Mechanism of dexamethasone in the context of *Toxoplasma gondii* infection

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SUMMARY

Toxoplasmosis is a serious zoonoses disease and opportunistic, and can be life-threatening. Dexamethasone (DEX) is widely used in the clinic for treatment of inflammatory and autoimmune diseases. However, long-term use of DEX is often easy to lead to acute toxoplasmosis in patients, and the potential molecular mechanism is still not very clear. The aims of this study were to investigate the effect of DEX on proliferation of Toxoplasma and its molecular mechanisms, and to establish the corresponding control measures. All the results showed that dexamethasone could enhance the proliferation of *Toxoplasma gondii* tachyzoites. After 72 h of DEX treatment, 566 (±7) tachyzoites were found in 100 host cells, while only 86 (±8) tachyzoites were counted from the non-treated control cells ($P < 0{\cdot}01$). Gas chromatography (GC) analysis showed changes in level and composition of fatty acids in DEX-treated host cells, and *T. gondii*. Fish oil was added as a modulator of lipid metabolism in experimental mice. It was found that mice fed with fish oil did not develop the disease after infection with *T. gondii*, and the structure of fatty acids in plasma changed significantly. The metabolism of fatty acid in the parasites was limited, and the desaturase gene expression was downregulated. These results indicate that the molecular mechanism of dexamethasone to promote the proliferation of *T. gondii* may be that dexamethasone induces the change of fatty acids composition of tachyzoites and host cells. Therefore, we recommend supplementation of fatty acid in immunosuppressive and immunocompromised patients in order to inhibit toxoplasmosis.

Key words: *Toxoplasma gondii*, dexamethasone, proliferation, fatty acid.

INTRODUCTION

Dexamethasone is an important synthetic glucocorticoid, and stress hormone. Due to their well-known anti-inflammatory properties, glucocorticoids have been widely used in a variety of clinical practice (Boumpas *et al*. 1993; Schmid *et al*. 1995; Rhen and Cidlowski, 2005; Vandevyver *et al*. 2013). In recent years, some reports suggested that the use of exogenous corticosteroids would cause the experimental animals to have more susceptibility to the pathogenic organisms, such as *Streptococcus pneumoniae*, *Plasmodium yoelii* and *Trichinella spiralis*. Meanwhile, studies have shown that glucocorticoids could regulate the activity of some of the fatty acid desaturase, involved in the conversion of fatty acid composition (Marra *et al*. 1988; Vrinten *et al*. 2007). Fatty acids are not only important components of biological membranes, but also involved in the essential process of signal transmission for cell growth and development (Coppens, 2013). So, the fatty acid compositions of the cell membrane are crucial for cells survival and normal growth (Shamseddin *et al*. 2015).

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As the most common intracellular parasites, *Toxoplasma gondii* often caused human and mammalian diseases (Baker and Antonovics, 2012). At present, the global toxoplasma infection has been more than one billion. Although toxoplasmosis is an opportunistic infectious disease, and a majority of human infections are asymptomatic, toxoplasmosis is life-threatening in immunocompromised patients with cancer, AIDS and organ transplant (Brindle *et al*. 1991; Parmley *et al*. 2002; Welti *et al*. 2007). In recent years, it is reported that patients who use dexamethasone (DEX) and other glucocorticoids for a long-term would have more chances to suffer from toxoplasmosis than those who did not use glucocorticoids (Morhun *et al*. 1996; Chandrasekar and Momin, 1997; Flaster *et al*. 2007; Wang *et al*. 2014). Wang *et al*. (2014) found that DEX could promote the proliferation of *T. gondii* in macrophages. However, the molecular mechanism between using glucocorticoids and severe toxoplasmosis is not well evident, so it is an urgent task to explore the pathogenesis of toxoplasmosis induced by glucocorticoids, and to promote a more efficacious and secure treatment for toxoplasmosis.

Fish oil is rich in eicosapentaenoic acid (EPA, C20:5*n*3), docosahexaenoic acid (DHA, C22:6*n*3) and other polyunsaturated fatty acids (PUFA), which could serve as an important food supplement

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and is beneficial to human health (Corporeau *et al*. 2006; Yang *et al*. 2011). Considering the effects of fish oil on metabolic pathways and lipid gene expression, adding fish oil appropriately may change the adverse effects of glucocorticoids, and may even prevent acute Toxoplasma infection.

In the present study, the aims were to understand the relationship between DEX and the proliferation of *T. gondii*, to explore the changes of fatty acid accumulation and composition in DEX-induced host cells and *T. gondii*, and to study the effects of supplementation of fish oil.

METHODS AND MATERIAL

Material and reagents

Special pathogen-free Kunming mice weighing from 18 to 20 g were purchased from the animal centre of Chongqing Medical University, China. Mice macrophage RAW-264 cell line was purchased from the Chinese Academy of Sciences, Shanghai, China. The *T. gondii* RH strain was provided by veterinary research institute, Lanzhou. Fatty acid methyl ester mixture, Percoll and dexamethasone were purchased from Sigma-Aldrich. Organic solvents and other chemicals of analytical grade were from Sangon Biotech, Shanghai, China. The reagents for analysis were GC-analytical grade. All medium components were purchased from Life Technologies (hyclone).

