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31

Inhibition of DNA Polymerase λ, a DNA Repair Enzyme, and Anti-Inflammation: Chemical Knockout Analysis for DNA Polymerase λ Using Curcumin Derivatives

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1. Introduction

DNA polymerase (i.e., DNA-dependent DNA polymerase [pol], E.C. 2.7.7.7) catalyzes the polymerization of deoxyribonucleotides alongside a DNA strand, which it "reads" and uses as a template (Kornberg & Baker, 1992). The newly polymerized molecule is complementary to the template strand and identical to the template's partner strand. Pol can add free nucleotides only to the 3′ end of the newly formed strand, meaning that elongation of the new strand occurs in a 5′ to 3′ direction.

The human genome encodes at least 14 pols that conduct cellular DNA synthesis (Bebenek & Kunkel, 2004; Hubscher et al., 2002). Eukaryotic cells contain 3 DNA replicative pols (α, δ and ε), 1 mitochondrial pol (γ), and at least 10 DNA repair and/or recombination-related pols (β, ζ, η, θ, ι, κ, λ, μ and ν, and REV1) (Friedberg et al., 2000; Takata et al., 2006). Pols have a highly conserved structure, which means that their overall catalytic subunits show little variance among species. Enzymes with conserved structures usually perform important cellular functions, the maintenance of which provides evolutionary advantages. On the basis of sequence homology, eukaryotic pols can be divided into 4 main families, termed A, B, X and Y (Friedberg et al., 2000). Family A includes mitochondrial pol γ, as well as pols θ and ν. Family B includes 3 DNA replicative pols (α, δ and ε) and pol ζ. Family X comprises pols β, λ and μ; and lastly, family Y includes pols η, ι and κ, in addition to REV1.

We have been screening for selective inhibitors of each pol derived from natural products including food materials and nutrients for more than 15 years (Mizushina, 2009; Sakaguchi et al., 2002). In our studies of pol inhibitors, we have found that selective inhibitors of pol λ, which is a DNA repair-related pol, have anti-inflammatory activity against 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation (Mizushina et al., 2003; Mizushina, 2009). Although tumor promoters such as TPA are classified as compounds that promote tumor formation (Hecker, 1978), they also cause inflammation and are commonly used as artificial inducers of inflammation in order to screen for anti-inflammatory agents (Fujiki & Sugimura, 1987). Tumor promoter-induced inflammation can be distinguished from acute inflammation, which is exudative and accompanied by fibroblast proliferation and granulation. The tumor promoter TPA is frequently used to search for new types of anti-inflammatory compound. TPA not only causes inflammation, but also influences mammalian cell growth (Nakamura et al., 1995), suggesting that the molecular basis of the inflammation stems from pol reactions related to cell proliferation. This relationship, however, needs to be investigated more closely.

In this review, we examine the relationship between pol λ inhibition and anti-inflammation using pol λ-specific inhibitors, such as chemically synthesized curcumin derivatives. On the basis of these results, the pol λ-inhibitory mechanism and anti-inflammation effects of monoacetyl-curcumin, which was the strongest pol λ inhibitor among the compounds tested, is discussed.

2. Effect of curcumin derivatives on the activities of mammalian pols

2.1 Pol assay for inhibitor screening

A pol activity assay to detect pol inhibitors was established by Mizushina et al. (1996a; 1996b; 1997). Purified mammalian pols α, β, γ, δ, ε, η, ι, κ and λ, which have high activity, were kind gifts from pol researchers around the world. As shown in Fig. 1, poly(dA)/oligo(dT)$_{18}$ (A/T = 2/1) and 2'-deoxythymidine 5'-triphosphate (dTTP) were used as the DNA template-primer and nucleotide (dNTP, 2'-deoxynucleotide 5'-triphosphate) substrate, respectively. The candidate inhibitors, which were low molecular weight organic compounds, were dissolved in distilled dimethyl sulfoxide (DMSO) at various concentrations and sonicated for 30 s. Aliquots (4 μL) of the sonicated samples were mixed with 16 μl of each enzyme (final amount 0.05 units) in 50 mM Tris–HCl (pH 7.5) containing 1 mM dithiothreitol, 50% glycerol and 0.1 mM EDTA, and pre-incubated at 0 °C for 10 min. These inhibitor–enzyme mixtures (8 μL) were then added to 16 μl of each of the enzyme standard reaction mixtures, and incubation was carried out at 37 °C for 60 min, except for *Taq* pol, which was incubated at 74 °C for 60 min. Activity without the inhibitor was considered 100%, and the remaining activity at each concentration of the inhibitor was determined relative to this value. One unit of pol activity was defined as the amount of enzyme that catalyzed the incorporation of 1 nmol of dNTP (i.e., dTTP) into synthetic DNA template–primers in 60 min at 37 °C under the normal reaction conditions for each enzyme (Mizushina et al., 1996b; 1997).

2.2 Mammalian pol inhibitory effect of curcumin derivatives

As described above, we are searching for natural inhibitors specific to each of the mammalian pols. A phenolic compound produced from a higher plant, a Japanese vegetable

