American Journal of Biomedical Sciences
ISSN: 1937-9080
nwpii.com/ajbms

UTL-5g Lowers Elevated Blood Levels of TNF-α and TGF-β and Increases Survival Rates in Animals Treated with LPS/D-(+)-galactosamine

Yiguan Zhang[1,2], Yongxin Tang[2], Ben Chen[3], Frederick Valeriote[4], Xiaohui Li[1], Jiajiu Shaw[3,*]

[1] Institute of Materia Medica & Department of Pharmaceutics, College of Pharmacy, Third Military Medical University, Chongqing, China
[2] New Phiaring Bio-medical Science Co., Ltd, Chengdu, China
[3] 21st Century Therapeutics, Ferndale, Michigan, USA
[4] Henry Ford Health System, Detroit, Michigan, USA
***Corresponding author:**
Jiajiu Shaw
21st Century Therapeutics, Inc.
Ferndale, Michigan
USA
Phone 1-248-545-0595
E-mail: jiajiuhaw@gmail.com

Received: 29 April 2014; | Revised: 20 May 2014; | Accepted: 8 June 2014

Abstract

N-(2,4-dichlorophenyl)-5-methyl-1,2-oxazole-3-carboxamide (UTL-5g) is a small-molecule chemoprotector against cisplatin and radioprotector against radiation. To further investigate its protective effects, we evaluated whether UTL-5g protects mice in a septic shock animal model. The two metabolites of UTL-5g, 5-methylisoxazole-3-carboxylic acid (Isox) and 2,4-dichloroaniline (DCA) were also evaluated side-by-side with UTL-5g. First, mice were pretreated with UTL-5g, Isox, and DCA before the *i.p.* injection of lipopolysaccharide (LPS)/D-(+)-galactosamine hydrochloride (D-Gal), respectively. Oral administration of both UTL-5g and Isox increased mouse survival while DCA did not, indicating that Isox is an active metabolite of UTL-5g while DCA is not. In the second study, mice were pretreated with UTL-5g or Isox individually by *i.p.* injection each at 30 mg/kg before LPS/D-Gal injection. The survival rates for both UTL-5g and Isox were better than those found for oral administration. In the third study, the same molar dose of UTL-5g and Isox by *i.p.* injection was used respectively and the results showed that UTL-5g had a better protective effect than Isox. In the fourth study, a protocol similar to the third study was used but blood samples were collected from the orbital plexus two hr after LPS/D-gal treatment. The results showed that UTL-5g lowered blood levels of both TNF-α and TGF-β elevated by LPS/D-gal. In summary, pretreatment of UTL-5g protected mice treated with LPS/D-Gal and the protection was related to the lowering of TNF-α and TGF-β levels elevated by LPS/D-Gal. In addition, UTL-5g appeared to be both an active drug and a prodrug wherein Isox is the active metabolite.

Keywords: UTL-5g, sepsis animal model, LPS/D-Gal, survival, TNF-α, TGF-β.

1. Introduction

Leflunomide is a disease-modifying anti-rheumatic drug (DMARD) but with significant potential side effects [1-5]. Based on a subtle but critical modification on the molecular scaffold of leflunomide, we designed, synthesized and, studied a series of UTL-5 compounds; several UTL-5 compounds have been shown to be TNF-α inhibitors and a particular compound, N-(2,4-dichlorophenyl)-5-methyl-1,2-oxazole-3-carboxamide (UTL-5g), is under preclinical development [6]. Although structurally similar to leflunomide, UTL-5 compounds have shown significantly different metabolic behaviors [7-9]. Leflunomide is metabolized by microsomes to become its major metabolite, teriflunomide (Fig. 1), by opening the isoxazole ring at the N-O bond [10]; however, UTL-5 compounds are metabolized by cleavage of the peptide bond but the isoxazole ring remains intact (Fig. 1). For example, UTL-5b is metabolized by rat microsomes to become 5-methylisoxazole-3-carboxylic acid (Isox) and 2-chloroaniline [8]; UTL-5g is quickly converted to Isox and 2,4-dichloroaniline (DCA) in the presence of esterase [7].