Parasite cultures

The RAW-264 cells were cultured on into 6-well plates (10^5 cells well^{-1}) beforehand in complete medium (RPMI-1640 medium + 3% fetal bovine serum, FBS) for 24 h at 37 °C and 5% CO_2. The RAW-264 cells were washed with medium and infected with tachyzoites (host cell: tachyzoites, 1: 10) in the complete medium. After 4 h, cells were washed again with the medium and incubated in the medium containing 0·1 μg mL^{-1} DEX. Cells were observed with an inverted fluorescence microscope after incubation. After incubation for 24, 48, 60 and 72 h, the number of tachyzoites per 100 cells was tested by Giemsa staining. At the same time, methyl thiazolyl tetrazolium (MTT, Beyotime Biotechnology, Cat. No. C0009) colorimetric assay distinguishes dead cells from live ones according to the method of Shamseddin *et al*. (2015). After 3 days of infection, scraping the monolayer cells, the cells were washed with sterile phosphate-buffered saline (PBS). The cell suspension was then passed through a 3·0 micron filter, centrifuging at 700 ***g*** for 10 min. Then the supernatant was excluded, and tachyzoites of *T. gondii* were harvested. The tachyzoites and host cells without DEX were used as the control group (CTR) (each treatment was on the same day and repeated three times).

Determination of phospholipid fatty acid compositions

After 72 h, RAW-264 cells (3×10^7 cells) and tachyzoites (3×10^7 cells) were collected for lipid extraction. The samples were detached using Percoll, and washed with phosphate buffer thoroughly, and collected by centrifugation (8 min at 10 000 ***g***). Phospholipids extraction and membrane fatty acid methyl esters (FAME) were prepared according to the method of Suutari *et al*. (1990). The upper part of that phospholipid extraction was aspirated for the further GC analysis. *In vivo* experiments, plasma samples (2 mL) were collected from dead mice or mice that were euthanazied (by exposure to ether following decapitation) with 10 days period. Plasma samples were collected in saline washed tubes containing heparin as anticoagulant. Fatty acids were measured by Suutari's method as described above. The FAME were subsequently analyzed by GC (GC-2010, Shimadzu, Kyoto, Japan). A fused capillary column, Supelco Nukol 30 m × 0·25 mm, was used, at a column temperature of 165 °C, an injection temperature of 250 °C, and a detector temperature of 250 °C. The chromatography peak was compared with that of the standards (Sigma) for fatty acid identification. Each fatty acid composition in the Tables 1, 2 and 4–6 is percentages of the total fatty acid content. All results are shown as an average of three independent experiments.

Delta-9 desaturase mRNA expression

Tachyzoites were harvested by filtration and total RNA was extracted from $2{\cdot}5 \times 10^8$ tachyzoite based on guanidinium thiocyanate according to the method of Chomczynski and Sacchi (1987). Total RNA was reverse transcribed to cDNA using the GeneRacer kit from Invitrogen (USA) and the cDNA (1 μL) was used as template for amplification of Delta-9 desaturase, and glyceraldehyde phosphate dehydrogenase (GAPDH) gene (used as internal standard) by reverse transcription -PCR (RT-PCR) with the following primers: Delta-9 desaturase, 5′-ACTT GCGGCGCTCATCGCTTGTG-3′ and 5′- GT CATGCACCAGCGAAGAGCTC-3′, producing a fragment of 465 bp; GADPH, 5′-AATGCKTCCT GYACCACCAACTGC-3′ and 5′-TTAGCCAWA TTCRTT GTCRTACCAGG-3′, producing an amplified fragment at 512 bp. PCR was performed on a thermal cycler with a predenaturation of 3 min at 95 °C, followed by 30 cycles of amplification (1 min at 95 °C , 1 min at 56 °C and 1 min at 70 °C) and a final extension at 72 °C for 8 min. All PCR products were detected by agarose gel electrophoresis.

Animal treatment

In this study, we used a total of 24 male mice, weighing from 18 to 20 g. All experiments were conducted

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Table 1. Effect of dexamethasone on *T. gondii* membranes fatty acid compositions

Fatty acid	T (%)	T + D (%)
c16:0	22·987 ± 0·002	18·381 ± 0·001**
c18:0	2·527 ± 0·003	2·451 ± 0·002
c18:1	2·402 ± 0·001	4·335 ± 0·001**
c18:2	0·789 ± 0·002	0·777 ± 0·001
c18:3	1·486 ± 0·003	0·757 ± 0·001*
c20:0	1·339 ± 0·001	1·009 ± 0·004*
c20:1	8·613 ± 0·003	7·742 ± 0·001*
c20:2	2·136 ± 0·002	2·161 ± 0·001
c20:4	2·545 ± 0·001	2·384 ± 0·002
c22:0	1·241 ± 0·001	1·377 ± 0·002

Note: Compared with *T. gondii* group, *$P < 0·05$, **$P < 0·01$.
T, *T. gondii*.
T + D, treated with *T. gondii* + dexamethasone.
Each fatty acid composition is percentages of the total fatty acid content (Each treatment was repeated three times).