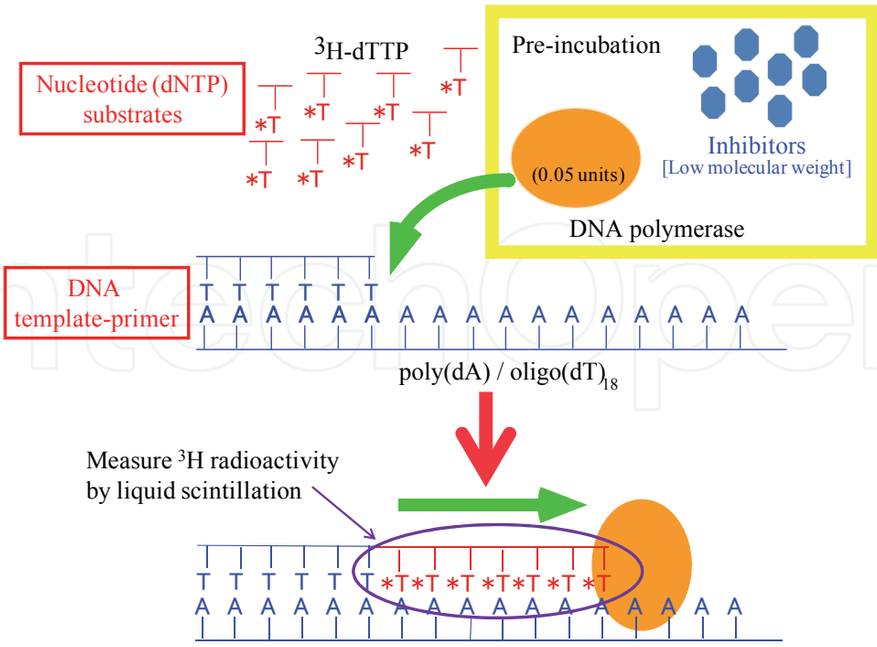


Fig. 1. Pol inhibitor assay scheme

(*Petasites japonicus*) collected from Akita prefecture, Japan, was found to inhibit pol λ activity selectively (Mizushina et al., 2002). The compound was purified and its chemical structure was analyzed, and it was identified as petasiphenol (compound **1** in Fig. 2) (Iriye et al., 1992). The three-dimensional relationship between pol inhibitors and the pol structure was investigated, which suggested that some phenolic compounds might be pol inhibitors. It was therefore tested whether commercial or easily obtainable phenolic compounds might also be pol λ-specific inhibitors. As a result, curcumin (diferuloylmethane, compound **2** in Fig. 2), which is the same type of phenolic compound as petasiphenol, and 13 chemically synthesized derivatives of curcumin (compounds **3–15** in Fig. 2) were prepared, and then tested for their inhibitory effects on mammalian pols.

The inhibition of four mammalian pols, namely calf pol α, human pol γ, human pol κ and human pol λ, by each compound at 10 μM was investigated. Pols α, γ, κ and λ were used as representatives of the B, A, Y and X families of pols, respectively (Bebenek & Kunkel, 2004; Hubscher et al., 2002; Takata et al., 2006). As shown in Fig. 3, petasiphenol (**1**) and curcumin (**2**) inhibited human pol λ activity. The inhibitory effect on pol λ of compounds **4**, **5**, **13** and **14** was stronger than that of curcumin (**2**). Compounds **6** to **11**, which do not have any enone moieties, did not affect pol λ activity; thus, the enone moiety, which is present in petasiphenol, might be important or essential for pol λ inhibition. As mentioned above, compounds **4**, **5** and **13**, which have one or more acetoxy moieties, strongly inhibited the activity of pol λ, and compound **13** (monoacetyl-curcumin) was the strongest inhibitor among the compounds tested. The one acetoxy moiety at position C4″ in monoacetyl-curcumin (13) might stimulate the inhibitory effect on pol λ. On the other hand, at 10 μM, none of the compounds inhibited the activity of calf pol α, human pol γ or human pol κ.

On the basis of these results, we concentrated on curcumin (**2**), which is a major food component, and monoacetyl-curcumin (**13**), which was the strongest inhibitor of pol λ among the curcumin derivatives tested, in the next part of this study.

