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Toxoplasma gondii infection in the peritoneal macrophages of rats treated with glucocorticoids

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32 **Abstract** It is well known that toxoplasmosis can be life-threatening to
33 immunocompromised individuals such as AIDS and organ transplantation patients.
34 Glucocorticoids (GCs) are widely used in the clinic for the treatment of autoimmune
35 diseases and organ transplantation resulting in acute toxoplasmosis in these patients.
36 However, the interaction and mechanism between the development of acute
37 toxoplasmosis and GC therapy are still unknown. The aims of this study were to
38 investigate the infection of *T. gondii* in the peritoneal macrophages of rats treated with
39 glucocorticoids. Our results showed that the growth rate of *T. gondii* RH strain was
40 significantly increased in the peritoneal macrophages of rats treated with
41 glucocorticoids *in vivo*. For instance, 242 (±16) tachyzoites were found in 100
42 macrophages from the rats treated with methylprednisolone (MP), while only 16 (±4)
43 tachyzoites were counted in the macrophages from the non-treated control rats 24
44 hrs after infection ($P<0.01$). We also demonstrated that a significant inhibition of
45 nitric oxide (NO) production was detected in the macrophages collected from the rats
46 post-treated with GCs with 12.90 μM (±0.99 μM) of nitrite production from the rats
47 treated with MP, while 30.85 μM (±1.62μM) was found in the non-treated control rats
48 36 hrs after incubation ($P<0.01$). Furthermore, glucocorticoids could significantly
49 inhibit the expression of inducible nitric oxide synthase mRNA and its protein in the
50 rat peritoneal macrophages. Our results strongly indicate that the decrease of NO in
51 the rat peritoneal macrophages is closely linked to the cause of acute toxoplasmosis in
52 the host. Additionally, there was a significant increase in the number of cysts
53 produced by the naturally cyst forming, *T. gondii* Prugniaud strain with an average of

54 2795 (±422) cysts of the parasite being detected in the brains of the rats treated with

55 dexamethasone, while only 1356 (±490) cysts were found in the non-treated control

56 animals (P<0.01). As rats and humans are both naturally resistant to *T. gondii*

57 infection, these novel data could lead to a better understanding of the development of

58 acute toxoplasmosis during glucocorticoid therapy in humans.

59

60 **Introduction**

61 As one of the most severe opportunistic infectious diseases, toxoplasmosis caused by

62 *Toxoplasma gondii* is life-threatening to immunocompromised patients with AIDS,

63 cancer and those who are under immunosuppressant treatment following

64 transplantation. A high mortality rate has been documented in immunocompromised

65 patients due to severe toxoplasmosis (caused by the re-activation of chronic infection)

66 (Montoya and Liesenfeld, 2004). However, the association between

67 immunosuppression using glucocorticoids and the occurrence of severe toxoplasmosis

68 is not well understood (Chandrasekar and Momin, 2003; De-Medeiros et al. 2001).

69 Glucocorticoids (GCs) are important modulators of immune cell functions

70 (Sternberg, 2006). Due to their well-known immunosuppressive and

71 anti-inflammatory properties, synthetic GCs are widely used in clinical practice and

72 are effective in the treatment of autoimmune disease (Sathasivam, 2008),

73 immunological rejection (Everson *et al*. 2003), atopic inflammatory disease

74 (Tarpataki , 2006) and acute critical illness (Minneci et al. 2004; Rady et al. 2006).

75 It has also been well documented that GCs can mediate immune responses by

76 affecting viability and function of various important immune cells including
77 macrophages, B cells, monocytes and granulocytes (Chen et al. 2010; Tsianakas et al.
78 2012). Amongst these cells, macrophages are considered the most ubiquitous in
79 mammalian tissues and play a central role in both innate and acquired immune
80 responses.