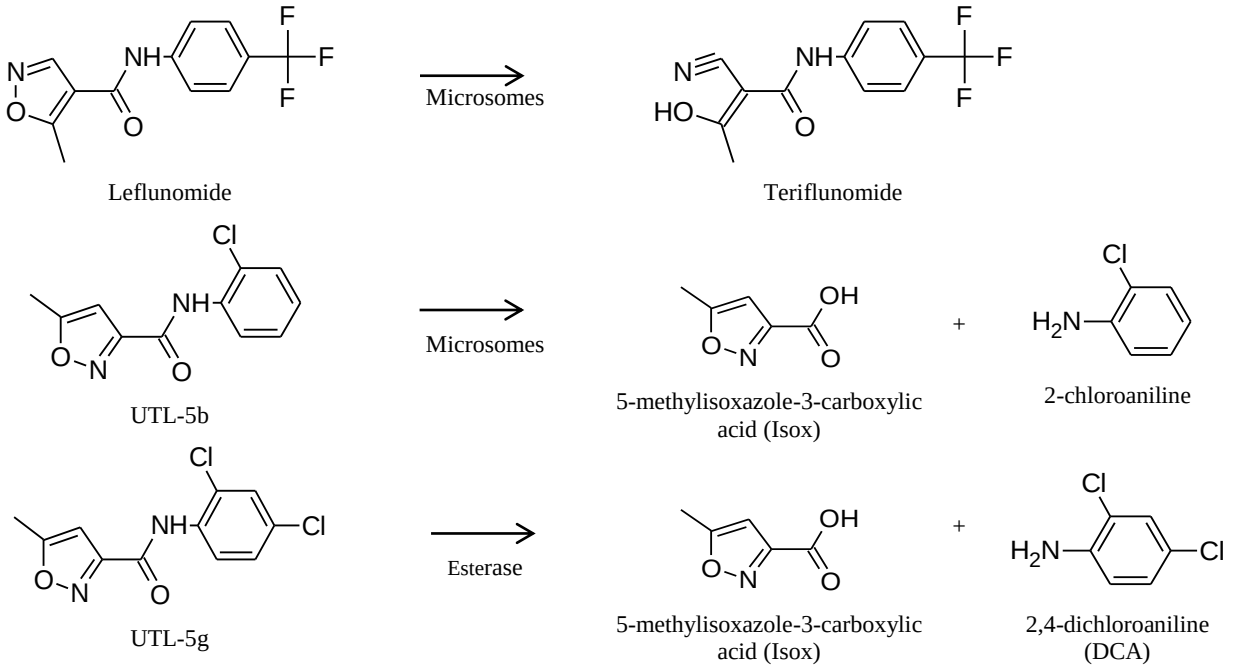


Figure 1. Structures of leflunomide, UTL-5b, -5g, and their respective metabolites

In terms of pharmacological effects, UTL-5 compounds are similar to leflunomide in that they are both anti-inflammatory and anti-arthritic [11-14]. However, UTL-5 compounds possess some protective effects which are significantly different from those of leflunomide. For example, UTL-5g reduces cisplatin-induced side effects by protecting kidney, liver, and platelets [6], thereby, increasing the tolerability of cisplatin [15]. In addition, UTL-5b and -5g reduce radiation-induced side effects in liver [16]. These pharmacological effects against chemotherapy/ radiation-induced side effects are different from those of leflunomide. For example, lefunomide is associated with certain liver toxicity and FDA issued prescribing warnings about potential hepatotoxicity [17]; in many cases, aspartate transaminase (AST) and alanine transaminase (ALT) levels are elevated [18], whereas UTL-5g

lowers AST/ALT levels elevated by cisplatin or radiation in our animal studies [6].

Therefore, it is of interest to further investigate the protective effects of UTL-5g and its metabolites. Because UTL-5g is a TNF-α inhibitor and lipopolysaccharide (LPS) is an endotoxin that promotes the secretion of pro-inflammatory cytokines including TNF-α [19, 20], we investigated whether UTL-5g and/or its metabolites protect mice from the lethal toxicity induced by LPS/D-(+)-galactosamine hydrochloride (D-Gal) in a septic shock animal model [21].

2. Materials and Methods

2.1. Materials

UTL-5g (HPLC purity >99%) was provided by 21st Century Therapeutics. Isox, DCA, and LPS were purchased from Sigma-Aldrich and used without further purification. CMC was purchased from Anhui Sunhe Pharmaceutical Excipients Co. (China). UTL-5g, Isox, and DCA were dispersed respectively in 1% carboxymethyl cellulose (CMC) for the animal treatment; UTL-5g and DCA maintained as suspensions in 1% CMC but the Isox preparation became a clear solution.

Kunming female mice (3-6 wk, 16-22 g) were purchased from the Third Army Medical University, Animal Testing Center (Chongqing, China). TNF-α Elisa kits were purchased from Ray Biotech (USA); TGF-β testing kits were purchased from DaKeWei Biotech, Beijing (China). All other reagents were purchased from Aldrich-Sigma unless otherwise specified.

2.2. Animal Study

In the first study, a total of 50 Kunming female mice were randomly divided into 5 groups: (1) vehicle control, (2) LPS/D-Gal, (3) UTL-5g and LPS/D-Gal, (4) Isox and LPS/D-Gal, (5) DCA and LPS/D-Gal. Isox and DCA were studied because they both are metabolites of UTL-5g [7]. For individual mice in groups 2-5, testing agents (UTL-5g, Isox, and DCA all at 60 mg/kg) were given by oral gavage respectively 1 hr before given LPS/D-gal (50 μg/kg and 600 mg/kg respectively) by *i.p.* injection.

In the second study, a total of 40 mice were used and divided into 4 groups: (1) vehicle control, (2) LPS/D-gal, (3) UTL-5g and LPS/D-Gal, and (4) Isox and LPS/D-Gal. In the control group, only vehicle was injected. For groups 2, 3, and 4, individual mice in groups were pretreated with corresponding agent 1 hr before given LPS/D-gal (50 μg/kg and 550 mg/kg respectively). Different from the first study, D-gal was reduced from 600 to 550 mg/kg; the doses of UTL-5g and Isox were reduced to one half (30 mg/kg each) and both were administered by *i.p.* injection. All animals were monitored for their body weights and survival.

In the third study, 6 groups of mice (10 mice/group) were used: (1) vehicle group, (2) LPS/D-gal, (3) UTL-5g (30 mg/kg or 0.11 mMole/kg) and LPS-D-gal, (4) UTL-5g (60 mg/kg or 0.22 mMole/kg) and LPS/D-gal, (5) Isox (14 mg/kg or 0.11 mMole/kg)-LPS/D-gal, (6) Isox (28 mg/kg or 0.22 mMole/kg)-LPS/D-gal. UTL-5g and Isox were administered by *i.p.* injection 1hr before given 50 μg/kg LPS and 550 mg/kg D-gal by *i.p.* injection. Again, all animals were monitored for their bodyweights and survivals.