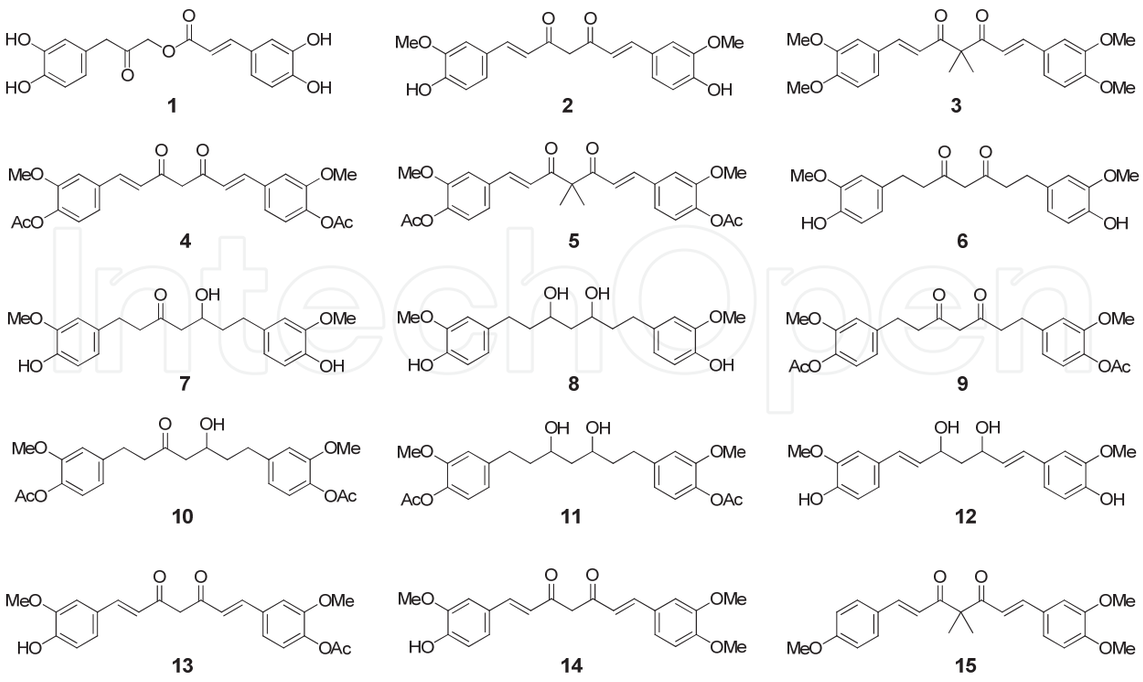


Fig. 2. Structure of curcumin derivatives. Compound **1**: petasiphenol; compound **2**: curcumin (diferuloylmethane); compound **3**: (1*E*,6*E*)-1,7-bis(3′,4′-dimethoxyphenyl)-4,4-dimethyl-1,6-heptadien-3,5-dione; compound **4**: (1*E*,4*Z*,6*E*)-1,7-bis(4′-acetoxy-3′-methoxyphenyl)-5-hydroxy-1,4,6-heptatrien-3-one (diacetyl-curcumin); compound **5**: (1*E*,6*E*)-1,7-bis(4′-acetoxy-3′-methoxyphenyl)-4,4-dimethyl-1,6-heptadien-3,5-dione; compound **6**: 1,7-bis4-hydroxy-3-methoxyphenyl-3,5-heptadione; compound **7**: 1,7-bis4-hydroxy-3-methoxyphenyl-5-hydroxy-3-heptanone; compound **8**: 1,7-bis4-hydroxy-3-methoxyphenyl-3,5-heptadiol; compound **9**: 1,7-bis4-acetoxy-3-methoxyphenyl-3,5-heptadione; compound **10**: 1,7-bis4-acetoxy-3-methoxyphenyl-5-hydroxy-3-heptanone; compound **11**: 1*E*,6*E*-1,7-bis4-acetoxy-3-methoxyphenyl-3,5-dihydroxyheptane; compound **12**: 1*E*,6*E*-1,7-bis4-hydroxy-3-methoxyphenyl-3,5-dihydroxy-1,6-heptadiene; compound **13**: (1*E*,4*Z*,6*E*)-7-(4′′-acetoxy-3′′-methoxyphenyl)-5-hydroxy-1-(4′-hydroxy-3′-methoxyphenyl)hepta-1,4,6-trien-3-one (monoacetyl-curcumin); compound **14**: (1*E*,4*Z*,6*E*)-1-(3′,4′-dimethoxyphenyl)-5-hydroxy-7-[4″-hydroxy-3″-methoxypheny]hepta-1,4,6-trien-3-one (monometyl-curcumin); compound **15**: 1*E*,6*E*-1-3,4-dimethoxyphenyl-4,4-dimethyl-7-4-methoxyphenylhepta-1,6-dien-3,5-diol.

3. Effect of curcumin (2) and monoacetyl-curcumin (13) on the activities of pols and other DNA metabolic enzymes

Curcumin (**2**) and monoacetyl-curcumin (**13**) were effective at inhibiting human pol λ activity, and the inhibition was dose-dependent with 50% inhibition observed at a concentration of 7.0 and 3.9 μM, respectively (Table 1). These compounds had no influence on the activities of not only DNA replicative pols such as calf pol α, human pol δ and human pol ε, or mitochondrial DNA replicative pols such as human pol γ, but also DNA repair-related pols such as rat pol β, human pols η, ι and κ. It is interesting that these compounds had no affect on the activity of pol β, because pols β and λ both belong to the

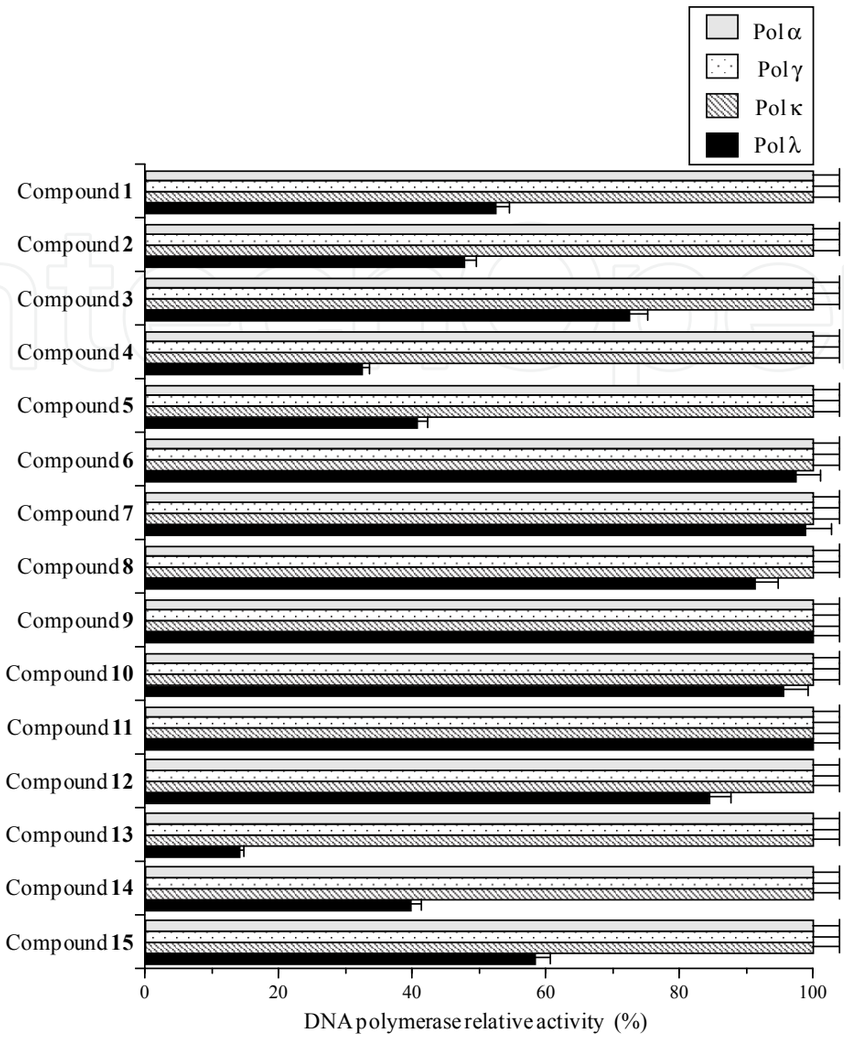


Fig. 3. Inhibitory effect of curcumin derivatives (compounds **1**–**15**) on the activities of mammalian pols. Each compound (10 μM) was incubated with calf pol α (B-family pol), human pol γ (A-family pol), human pol κ (Y-family pol) and human pol λ (X-family pol) (0.05 units each). Pol activity in the absence of the compound was taken as 100%, and the relative activity is shown. Data are shown as the mean ± SE (n=3).

X-family of pols, and the three-dimensional structure of pol β is thought to be highly similar to pol λ (Garcia-Diaz et al., 2004).

Curcumin (**2**) and monoacetyl-curcumin (**13**) also had no inhibitory effect on cherry salmon (fish) pols α and δ, cauliflower (higher plant) pol α, prokaryotic pols such as the Klenow fragment of *E. coli* pol I, *Taq* pol and T4 pol, and other DNA metabolic enzymes such as calf DNA primase of pol α, human immunodeficiency virus type-1 (HIV-1) reverse transcriptase, T7 RNA polymerase, T4 polynucleotide kinase and bovine deoxyribonuclease I. Therefore, these phenolic compounds were specific inhibitors of human pol λ among the pols and DNA metabolic enzymes tested. Petasiphenol (**1**) also selectively inhibited the activity of eukaryotic pol λ such as curcumin (**2**) and monoacetyl-curcumin (**13**) (Mizushina et al., 2002).

