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Lipopolysaccharide enhancement of 12-*o*-tetradecanoylphorbol 13-acetate-mediated transformation in rat glioma C6, accompanied by induction of inducible nitric oxide synthase

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Abstract

Lipopolysaccharide (LPS) from Gram-negative bacterial has been identified as an important molecule involved in the inflammatory process through inducing nitric oxide (NO) production. However, the effect of LPS in carcinogenesis is still undefined. In the present study, the biological effect of LPS was examined in 12-*o*-tetradecanoylphorbol 13-acetate (TPA)-treated rat glioma cells C6. Results of MTT assay showed that LPS and TPA exhibited no significant cytotoxicity in glioma C6 cells. Interestingly, transformation foci were found in LPS/TPA-treated glioma C6 cells, but not in LPS- or TPA-treated cells. The transformation foci induced by LPS/TPA were also observed in the absence of serum. It indicates that induction of transformation foci formation by LPS and TPA is independent on the serum in glioma C6 cells. Induction of iNOS gene expression and NO production was examined in LPS/TPA-treated cells, but not obvious in LPS- or TPA-treated cells. NO donor sodium nitroprusside (SNP) induces transformation in glioma C6 cells in according with elevating NO production. In addition, LPS/TPA induces metalloproteinase 9 (MMP9) activity by gelatin activity assay in gel. Wogonin and quercetin but not rutin, inhibitors of iNOS gene expression and NO production induced by LPS, showed the significant inhibition on LPS/TPA-induced transformation foci formation, accompanied by inhibiting iNOS gene expression, NO production and MMP9 activity. Results of the present study provide scientific evidences to link the inflammatory responses and carcinogenesis, and suggest that NO derived from inflammation may contribute to the progression of carcinogenesis; natural products with anti-inflammatory effects such as wogonin and quercetin possess the ability to block transformation induced by LPS/TPA.

Keywords: LPS; TPA; NO; INOS; Transformation; Glioma C6

Abbreviations: MTT, (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; LPS, lipopolysaccharide; TPA, 12-*o*-tetradecanoylphorbol 13-acetate; MMP9, metalloproteinase 9; NO, nitric oxide; iNOS, inducible nitric oxide synthase; ODC, ornithine decarboxylase; PKC, protein kinase C; MAPK, mitogen activated protein kinases

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1. Introduction

There are three stages in carcinogenesis including initiation, promotion, and progression (Lin et al., 1999; Vineis, 2003). Initiation is an irreversible process to produce latent tumor cells, and the initiated cells reserve the normal physiological functions. Promotion, a reversible event, is the most important stage in carcinogenesis, and transforms the latent tumor cells to carcinoma cells. Progression is the final stage of carcinogenosis, and malignant transformation of carcinoma in situ to invasive carcinoma. During the carcinogenesis, transformation is an essential event, and characteristics of transformation including morphological changes, adhesion-independent growth, and induction of metalloproteinase (MMP) activity have been identified (Impola et al., 2003; Govindarajan et al., 2003). Transformation has been performed in several cell systems and regarded as an important method for the screening the potential agents including mutagens or promoters or anti-carcinogens (Tsuchiya and Umeda, 1997). However, the in vitro assay method requires a long period of cultivation for the transformation foci, and the transformation frequency is relatively low, which requires the use of many dishes, thus increasing the cost. Therefore, development of an effective in vitro transformation model is deserved for study. 12-*o*-tetradecanoylphorbol 13-acetate (TPA) is a well-known tumor promoter in mouse skin models (Mulherkar et al., 2003; Wei et al., 2003). On the basis of several studies, TPA treatment was able to activate protein kinase C, ornithine decarboxylase (ODC) activity and induce immediate early gene expression such as c-Jun, c-fos through reactive oxygen species production (Kuo et al., 1993; Mwimbi et al., 2002), Induction of colony formation in NIH3T3 by long-term TPA treatment has been demonstrated in our previous study through activating PKC kinase and MAPK cascade (Ko et al., 2002).

Bacterial endotoxin lipopolysaccharide (LPS), an outer membrane constituent of all Gram-negative bacteria, is a complex glycolipid composed of a hydrophilic polysaccharide moiety and a hydrophobic domain known as lipid A. LPS is a potent activator of innate immune responses that result in the production of pro- and anti-inflammatory mediators in several cell types (Karaghiosoff et al., 2003; Sato et al., 2003). LPS binding to membranous receptors including CD14 or Toll-like receptors, inflammatory signals will be transduced from membrane to cytoplasm and nuclear, followed by activating several genes' expression such as inducible nitric oxide synthase (iNOS) (Nadeau and Rivest, 2002; Triantafilou and Triantafilou, 2002). Nitric oxide (NO) is one of the molecules induced by LPS, and several previous studies indicated that NO participates in the cause of human diseases such as sepsis and neuron degeneration (Price et al., 2002; Estevez et al., 1998). At least three isoforms of nitric oxide synthases (NOS) including nNOS, eNOS, and iNOS involved in NO production have been identified. Both nNOS and eNOS (cNOS) are constitutive and their activation is Ca^{2+}-dependent. Sustained release of NO from cNOS plays a role in keeping the vasculture in an active state of vasodilation, and several neurotransmitters such as acetylcholine and bradykinin triggered cNOS-mediated NO production through increasing intracellular Ca^{2+} (Xu et al., 1996; Schneider et al., 2002). On the other hand, iNOS in macrophages and hepatocytes is inducible and Ca^{2+}-independent. In the presence of extracellular stimulus such as vial infection or bacterial infection, NO can be produced quantitatively through activating iNOS gene expression and cause deleterious and pathophysiological effects. A large amount of NO production by LPS has been shown by activation of iNOS gene expression in several previous studies, and compounds inhibited LPS-induced NO production reserve the potential for anti-inflammation (Chen et al., 2000, 2001a,b; Shen et al., 2002). Although the role of NO induced by LPS played in inflammation has been studied widely, the effect NO and LPS in carcinogenesis is still undefined.