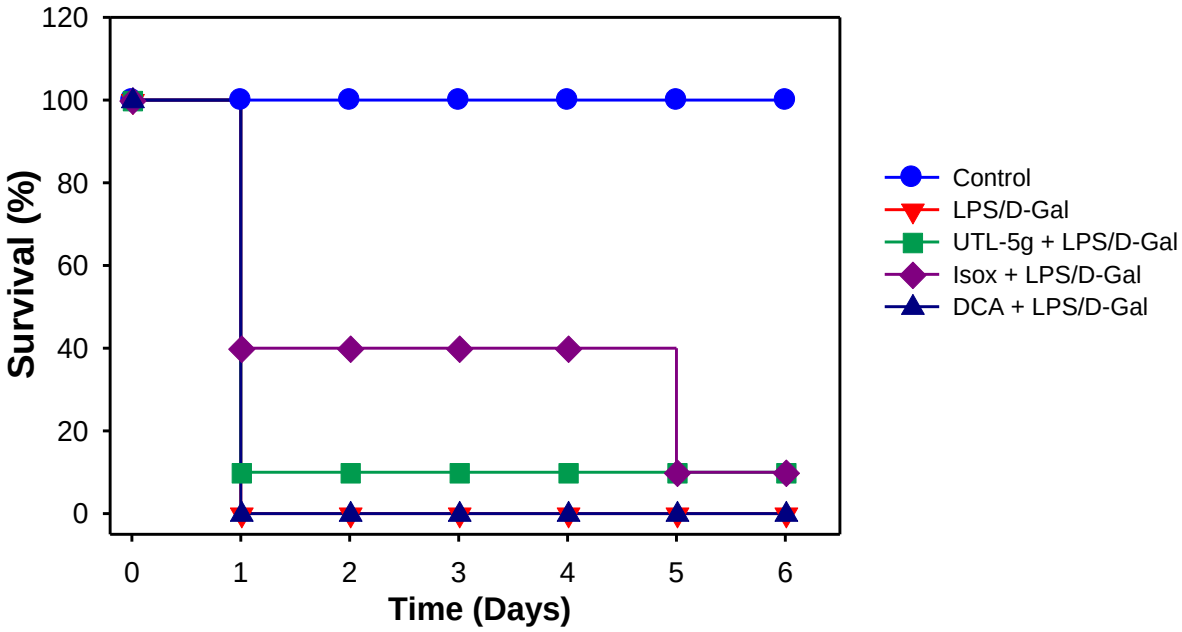
In the last study, Kunming female mice (5 mice/group) were treated in the manner identical to the third study, but blood samples were collected from the orbital plexus two hr after LPS/D-Gal treatment. Mouse plasma samples were prepared and stored at -70 ºC until analyzed by commercially available ELISA kits for levels of both TNF-α and TGF-β according to the manufactures' instructions.

3. Results

3.1. Animal study #1

As shown in Fig 2a, on Day 1 (24 hr after given LPS/D-gal), in the group without pretreatment, all 10 mice died; in the group pretreated with oral UTL-5g (at 60 mg/kg), 1 mouse survived; in the group pretreated with metabolite #1 (Isox) at 60 mg/kg, 4 mice survived; in the group pretreated with metabolite #2 (DCA) at 60 mg/kg, all 10 mice died.

(a)



(b)

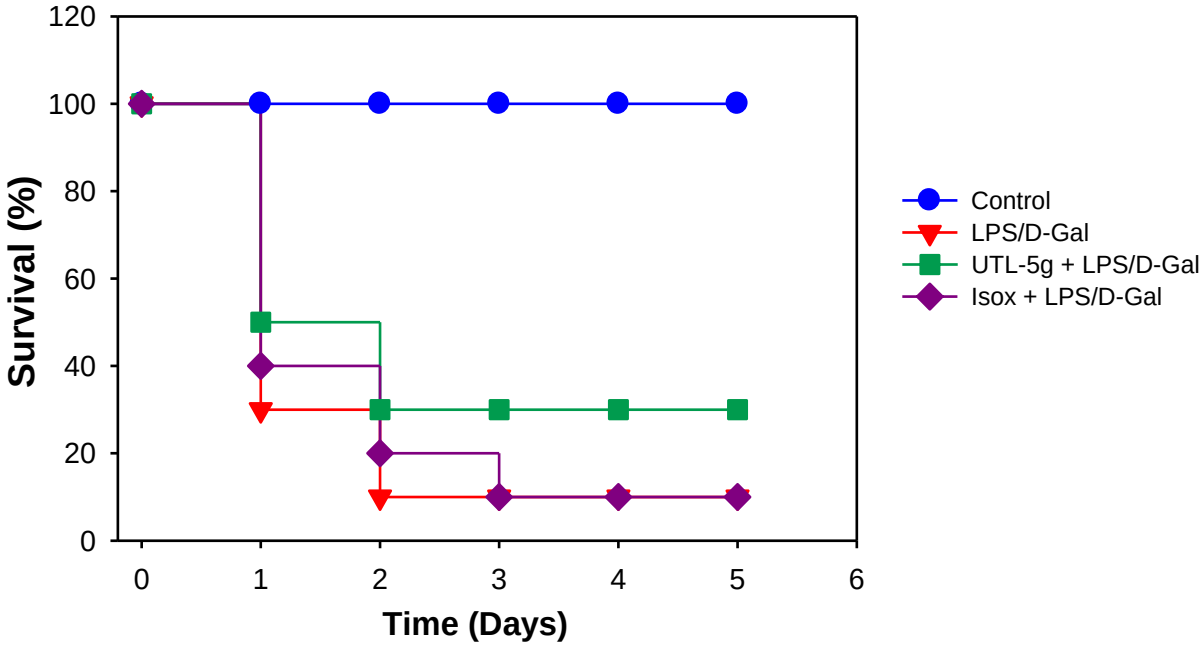


Figure 2. Effects of UTL-5g/Isox pretreatment on mouse survival rates
(a) Mouse survival rates following pretreatment by UTL-5g and Isox (both at 60 mg/kg) by oral gavage before LPS/D-Gal administration (50 μg/kg and 600 mg/kg); **(b)** Mouse survival rates following pretreatment by UTL-5g and Isox (both at 30mg/kg) by *i.p.* injection before LPS/D-Gal administration (50 μg/kg and 550 mg/kg).