81 Activated macrophages acquire competence for enhanced anti-microbial activity
82 including nitric oxide (NO) production (Cape and Hurst, 2009; Severin et al. 2010;
83 Silva, 2010). NO is recognized as a major regulatory molecule of the immune system
84 and a principal cytotoxic mediator of activated immune effector cells which plays a
85 critical role against pathogen infections (Adams et al. 1990; Li et al. 2012)

86 However, what is still not clear is the relationship between the effects of GCs on
87 the function of macrophages and the development of acute toxoplasmosis from
88 chronic infections. Infection of humans, with *T. gondii*, usually shows a chronic
89 profile which is similar to that found in rats (Sumyuen et al. 1996; Da-Silva et al.
90 2010; Dubey, 2011; Li et al. 2012). Therefore, the rat model has been suggested to be
91 an ideal model to study the development of acute toxoplasmosis from chronic
92 infection in humans. The aims of this study were to understand the effect of GCs on
93 the infection of rat peritoneal macrophages with the *T. gondii* RH strain and the effect
94 on cyst formation of the naturally cyst forming *T. gondii* Prugniaud strain in the brains
95 of rats treated with GCs. Results from this work will provide useful data to better
96 understand human toxoplasmosis from the individuals who were treated with GCs.
97

98 **Materials and methods**

99 Animals and parasites

100 Special pathogen free Sprague Dawley (SD) rats weighing from 180 to 220 g were
101 used for the source of peritoneal macrophages and BALB/c mice used for the
102 maintenance of the *T. gondii* RH were purchased from the experimental animal center
103 of Guangdong province, China. Special pathogen free F344 rats were purchased from
104 Vital River Laboratories (Beijing, China). BALB/c mice were intraperitoneally (i.p.)
105 inoculated with 1×10^6 tachyzoites of *T. gondii* RH-GFP (Nishikawa et al. 2003).
106 Tachyzoites were harvested and isolated from the infected animals and were
107 resuspended in RPMI-1640 medium supplemented with 10% FBS. *T. gondii*
108 Prugniaud strain was maintained in NIH mice. Cysts were isolated from the brains of
109 infected animal when they were required. Protocols for the use of animals were
110 approved by the Institutional Review Board of Animal Care of Sun Yat-Sen
111 University (973 project, #2010CB530000).

112

113 Peritoneal macrophage isolation and cultivation

114 Methods for isolation of macrophages from GC treated and non-treated rats were used
115 as previously described (Li et al. 2012; El-Mahmoudy et al. 2002). The pelleted
116 peritoneal macrophages were resuspended in RPMI-1640 medium containing 10%
117 fetal bovine serum (FBS) and penicillin (100 U/ml) after washing. Final
118 concentrations of 2×10^5 cells in 200 μl and of 5×10^6 cells in 2.5 ml were seeded
119 into each well in 96-and 6-well tissue culture plates (Corning, USA) respectively and

120 were incubated at 37°C, 5% CO_2, 95% air for 2 hrs. The macrophages were then
121 carefully washed three times with FBS free RPMI-1640 medium to remove the
122 non-adherent cells and incubated with the test compounds indicated in the results.
123
124 Animal treatments
125 For the experimental treatment with GCs, 8 rats in each of four groups were used in
126 this study. In the control group, each rat was intramuscularly (i.m.) injected with 0.2
127 ml saline solution, while in the other three groups, each animal was intramuscularly
128 injected with dexamethasone (DXM), hydrocortisone sodium succinate (HSS) and
129 methylprednisolone (MP) at 1.5 mg/kg/d, 20 mg/kg/d and 20 mg/kg/d respectively for
130 7 days (Da-Silva et al. 2010; Dimitriu et al. 2008).
131
132 *T. gondii* infection in macrophages collected from rats treated with GC
133 The macrophages harvested from the rats treated with GCs mentioned above, were
134 challenged with tachyzoites at a ratio of tachyzoite/macrophage of 1:1 at 12 hrs post
135 incubation. Extracellular tachyzoites were then washed out after 1 hr of contact with
136 macrophages and the time was then defined as 1 hr. The macrophages were
137 continuously cultured in RPMI-1640 contained FBS and penicillin. Macrophages
138 were observed under an inverted fluorescent microscope at 1, 12 and 24 hrs and the
139 numbers of cells infected with *T. gondii* as well as the number of parasites per 100
140 macrophages were counted. Controls were carried out using the macrophages
141 collected from the non-GC treated rats using an identical protocol.

