



Journal homepage:
<http://www.bsu.edu.eg/bsujournals/JVMR.aspx>
 Online ISSN: 2357-0520 Print ISSN: 2357-0512



Original Research Article

Pharmacokinetic interaction of tulathromycin with Flunixin meglumine after intravenous injection in goats

M. Adam, M. A. Tohamy, S.E. El-Sadek and Abeer M. Radi

Pharmacology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt

ABSTRACT

The pharmacokinetic aspects of tulathromycin (2.5 mg/kg b.w.) were studied following intravenous administration alone and in combination with flunixin meglumine (2.2 mg/kg b.w) in apparently healthy goats. Tulathromycin concentrations in serum were determined by microbiological assay technique using *Bacillus subtilis* (ATCC 66343) as test organism. The half-lives of distribution and elimination ($t_{0.5(a)}$ and $t_{0.5(p)}$) were 0.071, 0.046 and 6.43, and 5.05 h. following intravenous injection of tulathromycin alone and in combination with flunixin, respectively. Volume of distribution at steady state (V_{dss}) was 0.249 and 0.96l/kg., mean residence time (MRT) was 6.27 and 5.99 h and total body clearance (Cl_B) was 0.046 and 0.17 l/kg/hr., respectively. It was concluded that flunixin significantly altered the pharmacokinetics of tulathromycin by increase its distribution and accelerate its elimination from body. Therefore care should be taken during use of tulathromycin in goats concurrently with flunixin.

ARTICLE INFO

Article history:

Received 11/ 2016
 Accepted 12/2016
 Online 12/2016

Keywords:

Pharmacokinetics,
 Tulathromycin,
 Flunixin,
 Intravenous, Goats.

*Corresponding author. *Pharmacology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt. Tel: 01007236177*

1. Introduction

Tulathromycin is a novel triamilide antimicrobial in the macrolide class shown to be safe and effective in cattle and swine to treat bacterial respiratory disease (**Benchaoui et al., 2004; Nowakowski et al., 2004; Evans, 2005**). Macrolide structure facilitates rapid distribution of these drugs from the blood stream into tissues (**Williams & Sefton, 1993**). Newer macrolides, such as azithromycin and tulathromycin, have increased lung tissue uptake and longer half-lives than older macrolides such as erythromycin (**Benchaoui et al., 2004**). In addition, the tripleionized form of tulathromycin produces displacement of the Mg^{2+} ions present in the outer cell wall of gram-negative bacterial pathogens, facilitating drug entry into these agents (**Evans, 2005**). As a macrolide, tulathromycin exerts its activity through binding to the 50S subunit of bacterial ribosomes and blocking peptidyltransferase which results in dissociation of transfer RNA (tRNA), cessation of peptide translocation, and blockage of protein synthesis (**Benchaoui et al., 2004; Evans, 2005; Villarino et al., 2013**). Although this drug is classified as bacteriostatic, it can also exhibit bactericidal activity at higher concentrations (**Benchaoui et al., 2004; Evans, 2005; Nowakowski et al., 2004; Villarino et al., 2013**). **Brunton et al. (2008)** recorded that in addition to impacting enhanced tissue and cellular penetration characteristic of all

macrolides, this novel structure (tulathromycin) conveys desirable antibacterial properties particularly against Gram negative respiratory bacteria. Tulathromycin is more efficacious injectable macrolide antibiotic used for the treatment of pneumonia of ruminants compared with other antibiotics in recent years (**Venner et al., 2007; Nutsch et al., 2005; Godinho et al., 2005; Skogerboe et al., 2005 and Robb et al., 2007**).

Non steroidal anti-inflammatory drugs (NSAIDs) are inhibitors of cyclooxygenase that catalyze the incorporation of molecular oxygen into arachidonic acid to produce prostanoids (eg, thromboxanes, prostacyclin, and prostaglandin) and are effectively administered for inflammation and pain. Flunixin is one of (NSAIDs) has been reported to reduce fevers and improve clinical signs of endotoxemia (**Anderson et al., 1986**). Flunixin has been widely used for their anti-inflammatory and analgesic properties to treat the musculoskeletal conditions and colic in equine. It was also used routinely in ruminant practice to treat mastitis, endotoxemia and pneumonia (**Zu-Gong et al., 2007**). It is well documented that concurrent administration of drugs together may alter the pharmacokinetic parameters of these drugs. In veterinary practice the administration of antibiotics and NSAIDs at the same time was more common. Therefore, the aim of the study was to compare the disposition kinetic of tulathromycin in goats after a single

intravenous administration alone and when administrated with flunixin meglumine.