In the present study we explored in vitro effect of LPS in the development of the transformation in the presence of TPA in glioma C6 cells. Results of the present study demonstrated that co-treatment of cells with LPS and TPA induced malignant transformation in accord with induction of NO production and MMP9 activity. Wogonin and quercetin, inhibitors of LPS-induced NO production, showed the significant inhibitory activities on LPS/TPA-induced transformation. Results of the present study provided the first scientific evidences to demonstrate that LPS and TPA co-treatment induced malignant transformation in glioma C6 cells, and NO may play an important role in the transformation mechanism.

2. Materials and methods

2.1. Cells

Glioma C6, a rat glioma cell line, was obtained from the American Type Culture Collection. Cells were cultured in RPMI-1640 medium supplemented with 2 mM glutamine, antibiotics (100 U/ml of penicillin A and 100 U/ml of streptomycin) and 10% heat inactivated fetal bovine serum (Gibco/BRL) and maintained at 37 °C humidified incubator containing 5% CO_2.

2.2. Agents

Antibodies including anti-iNOS and anti-α-tubulin were purchased form Santa Cruz (Santa Cruz, CA). Wogonin, querecetin, rutin, TPA, LPS, naphylethylenediamine dihydrochloride and sulfanilamide were purchased from Sigma (St. Louis, MO). These compounds were dissolved in dimethyl sulfoxide (DMSO) before adding them to the culture. The final concentration of DMSO was below 0.5% of the medium.

2.3. Cell viability

Cell viability was assessed by MTT staining as described by Mosmann (Mosmann, 1983). Briefly, glioma C6 cells were plated at a density of 10^4 cells per well into 24-well plates under different treatment for different time points. At the end of treatment, 20 μl of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) (10 mg/ml) was added, and cells were incubated for a further 4 h. Cell viability was obtained by scanning with an ELISA reader (Molecular Devices) with a 600-nm filter.

2.4. Nitrite assay

The nitrite concentration in the medium was measured as an indicator of NO production according to the Griess reaction (Chen et al., 2001a). One hundred microliters of each supernatant was mixed with the same volume of Griess reagent (1% sulfanilaminde in 5% phosphoric acid and 0.1% naphthylethylendiamine dihydrochloride in water). Absorbance of the mixture at 550 nm was determined with an ELISA plate reader (Dynatech MR-7000; Dynatech Laboratories). Nitrite in medium without cells has been determined by Griess reaction, and the amount of nitrite in serum-containing medium and serum-free medium without cells is below 2 μM.

2.5. Transformation assay

Glioma C6 cells (10^4 per well) were treated with LPS, TPA, or LPS/TPA for 24 h, and the cells were fixed with ethanol, following by staining with 10% Giemsa-solution and rinsed three times with water. The transformed foci were detected by microscopic observation.

2.6. Western blots

Total cellular extracts were prepared according to our previous papers (Chen et al., 2002a,b), separated on 8% SDS-polyacrylamide min gels, and transferred to immobilon polyvinylidene difluoride membrane (Millipore). The membrane was incubated with 1% bovine serum albumin and then incubated with anti-iNOS, and anti-α-tubulin antibodies respectively (Santa Cruz, CA). Expression of iNOS and α-tubulin protein was detected by NBT and BCIP staining (Sigma Chemical Co.).

2.7. Zymography

Enzymatic activity of MMP proteins was determined by zymography as reported previously (Paemen et al., 1996). Briefly, after 24 h incubation of glioma cells with LPS, TPA or LPS/TPA, aliquots of the conditioned media containing 50 mg of protein were mixed with loading buffer and proteins were separated under nonreduction conditions in 10% polyacrylamide gels containing with 1 mg/ml gelatin (sigma). Then, the gel were washed with 2.5% Triton X-100 and incubated at 37 C for 24 h in 50 mM Tris–HCl (pH7.5) buffer containing 0.15 M NaCl, 5 mM $CaCl_2$, 1 mM ZnSO4 and 40 mmo1/l NaN_3. After staining with Coomassie brilliant blue, MMP activity was evident as clear bands against a blue background.

2.8. Statistical analysis

Results are expressed as the means ± S.E.M. of indicated number of experiments. Statistical significance was estimated using student's t test for paired

observations. A possibility value of less than 0.05 was considered significant.