142

143 Measurement of nitrite concentration

144 The NO production was evaluated indirectly by measuring nitrite concentration in

145 macrophage cultural supernatants as described previously (Green *et al*. 1982; Li et al.,

146 2012). Supernatant (150 μl) was collected from each well at intervals of cultivation

147 and mixed with an equal volume of Griess reagent on a 96-well flat-bottomed plate

148 (Corning, USA). Absorbance at 540/550 nm was measured in an ELISA reader.

149 Nitrite concentration was calculated based on a standard curve using sodium nitrite

150 diluted with the same medium used for the cultivation of macrophages.

151

152 RNA extraction and assessment of iNOS mRNA expression by reverse transcription

153 polymerase chain reaction (RT-PCR)

154 Total RNA from macrophages collected from rats treated or non-treated with GCs

155 were extracted using Trizol reagent (Invitrogen, Carlsbad, USA) according to the

156 manufacturer's instructions. Total RNA was reversed to cDNA using a set of oligo

157 (dT) primer and Super Script^{TM} III First-Strand Synthesis System (Invitrogen,

158 Carlsbad, USA) and the cDNA (1 μl) was used as template for amplification of iNOS

159 and GAPDH gene (used as internal standard) by PCR with the following primers:

160 iNOS, 5'-CTA CCT ACC TGG GGA ACA CCT GGG-3' and 5'-GGA GGA GCT

161 GAT GGA GTA GTA GCG G-3', producing a fragment of 442 bp; GADPH, 5'-AAT

162 GCK TCC TGY ACC ACC AAC TGC-3' and 5'-TTA GCC AWA TTC RTT GTC

163 RTA CCA GG-3, producing an amplified fragment at 513 bp. The PCR products were

164 analyzed by agarose gel electrophoresis.

165

166 Expression analysis of iNOS by western blotting

167 Cells were lysed in SDS loading buffer, fractionated in SDS-PAGE and transferred

168 onto the immunoblot polyvinylidene difluoride membrane (Pall, USA). The

169 membrane was probed using the rabbit polyclonal iNOS antibody (Thermo, USA)

170 (Santa Cruz, USA). β-tubulin was detected with anti-β-tubulin antibody (NOVUS,

171 USA) as a control. Horseradish peroxidase-labeled secondary antibodies (Cell

172 Signaling, USA) and a DAB (3,3’,5,5’-tetramethylbenzidine) Detection Kit (Tiangen,

173 China) were used for antibody detection.

174

175 Infection of *T. gondii* Prugniaud strain in F344 rats treated with or without GC

176 Twenty F344 rats were used in this experiment. Each animal was infected by feeding

177 300 cysts of the *T. gondii* Prugniaud strain. They were randomly divided into two

178 groups. Animals in Group I were treated with DXM (2 mg/kg/every two days), while

179 rats in Group II (control) were treated with PBS only. Animals in the groups were

180 treated with DXM or PBS for 4 weeks starting after infection. All animals were

181 sacrificed two months after infection and cysts were counted from the homogenized

182 brain tissue of each rat. The reason that DXM was chosen for this work was this

183 compound is more commonly used in the clinic than other compounds.

184

185 Statistical analysis

186 All data were obtained from the experiments repeated at least three times and were

187 shown as the mean ±S.D. Statistical analysis of the data for multiple comparisons was

188 performed by one-way ANOVA. P<0.05 was considered statistically significant and

189 P<0.01 was considered highly significant.

190

191 **Results**

192 Replication of *T. gondii* RH strain in the peritoneal macrophages of rats treated with

193 GCs

194 Table 1 shows the replication of *T. gondii* RH strains tachyzoites in the peritoneal

195 macrophages isolated from the rats injected intramuscularly with GCs

196 (dexamethasone, DXM; hydrocortisone sodium succinate, HSS and

197 methylprednisolone, MP). A difference in infection rate was not found across all 4

198 groups at 1 hr post infection. However, significant differences were observed between

199 the GC treated and non-GC treated group at 12 and 24 hrs post infection (P<0.01).