Table 2. Effect of dexamethasone on RAW264 cells membranes fatty acid compositions

	Infected cells (%)	Infected cells + DEX (%)
c14:0	8·445 ± 0·001	10·301 ± 0·002**
c16:0	7·281 ± 0·001	6·549 ± 0·001*
c18:0	8·168 ± 0·002	9·519 ± 0·001*
c18:1	5·099 ± 0·001	8·628 ± 0·001**
c18:2	1·407 ± 0·001	2·326 ± 0·003*
c18:3	1·165 ± 0·002	0·950 ± 0·001
c20:0	1·340 ± 0·002	0·950 ± 0·001*
c20:1	1·719 ± 0·001	1·345 ± 0·002*
c20:2	2·234 ± 0·002*	2·323 ± 0·001
c20:3	0·121 ± 0·001	0·311 ± 0·003*
c20:4	ND	0·848 ± 0·002*
c22:0	6·600 ± 0·002	ND
c22:1	3·090 ± 0·002	2·561 ± 0·001*
c22:2	3·403 ± 0·002	3·549 ± 0·001
c22:6	ND	8·555 ± 0·001**
SFA/PUFA	3·821	1·448*

Note: Compared with infected cells group, *$P < 0·05$, **$P < 0·01$.
SFA/PUFA, saturated fatty acids/polyunsaturated fatty acids.
ND, not detected.
Each fatty acid composition is percentages of the total fatty acid content (Each treatment was repeated three times).

according to the China Laboratory Animal Welfare Ethics Committee guidelines on care, handling and use of laboratory animals and approved by the local ethics committee. The mice were distributed among four groups and housed in four cages (6 animals each): CTR, treated with mineral oil, saline and *T. gondii*; DEX groups, treated with mineral oil, *T. gondii* and dexamethasone; DFT groups, treated with fish oil, *T. gondii* and dexamethasone; and FT groups, treated with fish oil, saline and *T. gondii*. The mice received daily intraperitoneal injections of DEX [1 mg kg^{-1} Body weight (BW) using1 mg mL^{-1} DEX solution] or 0·85% saline (1 mL kg^{-1} BW). Fish oil (1 g kg^{-1} BW) and mineral oils (1 g kg^{-1} BW) were administrated orally by gavage, for four consecutive days. On the 5th day, the mice were intraperitoneally inoculated with 1×10^6 tachyzoites of *T. gondii*. Saline and mineral oil were used as a control for DEX and fish oil, respectively. The dose of DEX was based on the study by Wu *et al.* (2010). The dose of fish oil was based according to the methods of Ferreira *et al.* (2013). BW, food intake and water intake were monitored daily until the day of euthanasia. On the 10th day, euthanasia was performed and blood and tissue samples were collected for further analysis.

Statistical analysis

All results were analysed using GraphPad Prism software 6.0. To determine statistically significant differences ($P < 0·05$ or $P < 0·01$) between control and treatment groups, the results which were analysed with *t* test should be used to compare two groups.

RESULTS

Effect of DEX on T. gondii *tachyzoite replication*

The effects of DEX on the proliferation of tachyzoites are shown in Fig. 1. There was an evident difference in the observation between the DEX -treated and non- DEX-treated group at 24, 48, 60

Table 3. Changes of body weight (g) and mice survival curve with treatment

Variable	Day	CTR	DEX	DFT	FT
Body weight (BW g^{-1})	4	17·2 ± 0·3	15·2 ± 0·3*	17·4 ± 0·4	17·4 ± 0·5
	7	15·4 ± 0·1	14·6 ± 0·2*	16·5 ± 0·3*	16·6 ± 0·3*
Time to appearance of disease (averages day)		7·5	6·1*	0	0
Survival time (averages day)		8·1	6·3*	>10**	>10**

Note: compared with control group, *$P < 0·05$, **$P < 0·01$.
(Each treatment was repeated three times).
CTR, Control groups, treated with mineral oil, saline and *T. gondii*.
DEX, DEX groups, treated with mineral oil, *T. gondii* and dexamethasone.
DFT, DFT groups, treated with fish oil, *T. gondii* and dexamethasone.
FT, FT groups, treated with fish oil, saline and *T. gondii*.

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Table 4. Effect of DEX on fish oil treated mice plasma fatty acid compositions

Fatty acid	FT (%)	DFT (%)
c16:0	14·689 ± 0·002	8·107 ± 0·004**
c18:0	10·314 ± 0·002	5·229 ± 0·001**
c18:1	7·603 ± 0·001	4·513 ± 0·003**
c18:2	10·532 ± 0·002	5·210 ± 0·004**
c18:3	4·213 ± 0·002	5·023 ± 0·003*
c20:0	2·046 ± 0·004	3·033 ± 0·003*
c20:3	4·512 ± 0·002	1·295 ± 0·001**
c20:4	2·633 ± 0·002	3·073 ± 0·004*
c22:6	2·046 ± 0·001	15·455 ± 0·003**
PUFA/SFA	0·88	3·69**
*n*6/*n*3	1·22	0·38**

Note: Compared with FT group, *$P < 0·05$, **$P < 0·01$.
FT, FT groups, treated with fish oil, saline and *T. gondii*.
DFT, DFT groups, treated with fish oil, *T. gondii* and dexamethasone.
PUFA/SFA, polyunsaturated fatty acids/aturated fatty acids.
*n*6/*n*3, omega-6/omega-3 polyunsaturated fatty acids.
Each fatty acid composition is percentages of the total fatty acid content (Each treatment was repeated three times).