3. Results

3.1. Effect of LPS, TPA, LPS/TPA in the viability of rat glioma C6 cells

The effect of LPS (1 mg/ml) in the presence or absence of TPA (400 ng/ml) in the viability of glioma C6 cells was evaluated by MTT assay. The viability of cells increased in a time-dependent manner without LPS or TPA. In the presence of LPS (1 mg/ml) or TPA (400 ng/ml) alone, the survival curve of cells was the same as that in control group. As the same part of experiment, glioma C6 cells under LPS (1 mg/mi) and TPA (400 ng/ml) (LPS/TPA) treatment was found in the same time-dependent pattern as detected in control, LPS- or TPA-treated group. These data suggested that LPS, TPA, LPS/TPA did not cause the alternation in the viability of rat glioma C6 cells (Fig. 1).

3.2. Transformation effect of LPS and TPA on the focus formation in rat glioma C6 cells

TPA is a well-known tumor promoter and activates the transformation in several cell types. In order to elucidate the biological effects of LPS and TPA in glioma C6 cells, cells were treated with different components for 24 h followed by Giemsa staining under microscopic observation. Result illustrated in Fig. 2 showed that morphology of cells did not show obvious alternation in LPS-treated cells, and TPA addition causes slight but significant transformation characteristics including cellular aggregation and deep basophilic staining by Giemsa solution. Interestingly, the addition of LPS with TPA enhanced TPA-induced transformation, and significant transformation foci formation was detected. It is appeared that LPS and TPA co-treatment was able to induce server cellular transformation and formation of dense multilayering cells, characteristics of transformation foci (Fig. 2).

3.3. Induction of NO production and iNOS gene expression in the presence of LPS and TPA in rat glioma C6 cells

Previous data provide a novel observation indicated that combination of LPS and TPA promotes the occurrence of transformation in rat glioma C6 cells. LPS is a well-known inflammatory inducer, and activation of iNOS gene expression and NO production have been identified in several cell types. In order to elucidate if iNOS induction involved in LPS/TPA-induced

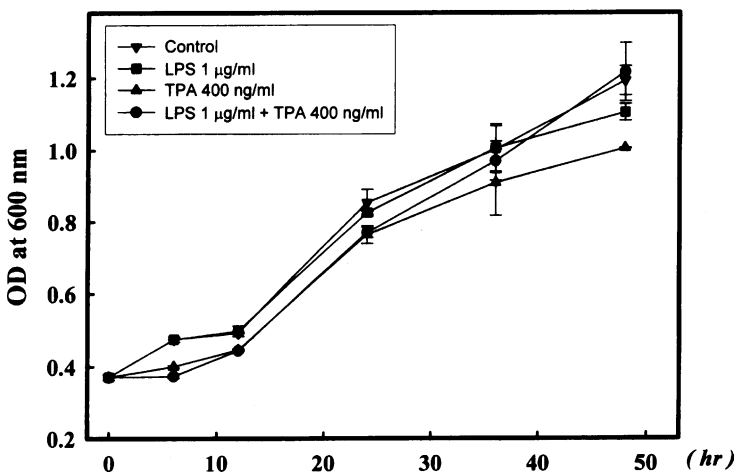

Fig. 1. Analysis of cell viability in LPS-, TPA-, or LPS/TPA-treated glioma C6 cells. Glioma C6 cells were plated into 24 well plates for 24 h and then treated with different concentrations including LPS (1 μg/ml), TPA (400 ng/ml) or LPS (1 μg/ml) plus TPA (400 ng/ml) (LPS/TPA) for different time periods. MTT was added into medium at the end of incubation for an additional 4 h. The viability of cells was detected by measuring the absorbance at a wavelength of 600 nm.