Material and Methods

Drugs: Tulathromycin 100 mg ml⁻¹ was supplied as an injectable solution (Draxxin®) by animal health division Pfizer Company, Cairo, Egypt. Flunixinmeglumine (Flunidyne) is a product of ArabcoMed, Egypt.

Animals: Ten apparently healthy, male and female Egyptian goats (3-9 months old and mean body weight of (12-23 kg) were used. Animals were obtained from a local market at Beni-Suef province, kept under good hygienic condition, fed barseem and free access to water.

Methods:

Experimental design: The animals were randomly divided into two groups (five goats each). Animals of first group were injected intravenously with tulathromycin in alone single dose of 2.5 mg kg⁻¹ (Clothier et al., 2011, Young et al., 2011; Grismer et al., 2014). While the 2nd group was intravenously injected with a single dose of tulathromycin and 2.2 mg kg⁻¹ flunixin (Konigsson et al., 2003) in the right jugular vein. Blood samples were collected via vein puncture from left jugular vein before and 0.083, 0.167, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12, 24, 48 and 72 hours post-administration. Blood samples were left to clot then centrifuged

at 3000 revolution per minute for 15 minutes to obtain clear serum that was kept frozen at -20 °C until assayed.

Drug bioassay

Samples were assayed by microbiological assay according to the method of Arret et al. (1971) using *Bacillus subtiles* (ATCC 6633) as a test organism. Standard tulathromycin concentrations of 0.078, 0.156, 0.3125, 0.625, 1.25, 2.5, 5, 10 and 20 ug ml⁻¹ were prepared in antibiotic-free goat serum and phosphate buffer saline (pH 8). The minimal detectable limit for the assay method was 0.078 ug ml⁻¹. Semi-logarithmic plots of the inhibition zone diameter versus standard tulathromycin concentrations in serum and phosphate buffer were linear with typical correlation coefficient of 0.992 (for the standard curve).

Pharmacokinetic analysis:

A computerized curve stripping program (R Strip; Micromath Scientific Software, Salt Lake City, UT, USA) was used to analyze the serum concentration-time curves for each individual animal using the statistical moment theory (Gibaldi and Perrier, 1982). Following IV injection, the serum concentration-time relationship was best estimated as a two-compartment open model system (Baggot, 1978), according to the following bi-exponential equation: $C_p = Ae^{-at} + Be^{-bt}$, where C_p is the

concentration of drug in the serum at time t ; A is the intercept of the distribution phase with the concentration axis expressed as $\mu\text{g ml}^{-1}$; B is the intercept of the elimination phase with the concentration axis expressed as $\mu\text{g ml}^{-1}$; a is the distribution rate constant expressed in units of reciprocal time (h^{-1});

p is the elimination rate constant expressed in units of reciprocal time (h^{-1}); and e is the natural logarithm base. Results were expressed as mean and standard error (S.E). Standard errors were calculated from the mean data according to **Snedecor and Cochran (1976)**.