3.2. Animal study #2

Results from the second study (Fig. 2b), using both UTL-5g and Isox at 30 mg/kg by *i.p.* injection, showed that, in the group without pre-treatment, 3 mice survived on Day 1 (24 hr), only 1 survived on Day 2, and maintained the same until Day 5. In the UTL-5g group, 5 mice survived on Day 1 (vs. 3 survived in LPS/D-gal group, a 66% increase in survival rate) and 3 survived on Day 2 (vs. 1 survival in LPS/D-Gal group) and maintained the same until Day 5. In the Isox group, 4 mice survived (vs. 3 survived in LPS/D-gal group, a 33% increase in survival) on Day 1, 2mice survived on Day 2 (vs. 1 survival in LPS/D-Gal group), and only 1 survived from Day 3 until Day 5.

As shown in Fig. 3, LPS/D-Gal treatment reduced the average body weights of mice by 7 % on Day 1 and UTL-5g lessened this weight-loss effect to only 3 % on Day 1. However, Isox did not lessen the weight-loss effect especially after Day 2 although Isox did increase the survival rate slightly; this could be because there was only one mouse survived after Day 2; thus the Isox data was not representative.

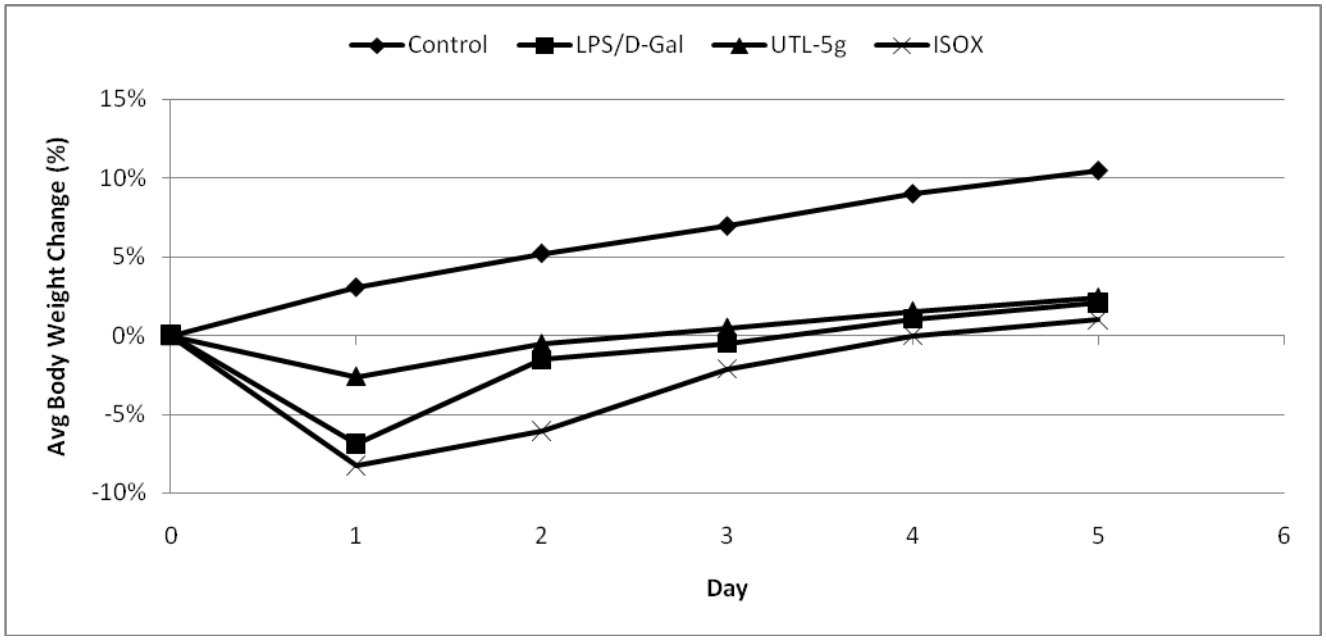


Figure 3. Mouse body weight change vs. time from the second study
Body weight profiles of four groups: (1) vehicle control, (2) LPS/D-Gal, (3) UTL-5g + LPS/D-Gal, and (4) Isox + LPS/D-Gal. UTL-5g and Isox were both given at 30 mg/kg by *i.p.* injection. Each data point represents the average of 10 mice on Day 0 and lower numbers (survivals) from Day 1 and after. Standard deviations are not shown to make it easier to distinguish the profiles among these four groups.