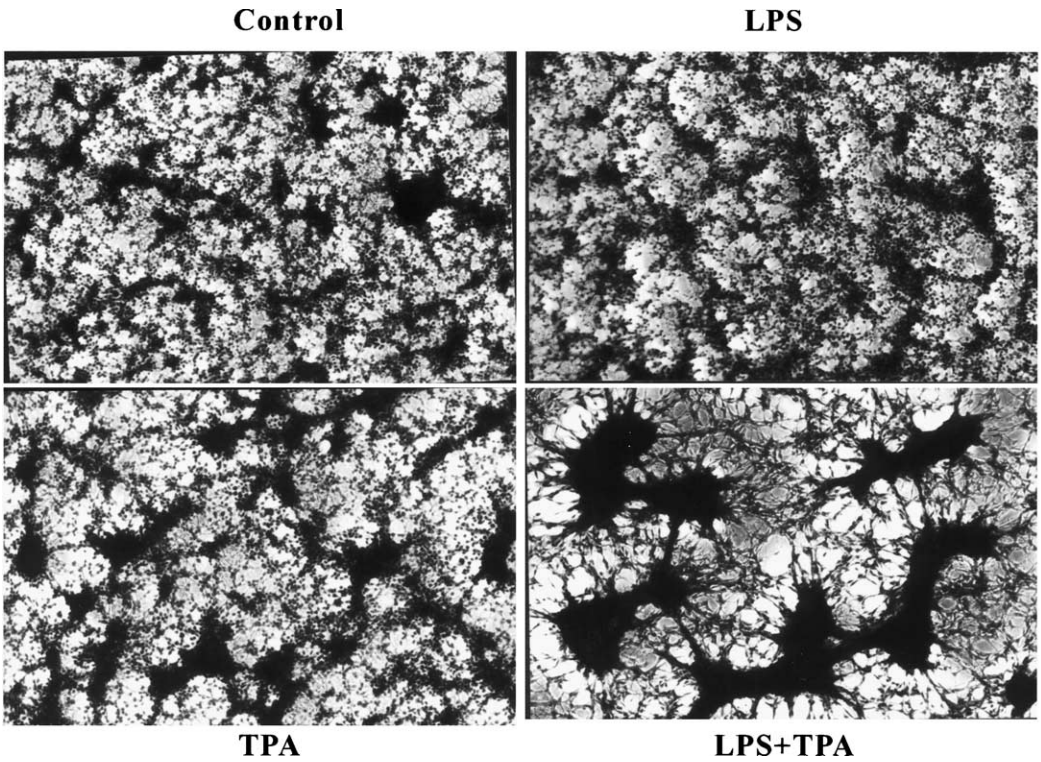

Fig. 2. Transformational morphology observed in LPS/TPA-treated glioma C6 cells. Cells were treated with LPS (1 μg/ml), TPA (400 ng/ml) or LPS (1 μg/ml) plus TPA (400 ng/ml) (LPS/TPA) for 24 h, followed by Giemsa staining. The transformational morphology was detected in LPS/TPA-treated cells under microscopic observation.

transformation, Western blot using specific antibodies to detect iNOS protein was performed. Results of Fig. 3A showed that LPS or TPA alone was unable to induce iNOS gene expression in C6 cells. However, addition of LPS with different doses of TPA showed the significant induction on iNOS protein expression in cells. In order to figure out if serum is required for LPS/TPA-induced iNOS gene expression and transformation, cells were treated with LPS/TPA for different time periods in the presence (+FBS) or absence of serum (SF), and the expression of iNOS was examined. Results of Fig. 3B showed that a time-dependent induction of iNOS protein by LPS/TPA in the presence or absence of serum, and the transformation foci formation in LPS/TPA-treated cells were also observed in the absence of serum (Fig. 3B and data not shown). It suggests that LPS/TPA-induced transformation and iNOS gene expression are independent on the occurrence of serum. INOS enzyme has been shown to catalyze the conversion of L-arginine to L-citrullin with NO production. NO will react with ROS to form both nitrite and nitrate, and the amount of nitrite in medium can be measured by Griess reaction. Our preliminary data show that reduction of nitrate to nitrite by nitrate reductase did not affect the result of NO detection in cells induced by LPS. Therefore, a direct measurement of nitrite in the medium by Griess reaction as a representative of NO production was performed in the present study. Results of Fig. 4A showed that LPS/TPA induces nitrite formation in rat glioma C6 cells in the presence or absence of serum, however LPS or TPA alone did not shown any inductive effect in cells (Fig. 4A). Additional, NO donor sodium nitroprusside (SNP) induces transformation in glioma C6 cells in the presence of TPA, but not LPS, by Giemsa staining under microscopic observation (Fig. 5A). And, a significant elevation of NO production was detected in SNP-treated cells, however TPA did not show any

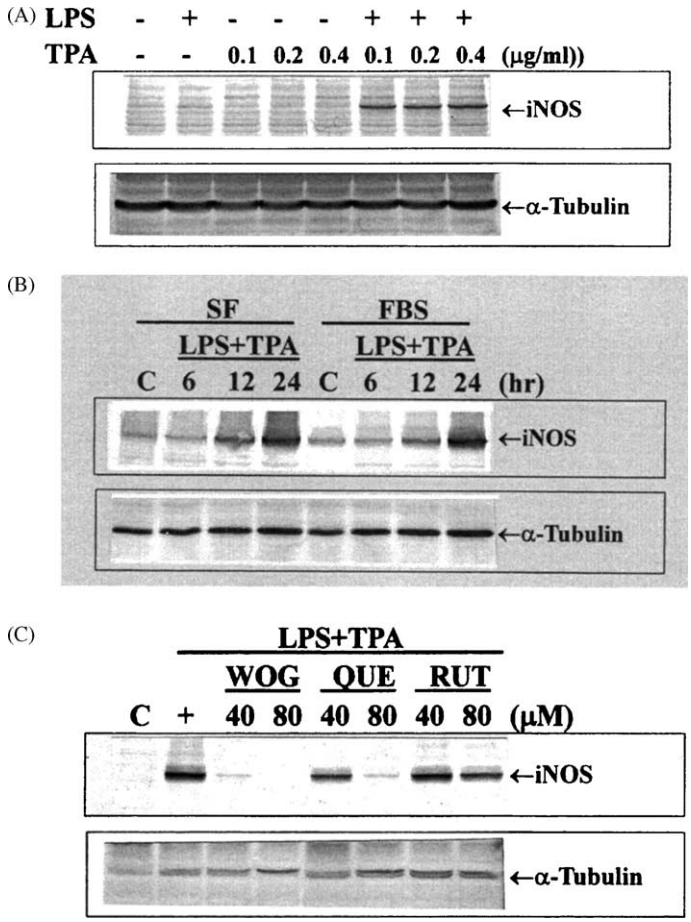

Fig. 3. Induction of iNOS gene expression in LPS/TPA-treated C6 cells in the presence or absence of serum. (A) Cells were treated with LPS (1 μg/ml), TPA (0.1, 0.2, 0.4 μg/ml) or LPS (1 μg/ml) plus different doses of TPA (0.1, 0.2, 0.4 μg/ml) for 24 h in the presence of 10% FBS, and the expression of iNOS and α-tubulin protein was examined using specific antibodies. (B) Cells were treated with LPS (1 μg/ml) plus TPA (400 ng/ml) (LPS/TPA) in the presence of 10% FBS (FBS) or absence of FBS (SF) for different time periods, and the expression of iNOS and α-tubulin protein was examined using specific antibodies. (C) Cells were treated with LPS (1 μg/ml) plus TPA (400 ng/ml) (LPS/TPA) in the presence of indicated flavonoids (40 and 80 μM) for 24 h, and the expression of iNOS and α-tubulin protein was examined using specific antibodies. WOG, wogonin; QUE, quercetin; RUT, rutin.

effect on SNP-induced NO production (Fig. 6). These data suggest that induction of iNOS gene expression and NO production is involved in LPS/TPA-induced transformation in C6 cells.