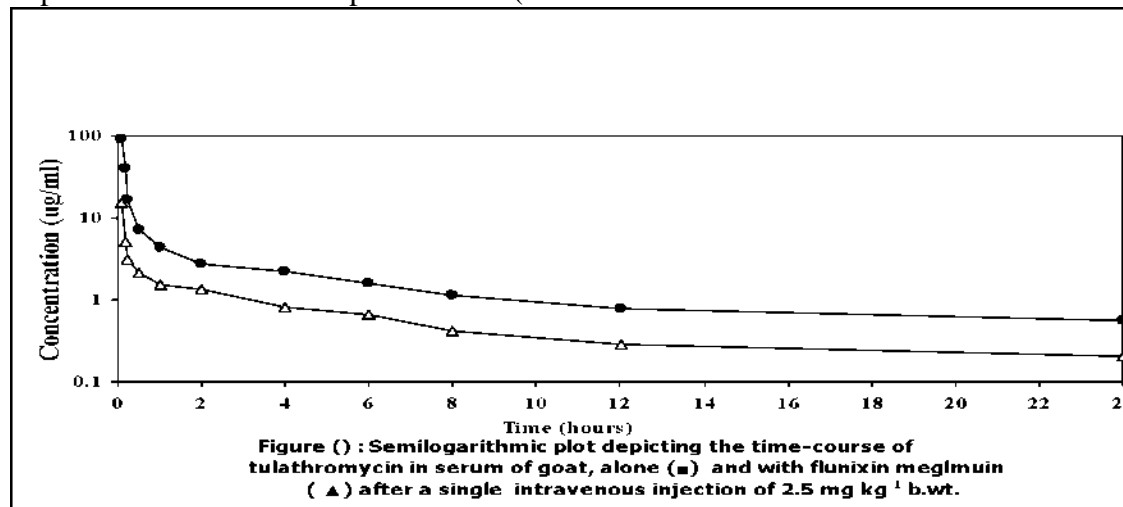


Figure (1): Semi-logarithmic graph depicting the time-concentration of tulathromycin in serum of goats after a single intravenous injection of $2.5 \text{ mg kg}^{-1} \text{ b.wt}$ alone (■) and with flunixin (▲).

Results:

Disposition of tulathromycin in serum after intravenous injection was best fitted by the 2-compartment open pharmacokinetic model (Figure 1). The pharmacokinetic parameters of tulathromycin following a single intravenous administration of $2.5 \text{ mg kg}^{-1} \text{ b.wt}$ alone and with flunixin are recorded in table (1). The results of the present study revealed that tulathromycin was rapidly

distributed following intravenous injection when administered with flunixin where the distribution half lives were ($t_{0.5(a)}$) 0.071 when given alone and 0.046 h with flunixin. The body clearance (Cl_B) was 0.46 and 0.17 l/kg.h , the volume of distribution at steady-state (Vd_{ss}) was 0.249 and 0.96 L/kg , respectively.

Table (1): Pharmacokinetic parameters of tulathromycin alone (of 2.5 mg kg⁻¹b.wt) and with flunixin (of 2.2 mg kg⁻¹b.wt) following a single intravenous injection in goats (n=5). (Mean ± SE)

Pharmacokinetic parameter	tulathromycin alone	Tulthromycin with flunixin
Cp (ug/h)	257.3±77.01	72.3±35.4
A (ug/ml)	252.8±76.5	70.3±35.1
a (h ⁻¹)	11.17±2.05	16.7±2.74
t _{0.5a} (min)	0.071±0.013	0.046±0.007
B (ug/ml)	4.51±0.6	2.0±0.36
P (h ⁻¹)	0.14±0.028	0.18±0.032
t _{0.5P} (h)	6.43±2.13	5.05±1.64
K ₁₂ (h ⁻¹)	6.34±0.84	11.78±0.61
K ₂₁ (h ⁻¹)	0.35±0.048	0.79± 0.12
K _e (k ₁₀) (h ⁻¹)	4.62±1.23	4.3±1.67
V _c (l/kg)	0.0132±0.003	0.061±0.015
Vd _{ss} (l/kg)	0.249±0.076	0.96±0.29
CL _B (l/kg/hr)	0.046±0.054	0.17±0.015
AUC (ug.h.ml ⁻¹)	57.1±7.11	15.45±1.48
AUMC (gg.h.ml ⁻¹)	78.97±6.3	24.71±1.95
MRT (h)	6.27±2.57	5.99±2.29

Cp° concentration at zero time (immediately after single IV injection); A, B zero-time intercepts of the biphasic disposition curve; a, P hybrid rate constants representing the slopes of distribution and elimination phases, respectively; k₁₂ first-order constant for transfer from central to peripheral compartment; k₂₁ first order constant for transfer from peripheral to central compartment; K_e elimination rate constant; t_{0.5(a)} distribution half-life; t_{0.5(P)} elimination half-life; MRT mean residence time; AUC area under serum concentration-time curve; AUMC area under moment curve; V_c apparent volume of the central compartment; Vd_{ss} volume of distribution at steady state; CL_B total body clearance. (*** P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05)

Discussion:

In veterinary medicine, antimicrobial and anti-inflammatory agents are commonly co-administered for management of pain and treatment of various infections. Following alone intravenous injection of tulathromycin in a single dose of 2.5 mg/ kgb.wt.in goats, the serum concentration time curve was best fitted by a two -compartment open model. Our result was disagreed with most of the results reported for tulathromycin in pigs (Benchaoui et al., 2004; Wange et al., 2012) rabbits (Abo-El-Sooud et al., 2012) and calves (Tohamy et al., 2011). The difference in kinetic parameters are relatively common and are frequently related to interspecies variation, age, breed, health status of the animals and/or the assay method used (Haddad et al., 1985). In the interaction study the results were consistent with that reported for the effect of flunixin on other antimicrobials as with sulphadimidine in horses by El-Banna (1999), enrofloxacin in rabbits (Elmas et al., 2008) and with the finding reported by (Tohamy, 2011) for orbifloxacin and flunixin in buffalo calves. But, differ from the findings recorded by (El-Hewaity, 2014) for flunixin and enrofloxacin in goats.

