.08 ± 2.69 pg/mL, p<0.001). After 168 hours (7 days), there was a decrease of 2.6 times lower than at 120 hours (9.20 ± 0.61 pg/mL, p<0.001). Although the concentration at 168 hours had the lowest value during the 7 days of observation, the value was still higher than the control. Overall, the experiment carried out for 7 days showed that there was an increase in *IL-6* concentration by 2 times, in the 0.5 hour and 120 hours periods.

In the case of *IL-10*, the increase in concentration that occurred in the first 0.25 hour after administration of I-131 was almost 1.5 times compared to controls (30.32 ± 3.22 vs 20.61 ± 1.59 pg/mL, p=0.03), followed by a decrease in *IL-10* concentration becomes 1.5 times (20.32 ± 1.46 pg/mL, p=0.02). Furthermore, in the period of 1 to 48 hours, there were insignificant fluctuations. However, the increase in *IL-10* concentration decreased significantly after 120 hours by 2.5 times compared to the 48 hours (24.08 ± 2.69 pg/mL, p<0.001). After 168 hours (7 days), there was a decrease of 1.46 times (19.32 ± 4.73 vs

Table 1. The *IL-6* and *IL-10* Concentration in Rats Blood Plasma Post Radioiodine Administration.

Group of treatment	IL-6		IL-10	
	Concentration (pg/mL)	P-value	Concentration (pg/mL)	P-value
Control	5.44 ± 0.95	-	20.61 ± 1.59	-
0.25 h	14.40 ± 2.52*	0.02	30.32 ± 3.22*	0.03
0.5 h	43.57 ± 5.28**	<0.001	20.32 ± 1.46*	0.02
1 h	14.38 ± 2.39**	<0.001	20.34 ± 3.19	1
3 h	11.38 ± 3.36	0.89	22.86 ± 1.65	0.98
24 h (1 day)	4.51 ± 1.16	0.11	27.26 ± 1.06	0.73
48 h (2 days)	9.64 ± 0.88	0.38	28.31 ± 5.63	1
120 h (5 days)	24.08 ± 2.69**	<0.001	19.32 ± 4.73*	0.05
168 h (7 days)	9.20 ± 0.61**	<0.001	11.90 ± 0.37	0.26

Note: *, The mean difference is significant at the 0.05 level; **, The mean difference is highly significant at the 0.01 level; *IL-6*, interleukin 6; *IL-10*, interleukin 10.

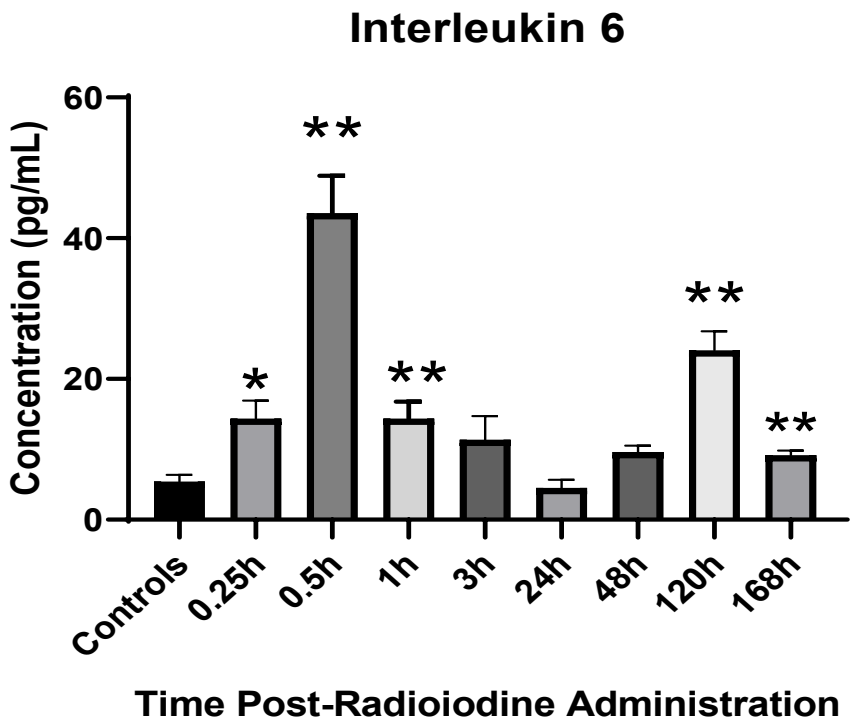

Figure 1. Effect of Radioiodine Administration to the *IL-6* Concentration.