3.4. iNOS inhibitors wogonin and quercetin suppress LPS/TPA induced transformation, accompanied by blocking NO production and iNOS gene expression

In order to demonstrate if induction of iNOS gene expression and NO production are essential events for LPS/TPA-induced transformation, wogonin, quercetin and rutin were used in the present study. Our previous studies demonstrated that both wogonin and quercetin, but not rutin, suppressed NO production through blocking iNOS gene expression (Chen et al., 2001a,b). Results of Fig. 6 showed that LPS/TPA induces transformation foci formation in cells, and both wogonin and quercetin suppress LPS/TPA-induced transformation without any cytotoxicity by MTT assay. In contrast to wogonin and quercetin, rutin did not exhibit any inhibitory effect on LPS/TPA-induced

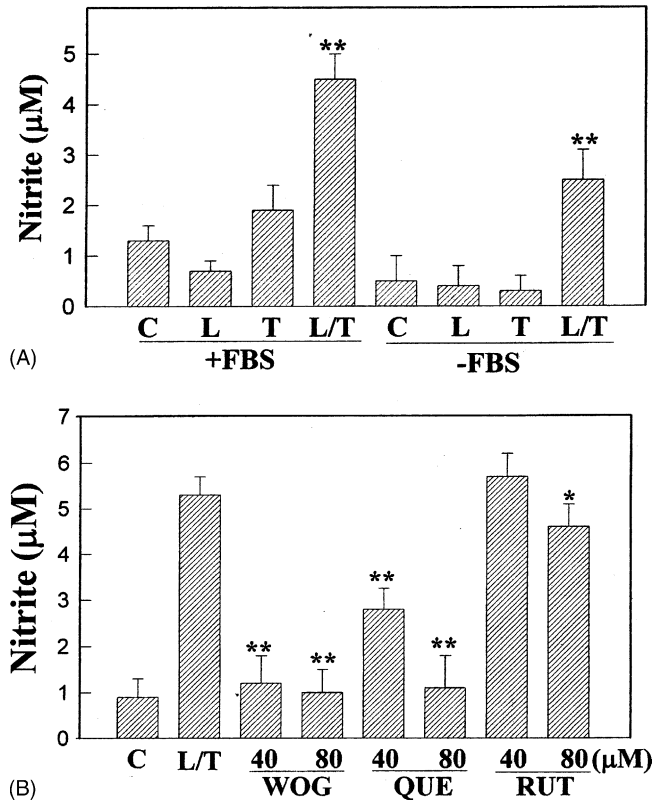

Fig. 4. LPS/TPA induced NO production in glioma C6 cells (A) Cells were treated with LPS (1 μg/ml; L), TPA (0.4 μg/ml; T), LPS (1 μg/ml) plus TPA (0.4 μg/ml) (L/T) in the presence (+FBS) or absence (−FBS) of 10% FBS, and the amount of NO production in the medium was measured by the Griess reaction. The amount of NO production was quantitatively assessed using $NaNO_2$ as a standard. Data were obtained from three independent experiments and are expressed as the mean ± S.E.. $^{**}P < 0.01$ indicates a significant difference from the LPS-treated group, as analyzed by Student's *t*-test. (B) Cells were treated with LPS (1 μg/ml) plus TPA (0.4 μg/ml) (L/T) in the presence of indicated flavonoids, and the amount of NO production in the medium was measured by the Griess reaction. WOG, wogonin; QUE, quercetin; RUT, rutin.

transformation. Furthermore, both wogonin and querecetin, but not rutin, inhibited LPS/TPA-induced iNOS gene expression and NO production by Western blot and Griess reaction, respectively (Fig. 3C and Fig. 4B). These data suggested that inhibition of LPS/TPA-induced transformation by wogonin and quercetin through blocking iNOS induction and NO production in rat glioma C6 cells.

3.5. Induction of MMP-9 activity in LPS/TPA-induced transformation in rat glioma C6 cells

Metalloproteinases (MMPs) have been demonstrated to be involved in the malignant transformation of tumor. In order to identify if activation of MMPs involved in LPS/TPA-induced transformation, an in gel gelatinase activity assay was performed in the study. In the presence of serum, a slight increase in MMP9 proteinase activity was detected due to a higher basal level of MMPs in serum-containing medium. In the absence of serum, MMP9 activity was induced significantly in LPS/TPA-treated cells, and wogonin and quercetin but not rutin showed the potent inhibitory activities on LPS/TPA-induced MMP9 activity. It is suggested that LPS/TPA induced transformation in according with activation of MMP9 activity in C6 cells (Fig. 7).