200 An example fluorescent micrograph demonstrating the presence of more

201 tachyzoites in the macrophages of rats treated with DMX than in those from the

202 non-treated control rats is shown in Figure 1B and 1D (at 1 and 24hrs). Similar results

203 were also observed in the macrophages collected from rats treated with HSS and MP

204 respectively (data not shown).

205

206 Nitrite production in the peritoneal macrophages of rats treated with GCs

207 The effect of GCs on NO production by peritoneal macrophages from rats was

208 determined by the measurement of nitrite concentration in cell culture supernatants as
209 a reliable marker for NO production. As shown in Table 2, by comparison to the
210 non-treated control, a significantly lower NO production was detected in the
211 peritoneal macrophages collected from the rats treated with GCs for 7 days ($P<0.01$).
212
213 Synergistic effects of *T. gondii* infection and GC treatment on NO production in rat
214 peritoneal macrophages
215 Table 3 shows the synergistic effect on the production of NO by rat peritoneal
216 macrophages when they were treated with GCs *in vivo* and infected with *T. gondii in*
217 *vitro*. Significantly less NO production was detected in the peritoneal macrophages of
218 rats treated with GCs and infected with *T. gondii* than that found in GC treatment or *T.*
219 *gondii* infection only ($P<0.01$). These results indicate that GC treatment and *T. gondii*
220 infection may have a synergistic effect on inhibiting NO production in the rat
221 peritoneal macrophages.
222
223 The mRNA expression and protein levels of iNOS in the peritoneal macrophages of
224 rats treated with GCs
225 Figure 2(A) shows the analysis of iNOS mRNA levels of the peritoneal macrophages
226 of rats treated with GCs by RT-PCR. The size of PCR products was estimated at the
227 correct size of 442 bp. Band intensities show lower levels of iNOS expression in the
228 peritoneal macrophages of rats treated with GCs. Figure 2(B) displays the analysis of
229 iNOS protein levels in the peritoneal macrophages of rats treated with GCs as

230 determined by Western blotting. Lower expression levels of iNOS were detected in
231 the peritoneal macrophages treated with GCs. Down-regulation of iNOS expression
232 by GC treatment was consistent with the reduction in nitrite level in the culture
233 supernatants from these macrophages.
234
235 Effects of DXM on cyst burden of the *T. gondii* Prugniaud strain in the brains of F344
236 rats
237 The Prugniaud strain of *T. gondii* is a type II strain and more readily forms cysts in
238 rodent brains than the type I RH strain. Figure 3 shows the number of cysts found in
239 the brains of F344 rats infected with the *T. gondii* Prugniaud strain and treated with
240 DXM. An average of 2795±422 cysts was found in the brain tissues from the rats
241 treated with DXM, while only 1356±490 cysts were detected from the control rats.
242 This result clearly indicated that DXM could significantly increase the number of
243 cysts in the brains of rats treated with this GC ($P<0.01$). Although obvious differences
244 in clinical signs were not observed between these two groups of rats, the significant
245 increase in cysts found in the brain of the rats treated with DXM clearly demonstrates
246 the effect of DXM on *T. gondii* cysts in F344 rats.
247
248 **Discussion**
249 Human infection with *T. gondii* can be obtained by ingestion of oocysts from
250 contaminated food and water, by eating undercooked or raw meat containing cysts
251 and by congenital transmission (Dubey, 2011). After the acute stage of disease

252 (tachyzoites), the parasite develops cysts (bradyzoites, latent stage) in a variety of
253 organs, but mainly in the brain and skeletal muscle cells, and establishes a chronic
254 infection (Lang et al. 2007). Immunosuppression found in AIDS, cancer and organ
255 transplantation patients can cause the reactivation of a latent infection resulting in
256 acute infection (Sibley, 1993; Skariah et al. 2010). Additionally, it has been suggested
257 that natural immunosuppression associated with pregnancy may also result in
258 reactivation of maternal infection followed by congenital transmission (Hide et al.
259 2009; Thomasson et al. 2011). However, little is known about the mechanism of the
260 reactivation of chronic infection of *T. gondii* within mammalian hosts although
261 weaker immunity has been suggested to be linked to such reactivation (Chandrasekar
262 and Momin, 2003; De-Medeiros et al. 2001).