Table 5. Effect of fish oil on mice plasma fatty acid compositions

Fatty acid	CTR (%)	FT (%)
c16:0	19·628 ± 0·002	14·689 ± 0·001**
c18:0	16·910 ± 0·001	10·314 ± 0·003**
c18:1	5·326 ± 0·003	7·603 ± 0·002**
c18:2	20·369 ± 0·002	10·532 ± 0·002**
c18:3	12·643 ± 0·001	4·213 ± 0·004**
c20:0	1·739 ± 0·004	2·046 ± 0·002*
c20:3	6·962 ± 0·002	4·512 ± 0·001*
c20:4	2·220 ± 0·003	2·633 ± 0·03*
c22:6	2·250 ± 0·001	2·046 ± 0·03
PUFA/SFA	1·47	0·88*
*n*6/*n*3	1·03	1·22*

Note: Compared with control group, *$P < 0·05$, **$P < 0·01$.
CTR, Control groups, treated with mineral oil, saline and *T. gondii*.
FT, FT groups, treated with fish oil, saline and *T. gondii*.
PUFA/SFA, polyunsaturated fatty acids/saturated fatty acids.
*n*6/*n*3, omega-6/omega-3 polyunsaturated fatty acids
Each fatty acid composition is percentages of the total fatty acid content (Each treatment was repeated three times).

and 72 h after infections ($P < 0·01$). At the same time, we saw the replication of tachyzoites in per 100 cells reached 566 after 72 h of treatment with DEX, and more tachyzoites in host cells treated with DEX were observed compared with the CTR (Fig. 2).

At the same time, as is shown in Fig. 3, the number of per 100 μL medium extracellular free tachyzoites also increased significantly in the experimental group. Statistically significant differences

Table 6. Effect of fish oil on DEX treated mice plasma fatty acid compositions

Fatty acid	DEX (%)	DFT (%)
c16:0	18·854 ± 0·003	8·107 ± 0·004**
c18:0	11·499 ± 0·002	5·229 ± 0·002**
c18:1	14·630 ± 0·003	4·513 ± 0·001**
c18:2	15·375 ± 0·001	5·210 ± 0·003**
c18:3	5·784 ± 0·002	5·023 ± 0·004*
c20:0	2·095 ± 0·002	3·033 ± 0·001*
c20:3	5·431 ± 0·002	1·295 ± 0·004**
c20:4	2·627 ± 0·001	3·073 ± 0·003*
c22:6	2·622 ± 0·002	15·455 ± 0·001**
PUFA/SFA	1·21	3·69**
*n*6/*n*3	1·3	0·38**

Note: Compared with DEX group, *$P < 0·05$, **$P < 0·01$.
DEX, DEX groups, treated with mineral oil, *T. gondii* and dexamethasone.
DFT, DFT groups, treated with fish oil, *T. gondii* and dexamethasone.
PUFA/SFA, polyunsaturated fatty acids/saturated fatty acids.
*n*6/*n*3, omega-6/omega-3 polyunsaturated fatty acids.
Each fatty acid composition is percentages of the total fatty acid content (Each treatment was repeated three times).

were observed between the experimental group and the CTR ($P < 0·01$). These results indicate that DEX has an enhancing effect on the growth rate of *T. gondii*.

Effect of DEX on fatty acid metabolism of T. gondii *tachyzoite*

The fatty acid composition of cells membrane of tachyzoites was analysed by GC, and the results are shown in Table 1. There were no new fatty acid peaks appearing in experimental group *T. gondii* compared with the CTR. However, the significant difference in the level of fatty acids was observed between the experimental and the control subjects. It can be seen from the table that the fatty acids C18:1 showed an increase of 80·5%, and fatty acids C16:0, C18:3 and C20:0 showed a decrease of 20·1, 49·1 and 24·6% respectively, in *T. gondii* tachyzoite after being cultured for 72 h in the medium containing DEX. This means that dexamethasone can change the fatty acid metabolism of *T. gondii* in the experimental group (Table 1).