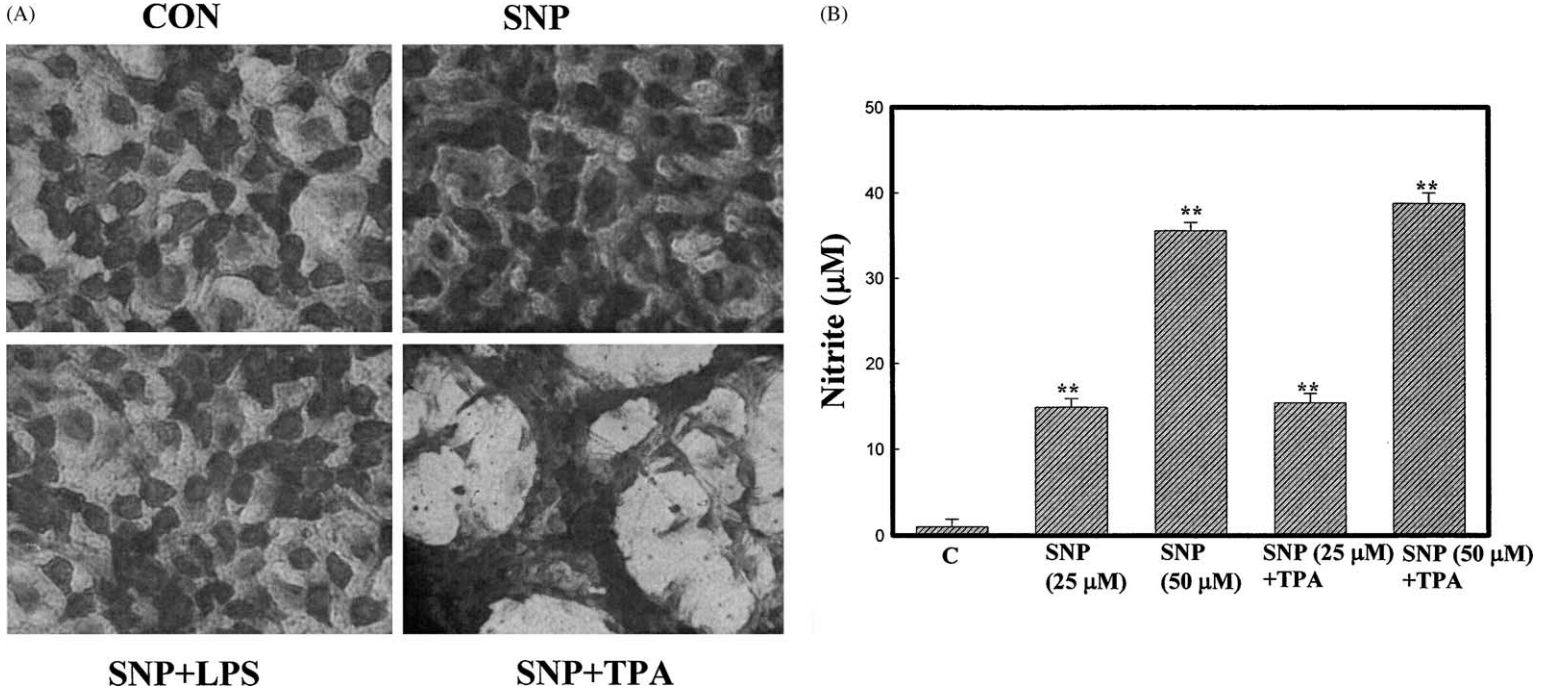

Fig. 5. NO donor sodium nitroprusside (SNP) enhances the transformation in the presence of TPA. (A) Glioma C6 cells were treated with SNP (50 μM), SNP (50 μM) plus TPA (400 ng/ml) (SNP + TPA), SNP (50 μM) plus LPS (1 μg/ml) (SNP + LPS) for 24 h, followed by Giemsa staining. The transformational morphology was detected in LPS/TPA-treated cells under microscopic observation. (B) Cells were treated with SNP (25 or 50 μM) in the presence or absence of TPA (400 ng/ml) for 24 h, and the production of nitrite in medium was measured by Griess reaction.