263 It is reported that the peritoneal macrophages of rat are naturally resistant to *T.*
264 *gondii* infection (Dubey, 2011) and recent studies have demonstrated that the
265 mechanism of such resistance is strongly linked to higher expression of inducible
266 nitric oxide synthase (iNOS) in the rat peritoneal macrophage (Li et al. 2012).
267 Interestingly, however, lower expression of iNOS was found in alveolar macrophages
268 of rat and they were demonstrated to be more sensitive, than those from the peritoneal
269 cavity, to infection with *T. gondii* RH strain (Zhao et al. 2013). GCs are known to
270 regulate the immunological reactions, mediate many aspects of homeostasis and play
271 a critical role in the systemic stress response. The powerful anti-inflammatory and
272 immunosuppressive properties of GCs have also made them an extremely valuable
273 therapy in patients with severe inflammatory and autoimmune disorders (Everson et al.

274 2003). Therefore, we proposed that GCs might decrease NO production in rat
275 peritoneal macrophages since it has been shown that the long term use of GCs in
276 mammalian hosts (rodents and humans) can result in the development of acute
277 toxoplasmosis from chronic infection (Montoya and Liesenfeld, 2004; De-Medeiros et
278 al. 2011). Understanding these mechanisms will greatly improve our knowledge of the
279 reasons why and how long term use of GCs in mammalian hosts including humans,
280 can cause acute toxoplasmosis.

281 In this study, we have adopted three GC agents that are all widely used in the
282 clinic and each has a physiological half-life and pharmacodynamic action. Our results
283 further support previous studies (Li et al. 2012) that *T. gondii* cannot multiply
284 efficiently in the normal rat peritoneal macrophages, but surprisingly we found that it
285 could grow well in the peritoneal macrophages of rats treated with GCs. Furthermore,
286 our results showed that the growth of *T. gondii* in the rat peritoneal macrophages was
287 linked to the reduction of NO production in macrophages treated with GCs. These
288 results are consistent with the results by Li and colleagues (Li et al. 2012) that they
289 demonstrated that a lower concentration of NO in the rodent peritoneal macrophages
290 is strongly linked to their susceptibility to *T. gondii* infection.

291 Some studies demonstrated that *T. gondii* infection could decrease NO
292 production in the peritoneal macrophages (Nishikawa et al. 2007) and it was
293 suggested as one of the reasons for the development of acute toxoplasmosis from
294 chronic infection. Our results also showed that *T. gondii* infection could partially
295 decrease NO production in the infected cells which was suggested as one of the

296 pathways used by the parasite to enable to escape the immune reaction in the host
297 (Seabra et al. 2002). However, we consider that it may not be the key reason for the
298 development of acute toxoplasmosis from chronic infection. Our data showed that the
299 synergistic interaction between *Toxoplasma* infection and GC treatment may be an
300 important driver for this transition. Based on the above analysis, it is reasonable to
301 propose that the down-regulation of NO could be a shared and generic mechanism
302 that could explain why macrophage resistance to *T. gondii* infection or control of
303 *Toxoplasma* activation is impaired during GC treatment.

304 Furthermore, we demonstrated that GCs could inhibit the expression of iNOS
305 mRNA and protein in rat peritoneal macrophages by RT-PCR and western-blotting
306 analysis. These data are supported by the results from previous studies which
307 demonstrated that DXM could inhibit the expression of iNOS in human hepatocytes
308 and alveolar macrophages (Geller et al. 1993; Xiang et al. 2000). Because of the
309 tremendous differences in iNOS activity between the peritoneal macrophages from
310 mice and rats (Li et al. 2012), the bias towards studies in mouse models needs to be
311 augmented with further studies on rats.