The mRNA expression levels of Delta-9 desaturase in the T. gondii *treated with DEX*

At the same time, we tested the expression of the Delta-9 desaturase gene in tachyzoite cells. In the experimental group, the expression of Delta-9 desaturase gene increased significantly. Figure 4 shows the analysis by RT–PCR of Delta-9 desaturase gene mRNA levels of the *T. gondii* treated with DEX. The size of RT–PCR products was estimated

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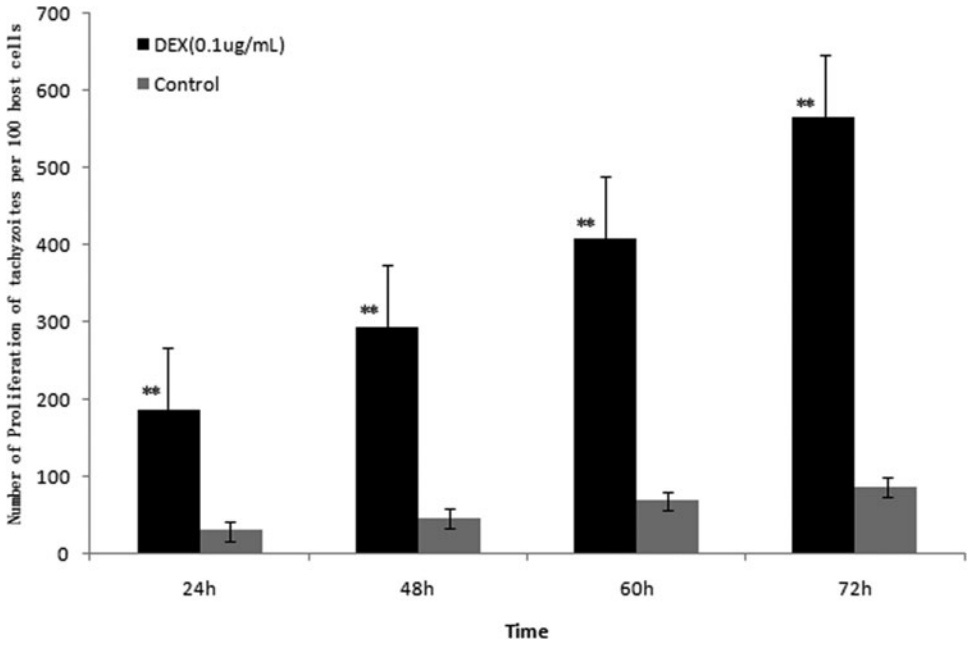

Fig. 1. Proliferation of tachyzoites per 100 host cells in cultures treated with DEX. After incubation for 24, 48, 60 and 72 h, the number of tachyzoites per 100 cells was tested by Giemsa staining and MTT colorimetric assay. Note: Compared with control group, $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

at the correct size of 465 bp. Band intensities of Delta-9 desaturase gene showed higher levels of expression in the *T. gondii* treated with DEX (Fig. 4).

Effect of DEX on fatty acid metabolism of RAW-264 cells

GC analysis of the membrane fatty acids of RAW-264 cells was performed to investigate the effects of DEX on cellular membranes. GC analysis of FAME showed that a high fatty acid peak corresponding to standard docosahexaenoic acid (C22:6*n*3), and arachidonic acid (C20:4*n*6) methyl ester were detected in DEX-treated RAW-264 cells, but were not detected in the CTR RAW-264 cells (Table 2). Table 2 showed the fatty acids C14:0, C18:1 showed an increase of 21·9, 69·2%, and fatty acids C20:0, C20:1 and C22:1 showed a decrease of 29·1, 21·8 and 17·1% respectively, in RAW-264 cell lines after being cultured for 72 h in the medium containing DEX. More importantly, the ratio of saturated fatty acids (SFA)/PUFA of the cell membrane decreased from 3·82 in the control cells to about 1·45 in DEX-treated cells, compared with the CTR statistically significant differences ($P < 0{\cdot}05$). Changes in fatty acid composition were probably consequences of membrane permeabilization due to the perturbing action of DEX.

Treatment with dexamethasone decreases mice BW

The initial BW of all experimental group mice was similar, but the weight was gradually decreased until euthanasia, and there was a significant difference in BW of the DEX and CTR groups. DEX and DFT, FT group also had significant differences in BW. The mice from both DEX and CTR groups died after being infected with *T. gondii*. Mice from DEX group died 4 days sooner than the CTR group. However, all mice from the DFT and FT groups survived the infection (Table 3).

Effect of DEX on mice plasma fatty acids metabolism

Figure 5 showed the fatty acids C18:1, C20:0 showed an increase of 174·6, 20·4% and fatty acids C18:0, C18:2 and C18:3 showed a decrease of 31·9, 24·5 and 54·3% respectively, in the mice plasma after being treated in the DEX. Compared with cells treated with DEX, the fatty acid composition of the two treatments was similar, and the amount of oleic acid and arachidonic acid increased. Table 4 showed that despite fish oil supplementation DEX treatment was still able to induce C20:0 to increase by 48·2%, and C18:0 and C18:2 decrease by 49·3 and 50·5%, respectively (Table 4). As can be seen from the data Table 4, and Fig. 5 the addition of DEX resulted into an increase over the amounts of arachidonic acid (AA, C20:4*n*6) and DHA (C22:6*n*3) in the mice.