Enzyme	IC_{50} value (μM)	
	Curcumin (**2**)	Monoacetyl-curcumin (**13**)
– Mammalian DNA polymerases –		
Calf DNA polymerase α	>100	>100
Rat DNA polymerase β	>100	>100
Human DNA polymerase γ	>100	>100
Human DNA polymerase δ	>100	>100
Human DNA polymerase ε	>100	>100
Human DNA polymerase η	>100	>100
Human DNA polymerase ι	>100	>100
Human DNA polymerase κ	>100	>100
Human DNA polymerase λ	7.0 ± 0.39	3.9 ± 0.25
– Fish DNA polymerases –		
Cherry salmon DNA polymerase α	>100	>100
Cherry salmon DNA polymerase δ	>100	>100
– Plant DNA polymerases –		
Cauliflower DNA polymerase α	>100	>100
– Prokaryotic DNA polymerases –		
E. coli DNA polymerase I	>100	>100
Taq DNA polymerase	>100	>100
T4 DNA polymerase	>100	>100
– Other DNA metabolic enzymes –		
Calf primase of DNA polymerase α	>100	>100
HIV-1 reverse transcriptase	>100	>100
T7 RNA polymerase	>100	>100
T4 polynucleotide kinase	>100	>100
Bovine deoxyribonuclease I	>100	>100

Table 1. IC_{50} values of curcumin (**2**) and monoacetyl-curcumin (**13**) for various pols and other DNA metabolic enzymes. The compounds were incubated with each enzyme (0.05 units). Enzyme activity in the absence of the compound was taken as 100%. Data are shown as the mean ± SE (n=3).

When activated DNA (i.e., bovine deoxyribonuclease I-treated DNA) and dNTP were used as the DNA template-primer and nucleotide substrate instead of synthesized DNA [poly(dA)/oligo$(dT)_{18}$ (A/T = 2/1)] and dTTP, respectively, the inhibitory effects of these compounds did not change.

4. Effect of curcumin derivatives on TPA-induced anti-inflammatory activity

As mentioned in the Introduction, TPA is known to cause inflammation and is commonly used in screens for anti-inflammatory agents (Fujiki & Sugimura, 1987). Curcumin (**2**) is known as an anti-TPA-induced inflammatory compound (Ammon & Wahl, 1991), but the other agents (compounds **1** and **3**–**15**) had not previously been tested for anti-TPA-induced inflammatory activity.

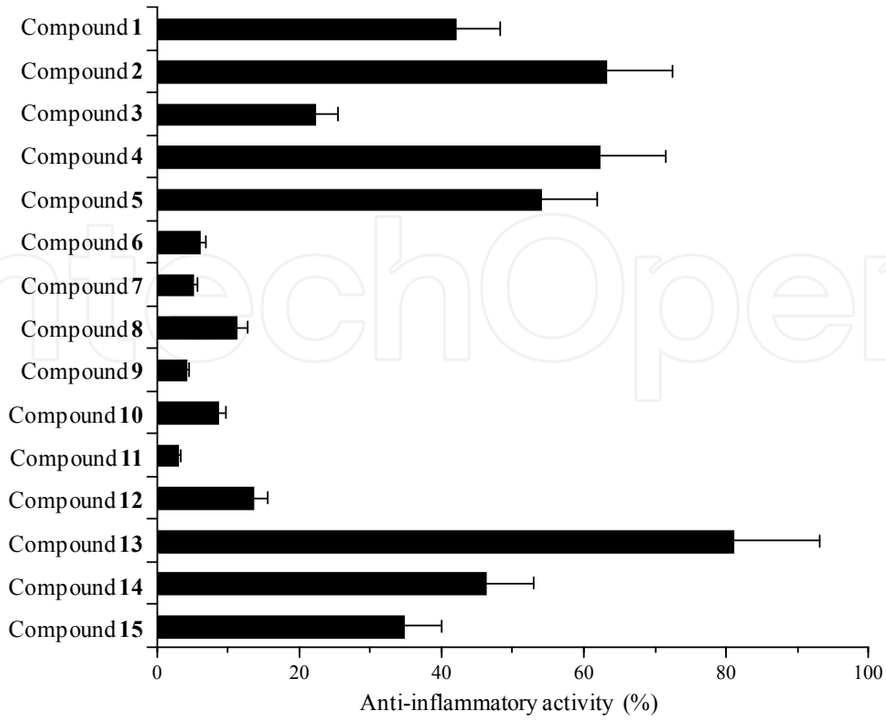


Fig. 4. Anti-inflammatory activity of curcumin derivatives (compounds **1–15**) toward TPA-induced edema on mouse ear. Each compound (250 μg) was applied to one of the mouse ears and, after 30 min, TPA (0.5 μg) was applied to both ears. Edema was evaluated after 7 h. The anti-inflammatory activity (%) is expressed as the percentage reduction in edema as compared with the non-treated ear. Data are shown as the mean ± SE (n=5).

Using an inflammation test in mice, the anti-inflammatory activity of these compounds was examined. The application of TPA (0.5 μg) to a mouse ear induced edema with a 241% increase in the weight of the ear disk at 7 h after application. As expected, curcumin (**2**) inhibited this inflammation at an applied dose of at least 250 μg (inhibitory effect (IE) = 63%) (Fig. 4). Petasiphenol (**1**), which was purified from Japanese vegetable (*Petasites japonicus*), was also an anti-inflammatory agent, although its effect was a third weaker than that of curcumin (**2**). Thus, both petasiphenol (**1**) and curcumin (**2**) could be potent inhibitors of inflammation caused by TPA. Interestingly, other curcumin derivatives also caused a marked reduction in TPA-induced inflammation: notably, the anti-inflammatory effect of monoacetyl-curcumin (**13**) was stronger than that of curcumin (**2**) with an IE of 81%, indicating that this compound possesses strong anti-inflammatory activity.