312 A key question that needs to be addressed is: what is the mechanism of NO
313 action that drives the transition from acute toxoplasmosis to the chronic infection? NO
314 is beneficial in minimizing pathogenesis in the host during chronic toxoplasmosis
315 (Hayashi et al.1996; Scharton-Kersten et al. 1997). In the absence of NO, chronically
316 infected rats and mice could develop lethal disease with increased *T. gondii* burden
317 and severe inflammation in the places where the parasites were located (Bohne et al.

318 1994; Scharton-Kersten et al. 1997). Some studies have shown that NO may serve as

319 a molecular trigger for stage conversion of *T. gondii* tachyzoites to bradyzoites

320 leading to a state of chronic infection in the host (Bohne et al. 1994). In most cases, *T.*

321 *gondii* causes asymptomatic infection in healthy individuals. However, GCs as

322 immunosuppressive agents could change the immune status of the host resulting in the

323 development of acute toxoplasmosis from a chronic infection (Djurkovic-Djakvoic

324 and Milenkovic, 2001). Our work demonstrated that GCs significantly reduced the

325 synthesis of NO in rat peritoneal macrophages which is similar to the effect of these

326 compounds on the hepatocytes of rats (Geller et al. 1993) and alveolar macrophages

327 of humans (Xiang et al. 2000). Furthermore, these studies provide novel data which

328 could enable a better understanding of the generation of acute toxoplasmosis.

329 Rats have long been considered the species of choice as a model for human

330 toxoplasmosis on account of the similarity of their resistance to *T. gondii* infection

331 with that found in humans (Derouin et al. 1995; Freyre et al. 2003; Dubey, 2011),

332 although much less work has been done than in the mouse model. It has been

333 demonstrated that activated rat peritoneal macrophages can markedly inhibit the

334 multiplication of *T. gondii in vitro* (Zhao et al. 2009). Cellular immunity appears to be

335 crucial in the control of *Toxoplasma* infection in mammalian hosts and, in particular,

336 the role of activated macrophages has been emphasized (Suzuki et al. 1988). Our

337 results showed that GCs have an enhancing effect on the growth of *T. gondii* in rat

338 peritoneal macrophages which further demonstrates the important role of NO in

339 macrophages in causing the host to be naturally resistant to *T. gondii* infection.

340 Interestingly, a recent study has revealed that acute toxoplasma infection also
341 increases endogenous production of GCs (Kugler et al. 2013). From the parasite
342 perspective and based on our observations, this mechanism this may serve to promote
343 further parasite growth. From the host perspective, Kugler et al (2013) show that this
344 endogenous GC production serves to invoke a CD4+ T cell response which they
345 propose limits collateral tissue damage and improves host survival. Our data would
346 support the role of GC dependent NO reduction in the parasite side of this
347 host-parasite evolutionary arms race.

348 In addition, our results demonstrated that significantly more *T. gondii* cysts
349 (Prugniaud strain) were found in the brains of rats treated with the glucocorticoid
350 dexamethasone (DXM). This result was consistent with our expectation. We consider
351 that the treatment of rat peritoneal macrophages with GCs causes changes in some
352 important physiological functions, for instance, a lower concentration of NO which
353 provides a suitable environment for the growth and multiplication of *T. gondii*. This
354 also potentially explains why acute infection of *T. gondii* is frequently found in human
355 patients treated with GCs.

356 In conclusion, we have analyzed possible mechanisms of the effect of GCs on
357 the resistance of rat peritoneal macrophages to *T. gondii* infection. These results not
358 only provide useful novel data for better understanding the protective mechanisms of
359 the host against this parasite but also reveal, indirectly, that patients who have been
360 administered with GCs following organ transplantation could potentially provide *T.*
361 *gondii* with a good opportunity to generate a new infection.

362

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367

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488 Tables and Figure legends

489 **Table 1** Proliferation of *T. gondii* in peritoneal macrophages of rats treated with GCs.