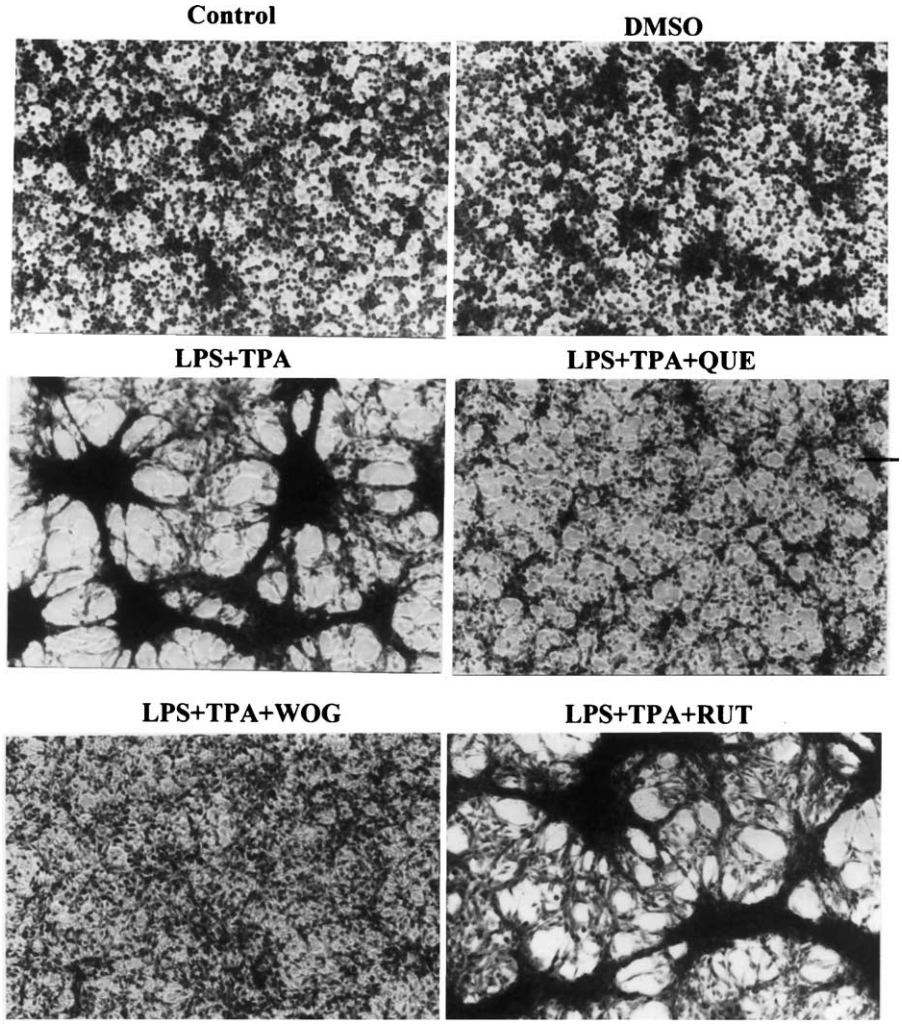

Fig. 6. Wogonin and quercetin, but not rutin, inhibition of LPS/TPA-induced transformation in glioma C6 cells. Cells were treated with LPS (1 μg/ml) plus TPA (0.4 μg/ml) in the presence or absence of DMSO (0.2%), quercetin (40 μM), wogonin (40 μM), or rutin (40 μM) for 24 h. The transformational morphology was detected by Giemsa staining under microscopic observation.

4. Discussion

The data demonstrated that LPS stimulates the formation of transformation foci in the presence TPA in glioma C6 cells. Induction of iNOS gene expression, NO production and MMP9 activity involve in LPS/TPA-induced transformation, and natural products wogonin and quercetin inhibit LPS/TPA-induced transformation in according with suppressing iNOS expression, NO production, and MMP9 activation. These data indicated the correlation between inflammatory responses and malignant tumoral transformation.

TPA has been widely used as a model compound for studying the mechanism of action of tumor promoters in several cells such as NIH3T3, BALB/c 3T3 and C3H10T1/2 cells (Wu et al., 1992; Chen et al., 1999; Maeda et al., 1999). In addition, Tsuchiya and Umeda reported that TPA induced expansion of transformants in BALB/c 3T3 cells (Tsuchiya and Umeda, 1997). It is suggested a relationship between TPA exposure and the appearance of transformed cells. In