Effect of fish oil on mice plasma fatty acids metabolism

Table 5 showed the plasma fatty acids C18:1, C20:0 showed an increase of 42·8, 17·7%, and fatty acids C16:0, C18:0, C18:2 and C18:3 showed a decrease of 25·2, 39, 48·3 and 66·7%, respectively, in the

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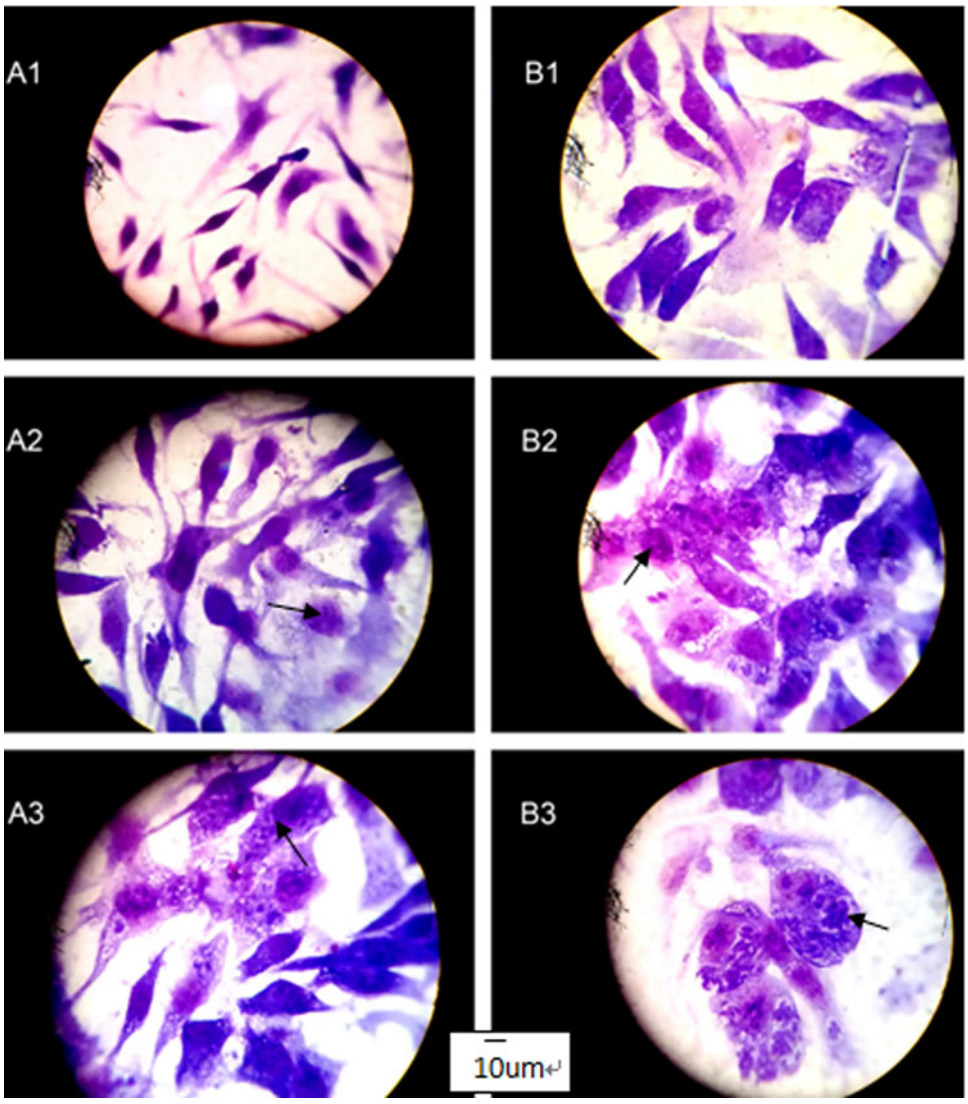

Fig. 2. Effect of DEX on intracellular replication of *T. gondii* RH strain tachyzoites. A1, A2, A3 were not treat with DEX. B1, B2, B3 were the RAW-264 cell infected *T. gondii* RH strain tachyzoites treat with DEX (0·1 μg mL^{-1}) for 48, 60 and 72 h. The more tachyzoites in host cells treated with DEX was observed compared with the control group. The arrows are pointing to *T. gondii* RH strain tachyzoites. (Giemsa staining)

plasma of blood obtained from FT mice. Mice plasma fatty acids treated with DEX and supplementation with fish oil are presented in Table 6, which shows the plasma fatty acids C20:0 showed an increase of 44·8, 20·4%,and fatty acids C16:0, C18:0, C18:1 and C18:2 showed a decrease of 57, 54·5, 69·2 and 66·1% respectively, in plasma of blood obtained from the DFT mice. Compared with cells treated with DEX, the amount of DHA increased evidently after DFT treatment.