490

491

	Number of *T. gondii*/100 cells		
GCs	1 h	12 hrs	24 hrs
DXM	35±3	55±8*	176±15#
HSS	39±5	81±12*	227±14#
MP	37±6	85±14*	242±16#
Control	39±5	23±4	16±4

492

493 SD rats were injected intramuscularly with a dose of GCs for 7 days, macrophages

494 were harvested and cultured for 12 hrs and then incubated with *T. gondii* at the ratio

495 of 1:1 (parasites/macrophages = 1:1). The extracellular *T. gondii* were washed from

496 the medium after 1 hr contact and the time was defined as 1 hr and the number of

497 parasites per 100 macrophages was counted at 1, 12 and 24 hrs after infection. * and #:

498 $P<0.01$ vs. control analyzed by one-way ANOVA. All values are expressed as the

499 mean ± S.D of each infected group mice (X±SD, n=3). Abbreviations: dexamethasone

500 (DXM), hydrocortisone sodium succinate (HSS) and methylprednisolone (MP).

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505 **Table 2** Nitrite production by peritoneal macrophages from rats treated with GCs.

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	Nitrite production (μM)		
GCs	12 h	24 hrs	36 hrs
DXM	9.78±0.37*	14.31±1.22*	17.99±1.08*
HSS	8.19±0.32*	11.05±0.74*	13.51±1.45*
MP	7.86±0.46*	10.65±0.98*	12.90±0.99*
Control	14.44±1.04	25.41±1.44	30.85±1.62

507

508 Peritoneal macrophages isolated from rats treated with GCs for 7 days were incubated

509 for 12, 24 and 36 hrs. NO_2^- production in the supernatant of the cell culture medium, a

510 reflection of NO production, was measured by the Griess reaction. *: P<0.01 vs.

511 control analyzed by one-way ANOVA (X±SD, n=3).

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516 **Table 3** Nitrite production by peritoneal macrophages isolated from rats injected

517 intramuscularly with GCs and subsequent infection with *Toxoplasma gondii*.

518

	Nitrite production (μM)	
Groups	12 h	24 h
DXM+*T. gondii*	7.70±0.79*	9.03±1.17*
HSS+*T. gondii*	6.37±0.97*	7.01±0.85*
MP+*T. gondii*	6.7±0.99*	7.22±0.85*
Control+*T. gondii*	12.02±1.12#	19.28±1.38#
Control	15.97±1.41	27.71±1.04

519

520 SD rats were injected intramuscularly with a dose of GCs for 7 days (see details in the

521 Materials and Methods). Peritoneal macrophages collected from the treated rats, were

522 cultured for 12 hrs and were then challenged with *T. gondii in vitro*. At 12 hrs and 24

523 hrs after infection, NO_2^- production in the supernatants was measured by the Griess

524 reaction.*：*P*<0.01 vs. control+*T. gondii*;#: *P*<0.05; the comparison between control+*T.*

525 *gondii* and control was analyzed by one-way ANOVA. All values are expressed as the

526 mean ± S.D of each infected group mice (X±SD, n=3).

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531 Figure legends:

532 **Fig. 1** Fluorescent micrographs of *Toxoplasma gondii* proliferation in the

533 macrophages of rats treated with DXM.

534 Macrophages collected from SD rats treated with DXM for 7 days and were cultured

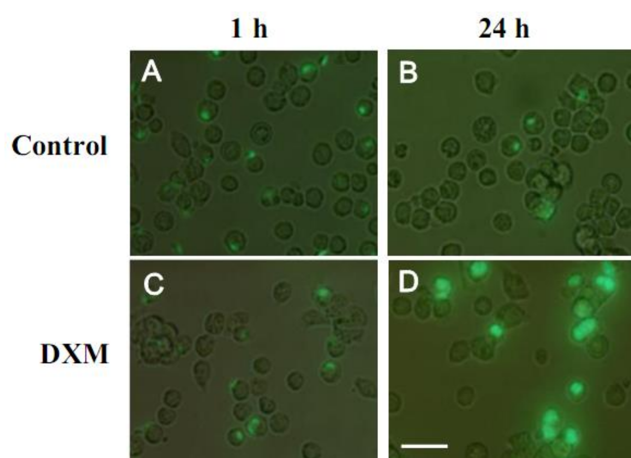
535 *in vitro* for 12 hrs before challenge with *T. gondii* tachyzoites at a ratio of *T. gondii*/

536 macrophage of 1:1. A and B: *T. gondii* in the normal control SD rat macrophages; and

537 C and D: *T. gondii* infection in the peritoneal macrophages of SD rats treated with

538 DXM. Similar results were also found in the macrophages of rats treated with HSS

539 and MP (Data not shown) (Scale is 50 μm)



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548 **Fig. 2** Comparison of iNOS expression levels from the peritoneal macrophages of rats

549 treated with GCs.