5. Structure–activity relationship of curcumin derivatives

Pol λ inhibition had a significant correlation (correlation coefficient = 0.9608) with anti-inflammatory activity, as shown by Fig. 3 and Fig. 4, which led us to speculate that TPA-induced inflammation may involve a process requiring pol λ, which is a DNA repair-related pol. Thus, to confirm whether there is a relationship between pol λ inhibition and anti-inflammation, the inhibitory effects of the curcumin derivatives (compounds **1–15**) on the two bio-activities were compared.

Among the fifteen curcumin derivatives tested, including curcumin (**2**) itself (Fig. 1), monoacetyl-curcumin (**13**) was the strongest inhibitor of both pol λ and anti-inflammation. Considering the structure of monoacetyl-curcumin (**13**) (Fig. 5), the essential moieties of the structure for these activities might be: <1> two enone moieties, <2> one hydroxyl group at position C4′, and <3> one acetoxy group at position C4″. These moieties are specific to monoacetyl-curcumin (**13**); therefore, these moieties are likely to be involved in the activities of both pol λ inhibition and anti-inflammation.

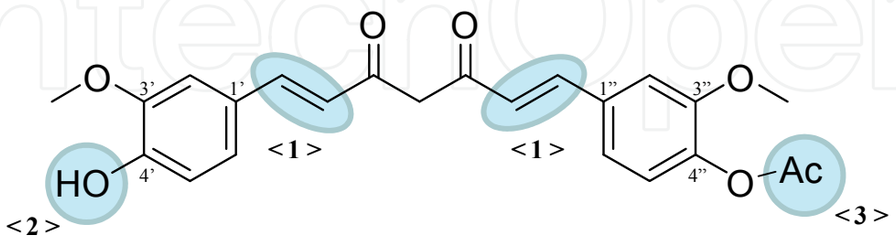


Fig. 5. Chemical structure of monoacetyl-curcumin (**13**). The functional groups likely to be essential for both pol λ inhibitory activity and anti-inflammatory activity in the curcumin derivatives are shown (<1> to <3>).

6. Inhibitory activity of curcumin (2) and monoacetyl-curcumin (13) against inflammatory responses in cultured cells

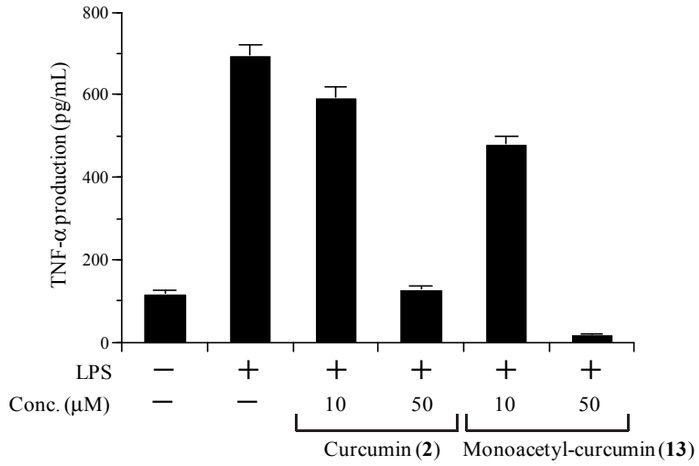
Next, because curcumin (**2**) and monoacetyl-curcumin (**13**) might be chemical knockout agents for DNA repair-related pol λ activity (Table 1), we used these compounds to investigate the anti-inflammatory mechanism of pol λ specific inhibitors in the murine macrophage cell line RAW264.7 treated with lipopolysaccharide (LPS or endotoxin), which stimulates macrophages to release inflammatory cytokines, interleukins (ILs) and tumor necrosis factor (TNF) (Hsu & Wen, 2002).

RAW264.7 cells were seeded on a 12-well plate at $1x10^5$ cells/well and incubated for 24 h. The cells were pre-treated with 10 or 50 μM curcumin (**2**) or monoacetyl-curcumin (**13**) for 30 min and then stimulated with 100 ng/mL of LPS. After 30 min or 24 h, the cell culture medium was collected to measure the levels of inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), nuclear factor-κB (NF-κB) and IκB. In RAW264.7 cells, cytotoxicity of these compounds at 50 μM was not observed (data not shown).

As shown in Fig. 6A, at 50 μM, curcumin (**2**) and monoacetyl-curcumin (**13**) both significantly suppressed LPS-stimulated production of TNF-α, and monoacetyl-curcumin (**13**) showed greater inhibition than curcumin (**2**). At 10 μM, monoacetyl-curcumin (**13**) still showed suppression of TNF-α production, although curcumin (**2**) at 10 μM did not significantly inhibit TNF-α production.

Next, the effect of monoacetyl-curcumin (**13**) on the expression level of pol λ protein in LPS-treated RAW264.7 cells was investigated. Fig. 6B shows that these macrophages underwent a more than 3-fold increase in the expression of pol λ after LPS stimulation, but this increase was suppressed by 50 μM monoacetyl-curcumin (**13**). These results suggest that there is the positive correlation between inflammatory induction by LPS and pol λ expression; thus, not only the DNA polymerization activity but also the protein expression of DNA repair-related pol λ is likely to be important in inflammation.

NF-κB is known to be the rate-controlling factor in inflammatory responses. Therefore, the inhibitory effects of curcumin (**2**) and monoacetyl-curcumin (**13**) on the LPS-induced nuclear translocation of NF-κB were examined in RAW264.7 cells. At 50 μM, curcumin (**2**) and monoacetyl-curcumin (**13**) both inhibited NF-κB nuclear translocation stimulated by 100 ng/mL of LPS, and the effect of monoacetyl-curcumin (**13**) was stronger than that of curcumin (**2**) (Fig. 6C). Stimulation with LPS results in activation of Toll-like receptor 4 and the downstream IκB kinases (IKKs), which in turn phosphorylate IκB, leading to degradation of IκB and translocation of NF-κB into the nucleus (Hashimoto et al., 2002). Therefore, the suppressive effects of curcumin (**2**) and monoacetyl-curcumin (**13**) on the LPS-induced phosphorylation of IκB were examined in RAW264.7 cells. By Western blot analysis, it was revealed that, at 50 μM, both curcumin (**2**) and monoacetyl-curcumin (**13**) significantly inhibited the LPS-induced phosphorylation of IκB (Fig. 6D). These results demonstrate that monoacetyl-curcumin (**13**)**,** as well as curcumin (**2**)**,** suppresses NF-κB nuclear translocation by inhibiting the phosphorylation of IκB.