28.31 ± 5.63, p<0.05). During 7 days of observation, the lowest *IL-10* concentration was obtained after 168 hours, but the value was not significantly different compared to controls (p>0.05).

Discussion

The results of this study demonstrated a strong correlation between exposure to radiation emitted internally by I-131 and changes in the immune response characterized by the production of the pro-inflammatory cytokine *IL-6* and the anti-inflammatory cytokine *IL-10* during 7 days of observation. The inflammatory reaction that occurs in immune cells shows an increase in the first 0.5 hours. After that, this reaction decreased until day 2. On day 5, the body again showed an inflammatory reaction with an increase in *IL-6* concentrations, and on day 7 the inflammatory response decreased again. The increase

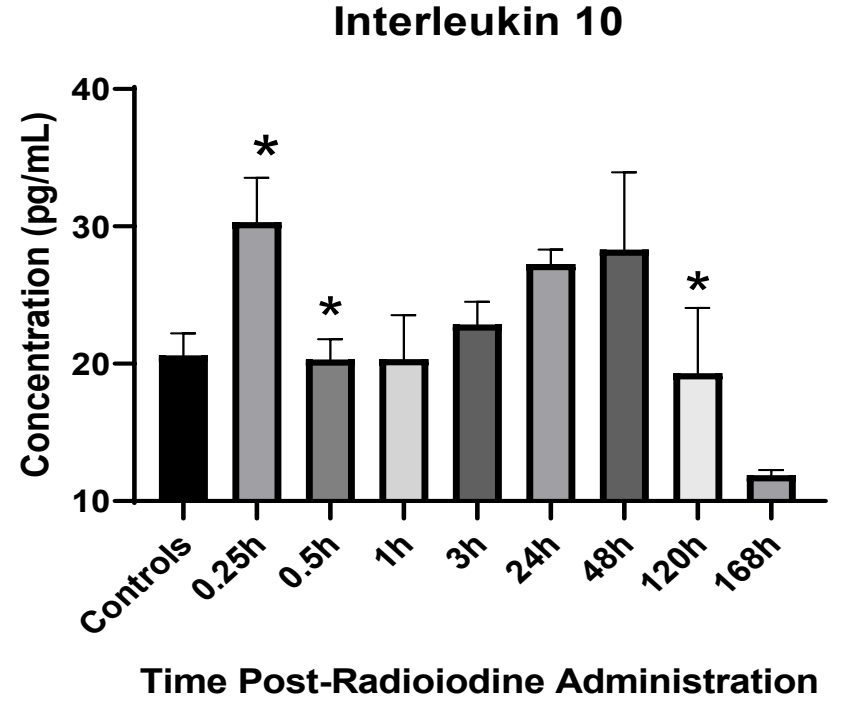

Figure 2. Effect of Radioiodine Administration to the *IL-10* Concentration.

in *IL-6* that occurs is a form of cell immune response to exposure to radiation emitted by I-131. There is a very strong relationship between inflammatory reactions and oxidative damage caused by radiation exposure to cells [18]. Exposure to radiation which has an indirect effect will form several types of ROS such as superoxide anion (O_2*), hydroxyl radical (OH*), and hydrogen peroxide (H_2O_2) molecules as a result of water radiolysis [19, 20]. These molecules will attack cells and damage the DNA. While, the direct effect that occurs is that exposure to gamma ionizing radiation will directly penetrate the cell to the nucleus and cause DNA damage in the nucleus [21]. Apart from damaging DNA, external exposure to radiation at a dose of 6 Gy can also damage lipids through the lipid peroxidation pathway in the spleen and liver by 2.3 times and 5.5 times, respectively [22].

Cell damage, in this case DNA damage, will trigger an inflammatory response from immune cells [23]. This inflammatory reaction is an effort made by cells to provide a response and as a danger signal. This is related to one of the functions of cytokines as important molecules that play a role in communication between cells [24]. The resulting danger signal due to DNA damage is then responded to by expressing DNA repair genes to form DNA repair proteins that are appropriate to the type of DNA damage that occurs. Several other members of the matrix protein family such as tenascin-C and osteopontin have also been reported to be involved in the inflammatory response and may function as danger signals [25].