550 Macrophages collected from SD rats treated with GCs for 7 days and were cultured *in*

551 *vitro* for 12 hrs. (A) RT-PCR analysis for the expression of iNOS mRNA. (B) Western

552 blotting analysis for the expression of iNOS protein.

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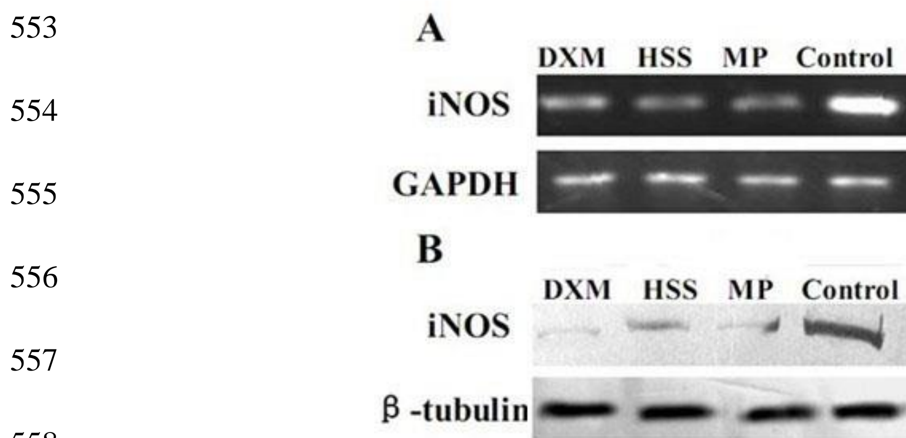

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571 **Fig. 3** Effect of DXM on the cyst burden of the *T. gondii* Prugniaud strain in the brain

572 of F344 rats.

573 Cyst burden from the brains of F344 rats infected with the Prugniaud (Pru) strain of *T.*

574 *gondii* and treated (B) and non-treated (A) with DXM. * indicates a significance value

575 of $P < 0.05$.

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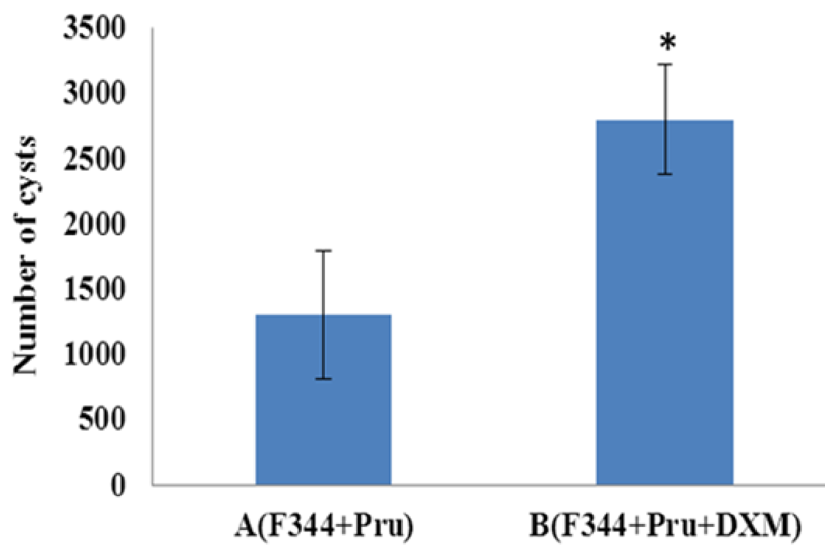

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