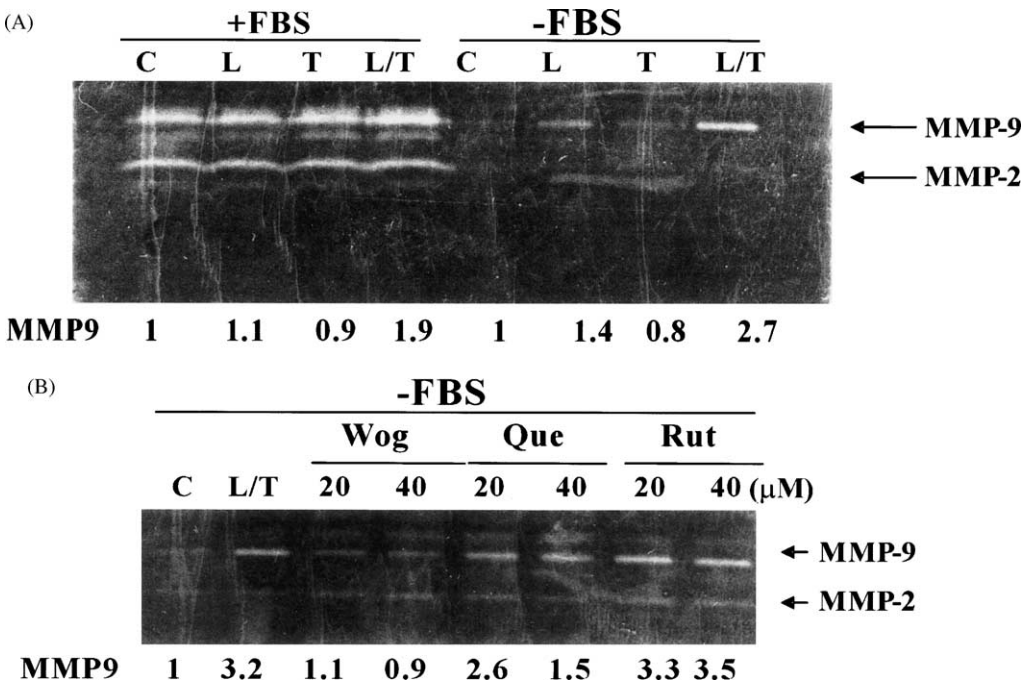

Fig. 7. Activation of MMP-9 activity in LPS/TPA-treated glioma C6 cells. (A) Cells were treated with LPS (1 μg/ml; L), TPA (0.4 μg/ml; T), LPS (1 μg/ml) plus TPA (0.4 μg/ml) (L/T) in the presence (+FBS) or absence (−FBS) of 10% FBS for 24 h, and the gelatinase activity in medium was examined by in gel gelatin digestion as described in the section of Materials and Methods. (B) Cells were treated with LPS (1 μg/ml) plus TPA (0.4 μg/ml) (L/T) in the presence of different doses (40 and 80 μM) of indicated flavonoids for 24 h, and the gelatinase activity in medium was examined. Wog, wogonin; Que, quercetin; Rut, rutin.

contrast to TPA, LPS is an endotoxin derived from Gram-negative bacterial, and has been shown as an important molecule in the cause of several human diseases such as sepsis and inflammation through induction of NO, PGE_2 and cytokine productions in macrophages, hepatocytes and fibroblasts. However, the effect of TPA and LPS in glioma cells C6 has not been clearly delineated. Syapin et al. indicated that maximal NO induction activity occurred in the treatment of glioma C6 with LPS and IFN-γ, but LPS alone was ineffective (Syapin et al., 2001). Militante et al. reported that exposure to LPS combined with TPA stimulates de novo synthesis of iNOS in glioma C6 cells (Militante et al., 1997), and Feinstein et al. also found that incubation of C6 cells with LPS did not stimulate NO production (Feinstein et al., 1994). These data suggested that LPS is an ineffective inducer for activating iNOS gene expression and NO production in glioma C6 cells, and addition of cytokines or TPA may potentiate NO production and iNOS gene expression in the presence of LPS. In addition to support the combination of TPA and LPS stimulates NO production in C6 cells, results of the present study also indicated that TPA and LPS significantly induced transformation in glioma C6 cells accompanied by induction of multi-layer of cells, NO production and MMP-9 activation. It is suggested that inflammatory responses induced by LPS may potentiate the transformation of C6 cells induced by TPA.

Metalloproteinases (MMPs) are a family of proteins including MMP1-13. Activation of MMPs has been found in malignant tumor cells, and tumor cells containing metastatic ability express higher level of MMPs activity. It indicates that MMPs involves in the malignant transformation of tumors. MMP-1 and MMP-9 have been shown to play important roles in tumor invasion and metastasis by facilitating the tumor microenvironment to enhance tumor growth. Therefore, agents inhibiting MMP-l or MMP-9 activity may reserve the potential to be anti-tumor

metastatic. Results of the present study indicate that LPS/TPA are able to increase MMP-9, but not MMP-1, activity in glioma C6 cells, and wogonin and quercetin showed the inhibition on LPS/TPA-induced MMP-9 activation. It suggests that inflammation induced by LPS potentiates metastatic activity of tumor induced by TPA and that wogonin and quercetin possess the anti-metastatic activity by blocking MMP-9 activity.

Flavonoids exist as either simple or complex glycosides in many plants, and humans are estimated to consume approximately 1 g flavonoids per day. Several biological functions of flavonoids such as apoptosis-inducing activity, free radical scavenging activity, and anti-tumorigenic activity have been identified (Lee et al., 2002; Shen et al., 2003; Lin et al., 2003; Chen et al., 2003). Wogonin has been examined to reserve several biological activities such as apoptosis-induction, suppression of NO production and neuronal protection. Quercetin and rutin (quercetin-3-*O*-rutinoside) are structurally related flavonoids, and exist extensively in diet, and the structural differences between them are rutin possesses a rutinose in at C3. Quercetin can be derived from rutin through hydrolyzation by glucosidase in the gastrointestinal tract, and has a wide range of biological activities such as inhibition of Na^+/K^+-ATPase, protein kinase C, tyrosine kinase, etc. Inhibition of LPS-induced iNOS gene expression by wogonin and querecetin, but not rutin, has been demonstrated in our previous studies (Chen et al., 2001a,b). Wogonin and quercetin have been shown to reserve antioxidant activity to block ROS-mediated damages. Both of them induced apoptosis in leukemia cells through activation of caspase 3 actiivty (Chen et al., 2001a; Shen et al., 2003). In the present study, wogonin and quercetin but not rutin perform inhibitory activities on LPS/TPA-induced transformation in according with suppressing iNOS gene expression and NO production. It suggests that wogonin and quercetin may prevent the occurrence of transformation and reserve the potential to be developed as tumor-preventive agents, and NO inhibition is involved. The alternative mechanisms involve in the inhibition of wogonin and queretin on LPS/TPA-induced transformation remain to be elucidated.

In conclusion, results of the present study provided scientific evidences to support that LPS/TPA induced transformation in glioma C6 cells, and activation of iNOS gene, NO production and MMP-9 activity was involved. Wogonin and quercetin are effective inhibitors of LPS/TPA-induced transformation through suppression of iNOS expression, NO production and MMP-9 activation. A possible link between inflammation and tumor transformation is delineated here.

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