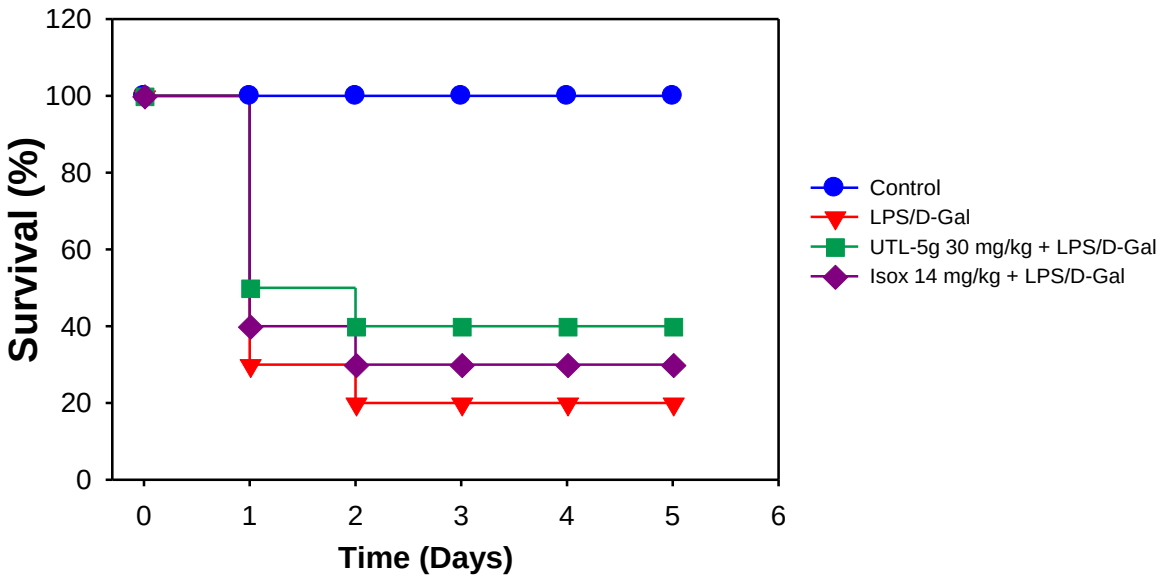
3.3. Animal study #3

Results from the third study (Fig. 4) showed that UTL-5g protection of animals from the lethal toxicity was dose dependent in that 5/6 mice survived by UTL-5g pretreatment at 0.11/0.22 mMole/kg on Day 1 whereas only 3 mice survived without pretreatment; the survival increase was dose dependent. Fig. 4(a) shows that at a lower dose (0.11 mMole/kg), UTL-5g has a better protective effect as compared to Isox. Fig. 4(b) shows that at 0.22 mMole, UTL-5g also has a much better protective effect and resulted in higher survivals.

As to the body weights, Fig. 5 shows that the average weight of the mice in the LPS/D-Gal group was the lowest as compared to all other groups and its nadir is on Day 2. At 0.11 mMole/kg (Fig. 5a), UTL-5g group did not lose

weight and started to gain weight from Day 2. At 0.22 mMole/kg (Fig. 5b), UTL-5g group did lose weight but started to recover from Day 2.At both doses (Fig. 5a and 5b), mice in Isox group lost some weights on Day 1 and 2, but started to gain back from Day 3.

(a)



(b)

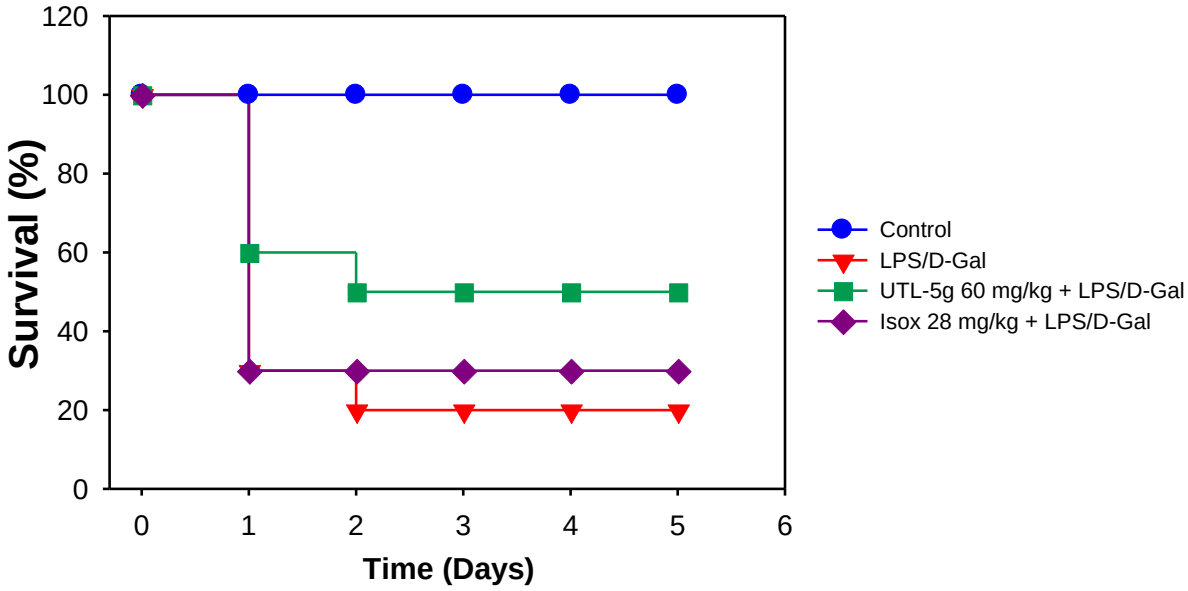
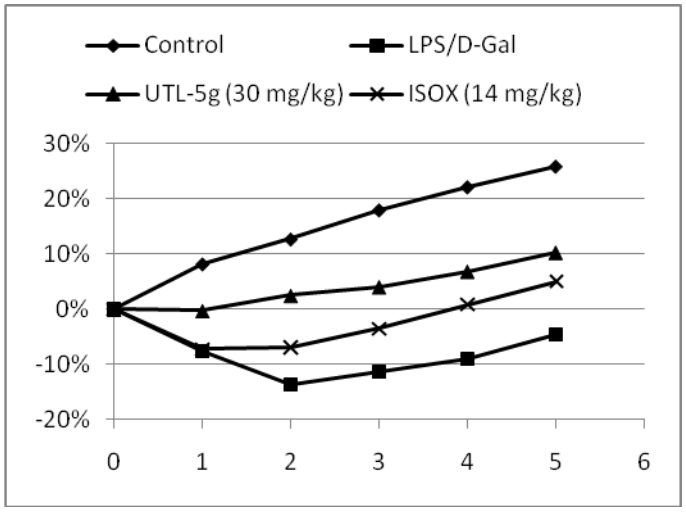


Figure 4. Survival rates with the pretreatment of UTL-5g and Isox at the same molar dose by *i.p.* injection **(a)** UTL-5g and Isox both at 0.11 mMole/kg (UTL-5g at 30 mg/kg vs. Isox at 14 mg/kg); **(b)** UTL-5g and Isox both at 0.22 mMole/kg (UTL-5g at 60 mg/kg vs. Isox at 28 mg/kg).