Beta and gamma radiation emitted by I-131 can change the number of immune cells and the function of the immune system of exposed tissue, especially increasing the number of macrophages and T lymphocytes (T cells) so that they are able to induce a number of inflammatory mediators, such as NF-κB, SMAD2/3, and several proinflammatory cytokines such as *TNF-α, IL-1, IL-2, IL-6, IL-33,* and *INF-γ* [18]. This condition will repeat itself as long as I-131 releases beta and gamma radiation into the body as shown in Figure 1. During 7 days of observation, there was 2 times increase in both *IL-6* and *IL-10* concentrations. In fact, a previous study states that exposure to high doses of ionizing radiation, for example in radiation disasters, inflammatory reactions will continue for years after exposure. This will disrupt the function and structure of the exposed organs [26, 27].

The increase in *IL-6* production in this study was in line with the production of the anti-inflammatory cytokine *IL-10* which also increased its production in the first 0.25 hours (Figure 2). This seems to be a response of immune cells to reduce or stop the inflammatory reaction through the release of anti-inflammatory cytokines such as *IL-4, IL-10, IL-11, IL-13*, and *TGF-β*. These cytokines have an important role in preventing excessive responses that can damage cells and tissues [28]. Results of other studies have confirmed the inhibitory effect of the anti-inflammatory cytokine *IL-10* by monocytes against the synthesis and secretion of the pro-inflammatory cytokine *IL-6* [29-31]. In addition, Frangogiannis [32] reported that the anti-inflammatory cytokine *IL-10*, members of the *TGF-β* family, and pro-resolve lipid mediators (such as lipoxin, resolvin, and protectin) can suppress pro-inflammatory signals.

Several studies have reported the mechanism of *Il-10* in suppressing *IL-6* production. First, inhibition through monocyte cells. *IL-10* produced by B cells, monocytes, and fibroblasts has been shown to play an immunosuppressive role against the monocyte-dependent proliferation of antigen-specific T-helper cells. *IL-10* plays a role in blocking *IL-6* production by monocyte cells activated with lipopolysaccharide (LPS). The second mechanism is the inhibition of *IL-6* production by T cells. The effects of *IL-I0* on T cells can be mediated through monocytes and *IL-2*-independent mechanisms [33]. In post-radiation treatment, *IL-10* plays a role in inhibiting radioactive fibrosis in radioactive skin injuries. *IL-10* has a protective effect against skin cell damage after exposure to ionizing radiation both in vitro and in vivo. In addition, *IL-10* plays a role in inhibiting radioactive fibrosis in radioactive skin injuries [34]. A study by Surniyantoro et al (2019) reported that external radiation exposure affects blood components. The increase in red blood cell and monocyte counts was significantly higher in-hospital radiation workers compared with controls, whereas white blood cell, hematocrit, mean blood cell volume and lymphocyte values showed significant decreases. The DNA damage also can be influenced by radiation exposure in medical radiation workers [35, 36].

In conclusion, we reported that the highest increase in *IL-6* and *IL-10* levels occurred respectively in the first 0.5 hours 8 times and in the first 0.25 hours 1.47 times compared to controls. Internal irradiation with radioiodine resulted in a significant increase in immune cells in exposed blood plasma characterized by the production of the cytokines *IL-6* and *IL-10*. This appears to be a response of immune cells to reduce or stop inflammatory reactions through the release of anti-inflammatory cytokines in an effort to prevent excessive inflammatory responses that can damage cells and tissues.

Author Contribution Statement

HNES designed the study, collected the samples, performed the experiment, processed the experimental data and statistical analysis, designed the tables and figures, prepared the manuscript, and revised for final publication. A, AB, MS, F, and RL assisted and accompanied during research activities and manuscript preparation. TK, DR, IS and IKHB helped in collection of blood samples. T and BBP helped during laboratory activities.

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Conflict of Interest

All of authors state that this study was conducted without any financial relationship construed as a potential conflict of interest.

Ethical Declaration

The ethical approval certificate for this present study was issued by Ethical Commission of Animal Care and Use, National Research and Innovation Agency of Indonesia (BRIN), on February 21, 2023 with certificate number 032/KE.02/SK/02/2023.

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