DISCUSSION

Patients with immunocompromised and immunosuppressive therapy can be extremely easy to suffer from reactivation of *T. gondii* infection. So, exploring the reasons for the proliferation of *T. gondii* in the body contributes towards the development of new drugs. Stress can suppress immune function, and leads to the outbreak of some latent infectious diseases. Therefore, we hypothesized that stress hormone may induce reactivation of Toxoplasma chronic infection, causing acute toxoplasmosis. Glucocorticoids are important stress hormone, which can regulate the immunity and anti-inflammatory. So, glucocorticoids are often used by patients with severe inflammatory diseases (Cheng *et al*. 2014). However, it is reported that glucocorticoids in patients can lead to reactivation and development of *T. gondii* (Chandrasekar and Momin, 1997). Wang *et al*. (2014) confirmed that the growth rate of *T. gondii* RH strain significantly increased in the peritoneal macrophages of rats treated with glucocorticoids *in vivo*. However, little is known about the mechanism of the reactivation of *T. gondii* with glucocorticoids act.

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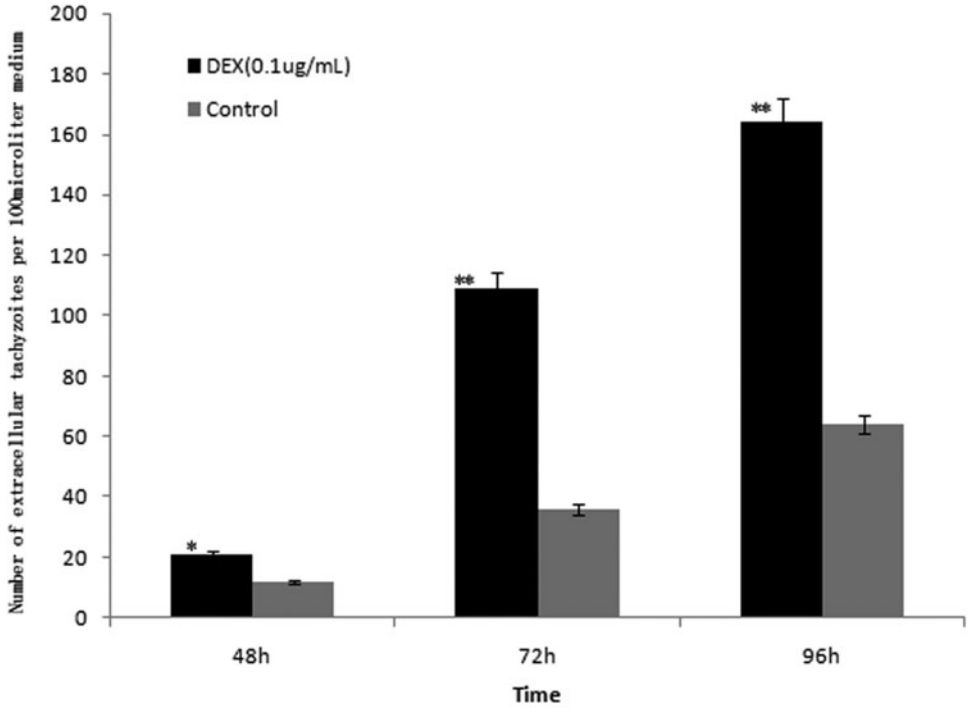

Fig. 3. Number of extracellular tachyzoites per 100 μL medium in cultures treated with DEX. Note: Compared with control group, $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

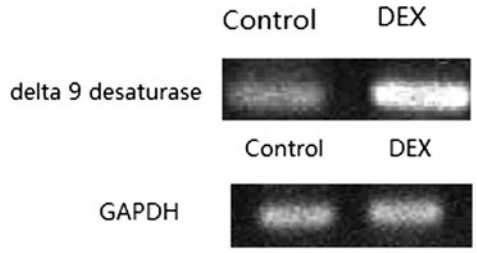

Fig. 4. RT-PCR analysis for the expression of Delta-9 desaturase mRNA.

In the present study, we adopted DEX to treat the cells and tachyzoites. Dye assay and MTT results showed that tachyzoites in the experimental group proliferated more quickly than the CTR, the number increasing five times of tachyzoites of the experiment after the 24 h. We also found that metabolic changes of fatty acids were observed in DEX treated tachyzoites, with a general increase in the amount of fatty acids in tachyzoites. At the same time, increase in the ratio of PUFA)/SFA in host cells was observed, especially the amount of oleic acid increased from 5·099 to 8·628. The result revealed that DEX could induce membrane PUFA of host cells and tachyzoites to change significantly. Hence, the main finding from this study is that DEX treatment can promote the proliferation of *T. gondii*, mainly probably because DEX can affect the metabolism of parasite and host fatty acid. On one hand, upregulated expression of desaturase gene of parasite could provide the membrane fatty acids for proliferation of parasites. On the other hand, regulation of host metabolism could provide the fatty acids for proliferation of parasite. When DEX was added, oleic acid, AA and DHA were significantly increased in both cells and animals. With the addition of fish oil, the amount of oleic acid decreased. This indicates that increased oleic acid plays an important role in the proliferation of *T. gondii*, and that the synthetic oleic acid plays an important role in the metabolic balance within the organism. Another notable finding is that supplementation of fish oil can alter the fatty acid metabolism of the host and parasite and plays an important role in reducing infection. When supplementing fish oil, we find the amount of oleic acid increased, and the amount of linoleic acid and linolenic acid decreased with respect to the control and DEX groups. This indicates that fish oil can lead to significant changes in the ratio of omega-6/omega-3 PUFA (*n*6/*n*3) in plasma fatty acids and again shows that increased oleic acid levels may have an important biological role in preventing infection. Of course, the role of oleic acid in intracellular anti-infection or induced infection needs to be studied and proved in the further studies. This information is liable to be useful in future studies and could come true by regulating fatty acid metabolism of the corresponding infection.