(a)



(b)

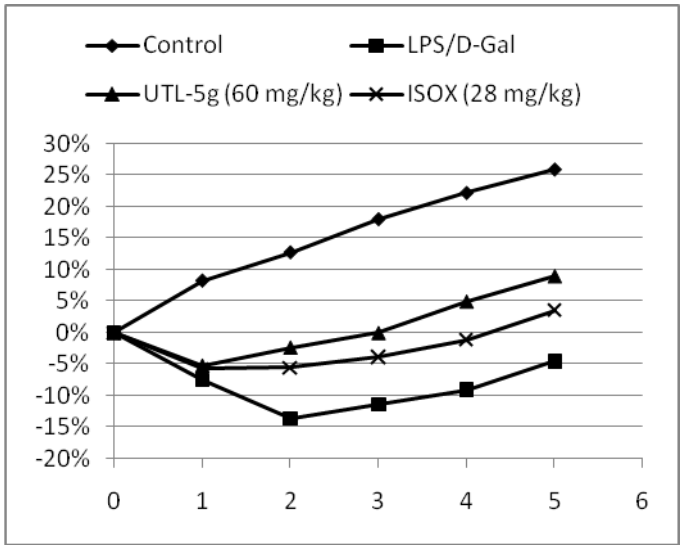
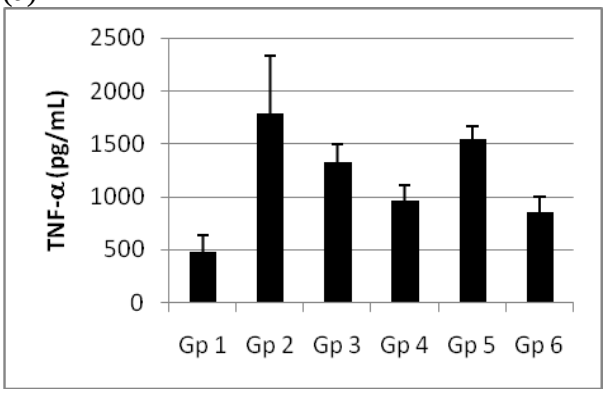


Figure 5. Average body weights for mice with and without pretreatment of UTL-5g and Isox
(a) comparison of the weight loss profiles of Control, LPS/D-Gal, UTL-5g at 30 mg/kg and Isox at 14 mg/kg (equal to UTL-5g in molar conc); **(b)** comparison of the weight loss profiles of Control, LPS/D-Gal, UTL-5g at 60 mg/kg and Isox at 28 mg/kg (equal to UTL-5g in molar concentration)

3.4. Animal study #4

As shown in Fig. 6, the final study demonstrated that TNF-α levels elevated by LPS/D-Gal (from 471 to 1782 ng/mL) were lowered by both UTL-5g and by Isox; essentially the same effects by UTL-5g and by Isox were observed for TGF-β although the reduction of TGF-β by Isox at 28 mg/kg was less than that at 14 mg/kg. In addition, the reductions of both TNF-α and TGF-β are generally parallel to the mouse survival pattern in the previous study.

(a)



(b)

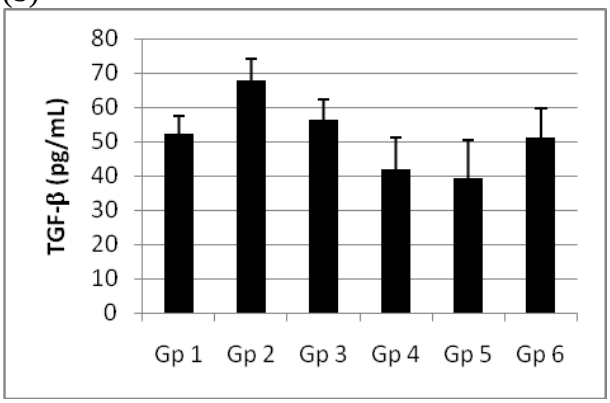


Figure 6. Plasma levels of TNF-α and TGF-β in mice with and without pretreatment of UTL-5g/Isox
Gp 1, vehicle control; Gp 2, LPS/D-Gal; Gp 3, UTL-5g 30 mg/kg + LPS/D-Gal; Gp 4, UTL-5g 60 mg/kg + LPS/D-Gal; Gp 5, Isox 14 mg/kg + LPS/D-Gal; Gp 6, Isox 28 mg/kg + LPS/D-Gal. For both TNF-α and TGF-β levels, $p < 0.05$ for Gp 2 vs. Gp 4. For Fig 6a, n = 5 for all groups; for Fig 6b, n = 5 for Gp 2 & 6, n = 4 for Gp 1, 3&4, and n = 3 for Gp 5due to insufficient plasma available.

4. Discussion

Results from the first animal study showed that both UTL-5g and Isox protected mice from the lethal toxicity induced by LPS/D-gal, but DCA had no protective effect against the lethality. The ability of both UTL-5g and Isox to increase mouse survival could be due to TNF-α inhibition at least in part because similar protective observations were reported previously for a structurally similar TNF-α inhibitor, UTL-5b [14]. In addition, UTL-5g is known to be quickly metabolized to produce Isox [7]. Therefore, it was not surprising to see that Isox also showed significant protective effect. However, in this first study, more animals survived under the treatment of Isox than under UTL-5g. This could be due to two additive factors: (1) Although the doses were the same, both at 60 mg/kg, the difference in molecular weights of UTL-5g and Isox (271 vs. 127) makes the effective molar conentration of Isox > 2 times higher than that of UTL-5g; (2) Although both UTL-5g and Isox were prepared in 1% CMC, UTL-5g stayed as a suspension whereas Isox was completely dissolved in 1% CMC; this could have resulted in a much lower oral bioavailability of UTL-5g as compared to Isox. Thus in the second animal study, both UTL-5g and Isox were administered by *i.p.* injection instead of oral administration.