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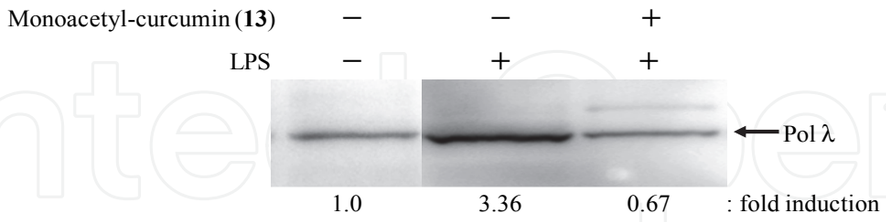

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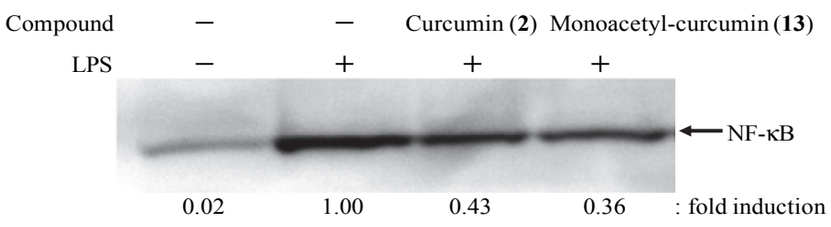

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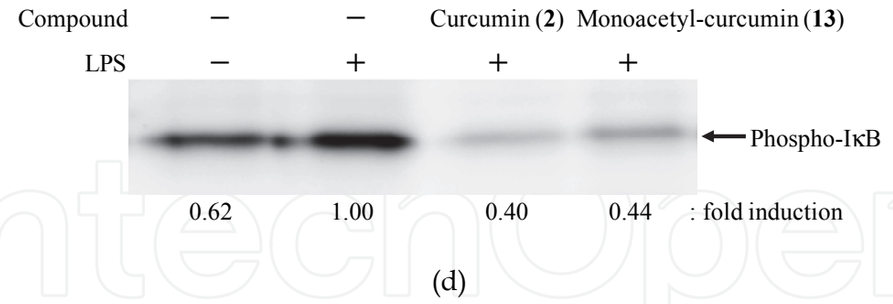

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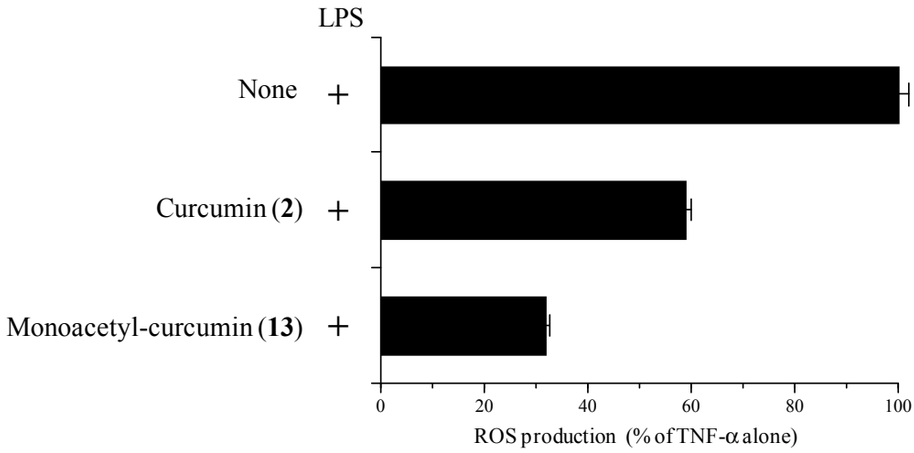


(e)

Fig. 6. Inhibitory activities of curcumin (**2**) and monoacetyl-curcumin (**13**) against inflammatory responses in the cultured murine macrophage cell line RAW264.7. (a) RAW264.7 cells were pre-treated with 10 or 50 μM curcumin (**2**) or monoacetyl-curcumin (**13**) for 30 min and then incubated with 100 ng/mL of LPS for 24 h. The TNF-α level in the culture medium was measured by ELISA. Data are shown as the mean ± SE (n=4). (b) RAW264.7 cells were pre-treated with 50 μM monoacetyl-curcumin (**13**), and then incubated with 100 ng/mL of LPS for 30 min. The expression level of pol λ was evaluated by Western blot analysis. The intensity of each band was analyzed, and the values relative to non-treatment with LPS are represented at the lower edge of the image. (c and d) RAW264.7 cells were pre-treated with 50 μM curcumin (**2**) or monoacetyl-curcumin (**13**), and then incubated with 100 ng/mL of LPS for 30 min. Nuclear translocation of NF-κB p65 (c) and the phosphorylation of IκB (d) were evaluated by Western blot analysis. The intensity of each band was analyzed, and the values relative to treatment with LPS alone are represented at the lower edge of the image. (e) RAW264.7 cells were pre-treated with 50 μM curcumin (**2**) or monoacetyl-curcumin (**13**) for 30 min, and then treated with 50 ng/mL of TNF-α and 4 μM DCFH-DA for 30 min. The fluorescent intensity of DCF, which indicates ROS production, was measured as described in a previous report (Corda et al., 2001). Data are shown as the mean ± SE (n=4).

Anti-oxidative activity has been reported to be linked to anti-inflammatory activity (Rahman et al., 2006). We therefore investigated the anti-oxidative activity of curcumin (**2**) and monoacetyl-curcumin (**13**) against the production of reactive oxygen species (ROS) induced by TNF-α. Measurement of intracellular ROS was performed according to the method of a previous report (Corda et al., 2001). In RAW264.7 cells, at 50 μM, the two compounds decreased the production of ROS by 50 ng/mL of TNF-α to 59.5% and 32.1%, respectively (Fig. 6E). These results suggest that both compounds possess anti-oxidative activity, but that monoacetyl-curcumin (**13**) has stronger activity than curcumin (**2**).