Many parasitic protozoa, including trypanosomes and toxoplasma have a high proportion of unsaturated fatty acids (Roberts *et al*. 2003; Ramakrishnan *et al*. 2012). The unsaturated fatty acid is not only the structural components of the membrane, but also takes part in the energy metabolism (Ramakrishnan

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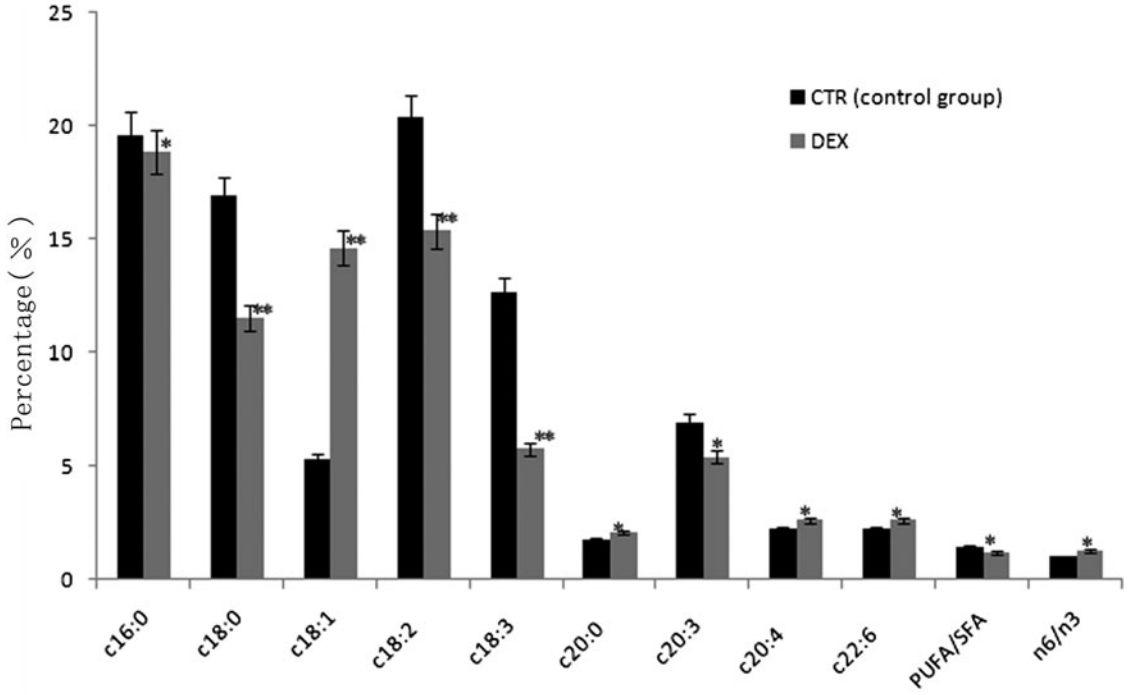

Fig. 5. Effect of dexamethasone on mice plasma fatty acid compositions. CTR, control groups, treated with mineral oil, saline and *T. gondii*. DEX. DEX groups, treated with mineral oil, *T. gondii* and dexamethasone. PUFA/SFA, polyunsaturated fatty acids/saturated fatty acids. *n*6/*n*3: omega-6/omega-3 polyunsaturated fatty acids. Note: Compared with control group, $*P < 0{\cdot}05$, $**P < 0{\cdot}01$.

et al. 2013). *Toxoplasma gondii* is adapted to thriving in the parasitophorous vacuole, within the cytoplasm of mammalian cells. Successful replication of *T. gondii* requires considerable amounts of lipids for membrane biogenesis. Therefore, proliferation rates of tachyzoites depend upon the rate of synthesis of fatty acids and new membranes (Formaglio *et al*. 2014; Ramakrishnan *et al*. 2015). At the same time, the proliferation of *T. gondii* tachyzoites also depends upon the interaction between the host and parasite fatty acid metabolism. Of course, the results of fatty acid metabolism interaction will also cause the host to increase inflammatory cytokine and calcium release from neutrophils, and induce parasite egress from the infected host cells (Mulvihill and Nomura, 2013; Arlia *et al*. 2016).

To sum up, change in fatty acid metabolism may be a key factor that induces the proliferation of *T. gondii* tachyzoites in cells. Therefore, we recommend appropriate supplementation of the corresponding fatty acids in immunosuppressive and immunocompromised patients to inhibit toxoplasma chronic infection. In the future, we will hope we can strengthen the role of individual fatty acids in infection research, and develop related fatty acid control drugs or fatty acid immune products.

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