Results from the second study again indicate that both UTL-5g and Isox protected mice treated with LPS/D-gal, but UTL-5g was more potent than Isox by *i.p*. injection at the same dose of 30 mg/kg. This observation supports our hypothesis that UTL-5g is both an active drug and a prodrug with Isox as the active metabolite of UTL-5g. We also used body weight as a general toxicity marker; indeed, UTL-5g lessened the weight-loss effect from LPS/D-Gal indicating its general protective effect against the toxicity induced by LPS/D-Gal. However, Isox did not lessen the weight-loss effect significantly.

The third animal study (Fig. 4) showed that UTL-5g protection of animals from the lethal toxicity (shown by the survival increase) was dose dependent. Although Isox also showed protective effect against the lethality induced by LPS/D-Gal, Isox was not as effective as UTL-5g at the same molar concentration further indicating that UTL-5g is likely both an active drug and a prodrug. Thus, it may be advantageous to use UTL-5g rather than Isox as a potential protective agent. As to the body weights, at 0.11 mMole/kg (Fig. 5a), UTL-5g group did not lose weight and started to gain weight from Day 2. At a higher dose of 0.22 mMole/kg (Fig. 5b), UTL-5g might have some negative effect itself in protecting body weight. Overall, UTL-5g and Isox both showed protective effect in body weight but UTL-5g is more effective.

The final study demonstrated that plasma levels of both TNF-α and TGF-β elevated by LPS/D-Gal were lowered by either UTL-5g or Isox although the reduction of TGF-β by Isox was not statistically significant. In addition, the reductions of both TNF-α and TGF-β are generally parallel to the mouse survival pattern in the previous study. This is logical because LPS-induced up-regulation of TGF-β receptor is associated with TNF-α expression and blockade of TGF-β signaling reduces the production of TNF-α [22]. In addition, elevated TNF-α levels induced by LPS are known to be associated with animal lethality [14, 23]. The only odd observation is that Isox at 14 mg/kg seemed to have a little better effect as compared to Isox 28 mg/kg, which can be seen in both the survival rates and the lowering of TGF-β levels, however, the difference is not statistically significant and could be partially due to experimental deviations.

It has been reported that both TNF-α and TGF-β levels are elevated in patients with sepsis [24-26]. In addition, blockade of TGF-β signaling significantly reduces the production of TNF-α by LPS-stimulated human monocyte-derived macrophages [22]. The present work on UTL-5g is in line with these reports and indicates that UTL-5g may be worthy of further investigation as a potential agent for sepsis.

This current study further expands the protective effects of UTL-5g in addition to our previous studies which showed that UTL-5g is chemoprotective and radioprotective. This work shows that UTL-5g increases the survival rates of mice treated by LPS/D-gal and its protective effects are related to the reduction of TNF-α/

TGF-β levels elevated by LPS/D-Gal. This is consistent with what we observed previously in the protective effect of UTL-5g against the toxicity of cisplatin in that elevated TNF-α levels were also lowered by UTL-5g [6]. Furthermore, our previous studies showed that a closely related TNF-α inhibitor, UTL-5b, significantly suppresses three genes that are relevant to the TNF-α pathway: Janus kinase 3 (JAK3), mitogen-activated protein kinase kinase kinase 2 (MAP3K2) and LPS-induced TNF-α factor (LITAF) [13, 14]. Based on the almost identical structural similarity between UTL-5g and UTL-5b, we believe that the mechanism of UTL-5g may be similar although the theory remains to be further verified.

Because Isox is an active metabolite of UTL-5g, it can be concluded that UTL-5g is also a prodrug. This conclusion is further supported by the facile conversion of UTL-5g into Isox [7]. However, Isox is not as potent as UTL-5g based on molecular concentration indicating that UTL-5g is also an active drug.

In summary, coupled with the chemoprotective effects of UTL-5g against cisplatin-induced side effects in kidney, liver, and platelets, as well as the radioprotective effects of UTL-5g in liver and lung, this study expands the protective range of UTL-5g potentially to LPS related diseases including sepsis [27, 28] and other immune-mediated diseases [29-31].

Acknowledgement

This work was supported by grants from National Institutes of Health, 2R44CA141749-02, and from Sichuan Science and Technology Support Plan, 2013-2015, No.2013SZ0049.

References

1. WHO, *Leflunomide: haematologic,hepatic and respiratory reactions.* WHO Drug Information, 2002. **16**: p. 205-68.
2. Shankaranarayana, s., C. Barrett, and P. Kubler, *The Safety of Leflunomide.* Australian Prescriber, 2012. **36**(1): p. 28-32.
3. Balague, C., et al., *Profiling of dihydroorotate dehydrogenase, p38 and JAK inhibitors in the rat adjuvant-induced arthritis model: a translational study.* Br J Pharmacol, 2012. **166**(4): p. 1320-32. DOI: 10.1111/j.1476-5381.2012.01836.x
4. Brent, R.L., *Teratogen update: reproductive risks of leflunomide (Arava); a pyrimidine synthesis inhibitor: counseling women taking leflunomide before or during pregnancy and men taking leflunomide who are contemplating fathering a child.* Teratology, 2001. **63**(2): p. 106-12. DOI 1002/1096-9926(200102)63:2<106::AID-TERA1017>3.0.CO;2-R
5. Sevilla-Mantilla, C., et al., *Leflunomide-induced acute hepatitis.* Dig Liver Dis, 2004. **36**(1): p. 82-4. doi: 10.1016/j.dld.2003.06.002
6. Shaw, J., et al., *The small-molecule TNF-alpha modulator, UTL-5g, reduces side effects induced by cisplatin and enhances the therapeutic effect of cisplatin in vivo.* J Exp Ther Oncol, 2011. **9**(2): p. 129-37.
7. Swartz, K., et al., *Using a simple HPLC approach to identify the enzymatic products of UTL-5g, a small molecule TNF-alpha inhibitor, from porcine esterase and from rabbit esterase.* J Chromatogr B Analyt Technol Biomed Life Sci, 2013. **940**: p. 1-6. DOI: 10.1016/j.jchromb.2013.09.021
8. Shaw, J., et al., *Metabolism studies of a small-molecule tumor necrosis factor-alpha (TNF-alpha) inhibitor, UTL-5b (GBL-5b).* Eur J Drug Metab Pharmacokinet, 2012. **37**(2): p. 83-9. DOI: 10.1007/s13318-011-0072-7
9. Song, Y., et al., *Comparison of Two Molecular Scaffolds, 5-Methylisoxazole-3-Carboxamide and 5-Methylisoxazole-4-Carboxamide.* Curr Pharm Des, 2013.