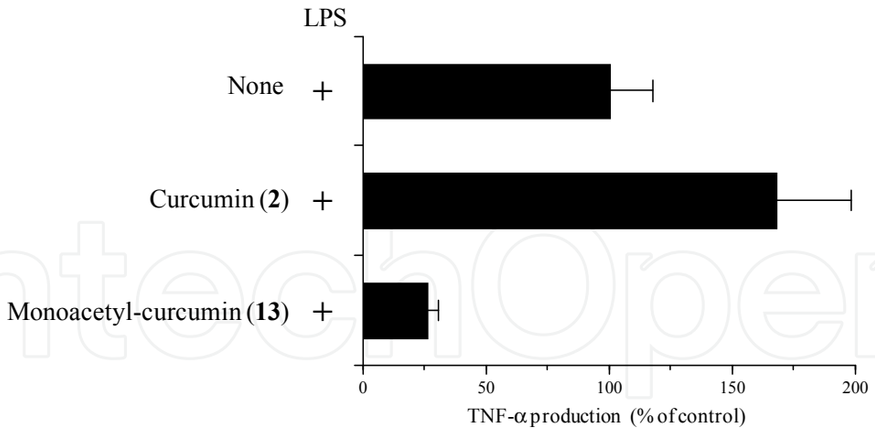
7. Inhibitory activity of curcumin (2) and monoacetyl-curcumin (13) against LPS-induced inflammation *in vivo*

To assess their anti-inflammatory effects *in vivo*, the inhibitory activity of curcumin (**2**) and monoacetyl-curcumin (**13**) against LPS-induced acute inflammation was investigated in mice (Fig. 7). As shown in Fig. 7A, treatment with 250 μg/kg (body weight, BW) of LPS increased the serum TNF-α level, and an oral injection of 100 mg/kg (BW) of monoacetyl-curcumin (**13**) significantly decreased the LPS-induced production of TNF-α to 36%. By contrast, curcumin (**2**) had no effect. Next, the inhibitory effects of these compounds on nuclear translocation of NF-κB in the liver were examined. Fig. 7B shows that LPS caused translocation of NF-κB into the nucleus, and monoacetyl-curcumin (**13**) blocked this nuclear translocation. Notably, curcumin (**2**) also inhibited nuclear translocation of NF-κB even though it did not block TNF-α production.

The serum levels of curcumin (**2**) and monoacetyl-curcumin (**13**) 2 h after oral administration were measured in the mice by liquid-chromatography mass spectrometry. The serum concentrations were below the detection limit and, thus, were less than 0.3 nM for both curcumin (**2**) and monoacetyl-curcumin (**13**) (data not shown). It has been reported that curcumin (**2**) is poorly absorbed in the body (Anand et al., 2007). Thus, a lower concentration of monoacetyl-curcumin (**13**) than of curcumin (**2**) might be able to decrease the serum TNF-α level in mice treated with LPS.

8. Discussion

Inflammatory mediators, such as TPA and LPS, quickly stimulate ROS (Hsu & Wen, 2002), and ROS are known to mediate oxidative DNA damage. As shown in Fig. 8, DNA repair pols such as pol λ induce protein expression and increase DNA polymerization activity to repair the damaged DNA. Furthermore, we consider that pol λ might have a great effect on inflammatory responses, such as TNF-α production, NF-κB activation, secretion of cytokines [e.g. interferons (IFNs) and interleukins (ILs) etc], tissue damage and cell death. The results summarized in this review suggest that inhibition of DNA repair by pol λ is related to anti-inflammatory pathways, and that pol λ-specific inhibitors such as monoacetyl-curcumin (**13**) might be chemotherapeutic drugs for inflammatory diseases. The detailed molecular mechanism underlying the correlation between DNA repair inhibition by pol λ and anti-inflammatory responses is not yet known; therefore, experiments with small interfering RNA (siRNA) targeting pol λ would help in further analyses.



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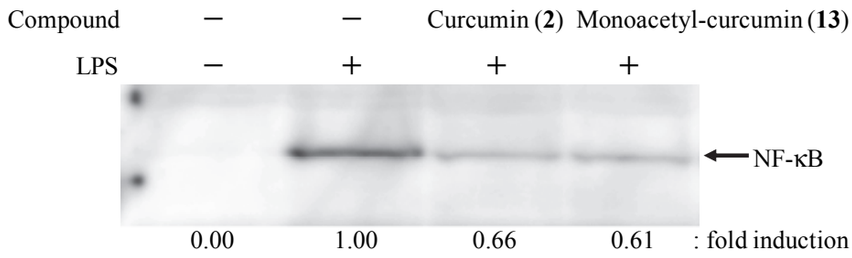

(b)

Fig. 7. The inhibitory activity of curcumin (**2**) and monoacetyl-curcumin (**13**) against LPS-induced inflammation *in vivo*. Male 8-week-old C57BL/6 mice were given an oral dose of 100 mg/kg (BW) of curcumin (**2**) or monoacetyl-curcumin (**13**) dissolved in corn oil or 200 μL of corn oil as a vehicle control. After 2 h, the mice were intraperitoneally injected with 250 μg/kg (BW) of LPS dissolved in phosphate-buffered saline (PBS) or 200 μL of PBS as a vehicle control. After 1 h, the mice were killed. (a) The TNF-α level in serum was measured by ELISA. Data are shown as the mean ± SE (n=4). The treatment with corn oil and LPS (positive control) was taken as 100% (TNF-α level, 728 pg/mL) and that with corn oil and saline (negative control) as taken as 0% (TNF-α level, 32 pg/mL). (b) NF-κB p65 in the nuclei of mouse liver cells was detected by Western blotting. The intensity of each band was analyzed, and the values relative to treatment with LPS alone are represented at the lower edge of the image.