10. FDA. *NDA 20905 Clinical Pharmacology and Biopharmaceutics Review(s)*. 1998 [cited 2013 February22, 2013]; Available from: http://www.fda.gov/ohrms/dockets/dockets/05p0127/05p-0127-cp00001-Tab-02-NDA-vol1.pdf.
11. Breedveld, F.C. and J.M. Dayer, *Leflunomide: mode of action in the treatment of rheumatoid arthritis.* Ann Rheum Dis, 2000. **59**(11): p. 841-9. DOI: 10.1136/ard.59.11.841
12. Fox, R.I., *Mechanism of action of leflunomide in rheumatoid arthritis.* J Rheumatol Suppl, 1998. **53**: p. 20-6.
13. Shaw, J., et al., *A Novel Leflunomide Analog, UTL-5b (GBL-5b), Suppresses JAK3, MAP3K2, and LITAF Genes.* Am J Biomed Sci, 2011. **3**(3): p. 218-227.
14. Shaw, J., et al., *Anti-inflammatory and Anti-arthritic Effects of a Novel Leflunomide Analogue, UTL-5b (GBL-5b).* Am J Biomed Sci, 2011. **3**(1): p. 31-39.
15. Shaw, J., et al., *The small-molecule TNF-alpha inhibitor, UTL-5g, delays deaths and increases survival rates for mice treated with high doses of cisplatin.* Cancer Chemother Pharmacol, 2013. **72**(3): p. 703-7. DOI: 10.1007/s00280-013-2236-4
16. Shaw, J., et al., *Pretreatment with a Small-Molecule Tumor Necrosis Factor-Alpha (TNF-á) Inhibitor, UTL-5g, Reduced Radiation-Induced Acute Liver Toxicity in Mice.* American Journalof Biomedical Sciences, 2012. **4**(2): p. 123-131. DOI: 10.5099/aj120200123
17. FDA. *Prescribing information,warnings of hepatotoxicity*. 2003; Available from: http://www.fda.gov/downloads/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/UCM168409.pdf.
18. Gupta, R., J. Bhatia, and S.K. Gupta, *Risk of hepatotoxicity with add-on leflunomide in rheumatoid arthritis patients.* Arzneimittelforschung, 2011. **61**(5): p. 312-6. DOI: 10.1055/s-0031-1296204
19. Levy, E., et al., *Distinct roles of TLR4 and CD14 in LPS-induced inflammatory responses of neonates.* Pediatr Res, 2009. **66**(2): p. 179-84. DOI: 10.1203/PDR.0b013e3181a9f41b
20. Wright, S.D., et al., *CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein.* Science, 1990. **249**(4975): p. 1431-3. DOI: 10.1126/science.1698311
21. Villa, P. and P. Ghezzi, *Animal models of endotoxic shock.* Methods Mol Med, 2004. **98**: p. 199-206.
22. Chen, Y., et al., *LPS-induced up-regulation of TGF-beta receptor 1 is associated with TNF-alpha expression in human monocyte-derived macrophages.* J Leukoc Biol, 2008. **83**(5): p. 1165-73. DOI: 10.1189/jlb.0807521
23. Tateda, K., et al., *Lipopolysaccharide-induced lethality and cytokine production in aged mice.* Infect Immun, 1996. **64**(3): p. 769-74.
24. Marie, C., J.M. Cavaillon, and M.R. Losser, *Elevated levels of circulating transforming growth factor-beta 1 in patients with the sepsis syndrome.* Ann Intern Med, 1996. **125**(6): p. 520-1. DOI: 10.7326/0003-4819-125-6-199609150-00034
25. Rigato, O., et al., *Tumor necrosis factor alpha (TNF-alpha) and sepsis: evidence for a role in host defense.* Infection, 1996. **24**(4): p. 314-8. DOI: 10.1007/BF01743367
26. Blackwell, T.S. and J.W. Christman, *Sepsis and cytokines: current status.* Br J Anaesth, 1996. **77**(1): p. 110-7. DOI: 10.1093/bja/77.1.110
27. Opal, S.M., et al., *Relationship between plasma levels of lipopolysaccharide (LPS) and LPS-binding protein in patients with severe sepsis and septic shock.* J Infect Dis, 1999. **180**(5): p. 1584-9. doi: 10.1086/315093
28. Shapira, L., et al., *Protection against endotoxic shock and lipopolysaccharide-induced local inflammation by tetracycline: correlation with inhibition of cytokine secretion.* Infect Immun, 1996. **64**(3): p. 825-8.
29. Charalambous, B.M., et al., *Role of bacterial endotoxin in chronic heart failure: the gut of the matter.* Shock, 2007. **28**(1): p. 15-23. DOI: 10.1097/shk.0b013e318033ebc5

30. Cavallo, T. and N.A. Granholm, *Bacterial lipopolysaccharide induces long-lasting IgA deficiency concurrently with features of polyclonal B cell activation in normal and in lupus-prone mice.* Clin Exp Immunol, 1991. **84**(1): p. 134-8.
31. Yoshino, S. and M. Ohsawa, *The role of lipopolysaccharide injected systemically in the reactivation of collagen-induced arthritis in mice.* Br J Pharmacol, 2000. **129**(7): p. 1309-14. doi: 10.1038/sj.bjp.0703166