As mentioned above, eukaryotic cells reportedly contain 14 pol species belonging to four families (Friedberg et al., 2000; Takata et al., 2006). Among the X family of pols, pol λ has an unclear biochemical function, although it seems to work in a similar way to pol β (Garcia-Diaz et al., 2002). Pol β is involved in the short-patch base excision repair (BER) pathway (Matsumoto & Kim, 1995; Singhal & Wilson, 1993; Sobol et al., 1996), as well as playing an essential role in neural development (Sugo et al., 2000). Recently, pol λ was found to possess 5'-deoxyribose-5-phosphate (dRP) lyase activity, but not apurinic/apyrimidinic (AP) lyase activity (Garcia-Diaz et al., 2001). Pol λ is able to substitute for pol β during *in vitro* BER, suggesting that pol λ also participates in BER. Northern blot analysis indicated that transcripts of pol β are abundantly expressed in the

testis, thymus and brain in rats (Hirose et al., 1989), whereas pol λ is efficiently transcribed mostly in the testis (Garcia-Diaz et al., 2000). Bertocci et al. reported that mice in which pol λ expression is knocked down are not only viable and fertile, but also display a normal hyper-mutation pattern (Bertocci et al., 2002).

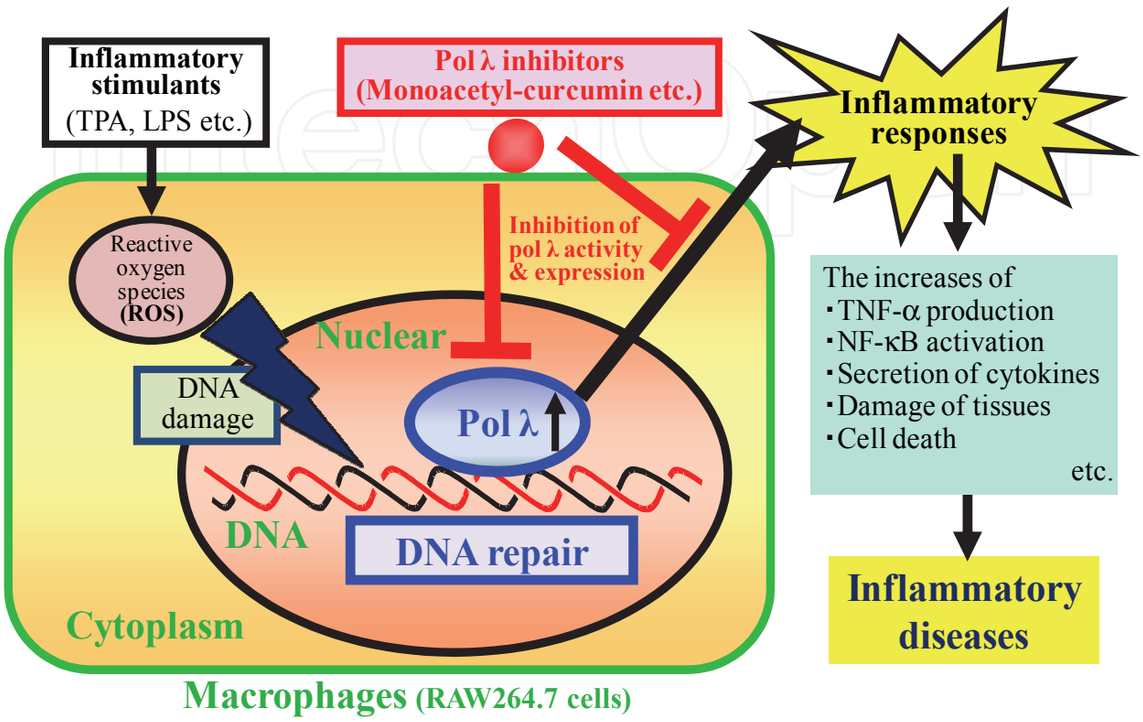

Fig. 8. The relationship between DNA repair by pol λ and inflammation

As well as causing inflammation, TPA influences cell proliferation and has physiological effects on cells because it has tumor promoter activity (Nakamura et al., 1995). Therefore, anti-inflammatory agents are expected to suppress DNA replication/repair/recombination in nuclei in relation to the action of TPA. Because pol λ is a DNA repair-related pol (Garcia-Diaz et al., 2002), our finding – that the molecular target of curcumin derivatives as monoacetyl-curcumin (**13**) is pol λ – is in good agreement with this expected mechanism of anti-inflammatory agents. As a result, any inhibitor of DNA repair-related pol λ might also be an inflammatory suppressor.

9. Conclusion

This review summarizes data showing that a major anti-inflammatory food compound, curcumin (**2**), selectively inhibits the activity of pol λ among 9 species of mammalian pols tested. Monoacetyl-curcumin (**13**) was the strongest inhibitor of pol λ among the 13 chemically synthesized derivatives of curcumin (**2**), suggesting that monoacetyl-curcumin (**13**) is a potent candidate for a functional compound. In addition, the inhibitory effects of monoacetyl-curcumin (**13**) on inflammatory responses in comparison to those of curcumin (**2**) *in vitro* and *in vivo* were investigated. Monoacetyl-curcumin (**13**) suppressed NF-κB activation induced by LPS and TNF-α in RAW264.7 murine macrophages. Moreover,

monoacetyl-curcumin (**13**) exerted inhibitory effects on TNF-α production and NF-κB activation in an animal model of LPS-induced acute inflammation. These results of the chemical knock out of pol λ by monoacetyl-curcumin (**13**) suggest that the inhibition of pol λ, which is a DNA repair-related pol, is related to anti-inflammatory processes.

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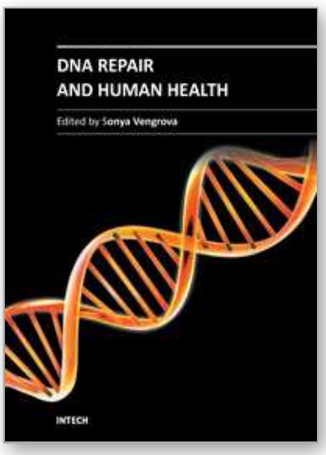

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Over the past decades, great advances have been made in understanding the cellular DNA repair pathways. At the same time, a wealth of descriptive knowledge of human diseases has been accumulated. Now, the basic research of the mechanisms of DNA repair is merging with clinical research, placing the action of the DNA repair pathways in the context of the whole organism. Such integrative approach enables understanding of the disease mechanisms and is invaluable in improving diagnostics and prevention, as well as designing better therapies. This book highlights the central role of DNA repair in human health and well-being. The reviews presented here, contain detailed descriptions of DNA repair pathways, as well as analysis of a large body of evidence addressing links between DNA damage repair and human health. They will be of interest to a broad audience, from molecular biologists working on DNA repair in any model system, to medical researchers.

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