The drug when administrated with flunixin was rapidly distributed with a distribution half life t_{0.5(a)} of 0.046 h and it was slightly lower than when tulathromycin given alone (0.071 h) this was agreed with (Tohamy et al., 2011)

(0.13) h for orbifloxacin and flunixin. The rapid distribution of tulathromycin when administered concurrently with flunixin is indicated by the low value of p (0.18 h) than that of a (16.76h) due to increased tissue distribution this is in accordance with that recorded by **Iqbal et al.(2009)** and **Tohamy(2011)**. Volume of distribution at steady state ($V_{d(ss)}$) = average of 0.96 L /kg) was in accordance with that reported by (**Tohamy, 2011**) for orbifloxacin and flunixin in buffalo calves (1.04 L/kg) and that observed by (**El-Hewaity, 2014**) (0.47 L/kg) for cefepime and flunixin in goats, this increase of $V_{d(ss)}$ over V_c indicated that the peripheral compartment is the major compartment for tulathromycin distribution at steady state.

Tulathromycin with flunixin was relatively rapidly eliminated with an elimination half life of $t_{0.5(p)}$ of 5.05 h.(this result was higher than that when administered alone (6.43 h) this findings were in accordance with that reported by (**Tohamy, 2011**) (4.95 h) for orbifloxacin and flunixin in buffalo calves, and (**El-Banna, 1999**) for sulphadimidine in horses. This rapid of elimination of the drug from the body is coincident with high rate of clearance ($CL_B=0.17$ L/ kg/ h), this result was agreed with that reported by **Tohamy(2011)** (0.15 L/kg/h) for orbifloxacin and flunixin in buffalo calves and that observed by **El-Hewaity (2014)** for cefepime in goats with flunixin (0.096 L/ kg/ h). The observed decrease in drug clearances as a result of co-administration of flunixin indicates

that these drugs interact during the elimination phase (**Ongio et al., 2005**). Tulathromycin was distributed when combined with flunixin in the central compartment with a volume of distribution (V_c =average of 0.061 L /kg), this was lower than that of (**Whitem et al., 1996**) for ciprofloxacin with flunixin in human (59.22 L /kg), (0.262 L /kg) for penicillin G with phenylebutazone (**Firth et al., 1990**) and for enrofloxacin with flunixin in rabbits (4.98 L/kg) (**Iqbal et al., 2009**). It was concluded that, the combination of flunixin with tulathromycin altered the kinetics of tulathromycin after intravenous injection in healthy goats.

References:

- Abo-El-Sooud, K.; Afifi, N. A. and Abd-El-Aty, A. M. (2012). Pharmacokinetics and bioavailability of tulathromycin following intravenous, intramuscular and subcutaneous administrations in healthy rabbits. *Vet. World*, 2012, 5(7):424-428.
- Anderson, K.L., Hunt, E. and Davis, B.J. (1991). The influence of anti-inflammatory therapy on bacterial clearance following intramammary *Escherichia coli* challenge in goats. *Veterinary Research Communications*, 15, 147-161.
- Arret, B.; Johnson, D.P. and Kirshbaum, A. (1971). Outline of details of microbiology assay of antibiotics. Second Revision, *J.Pharm.Sci.*, 60:1690-1694.
- Baggot, J.D. (1978). Some aspects of

clinical pharmacology in veterinary medicine, *J.vet.Pharmacol.Ther* (1): 5-17.

Benchaoui, H.A., Nowakowski, M., Sherington, J., Rowan, T.G., and Sunderland, S.J. (2004). Pharmacokinetics and lung tissue concentrations of tulathromycin in swine. *Journal of Veterinary Pharmacology and Therapeutics*, 27, 203-210.

Brunton, L.L.; Laz, J.S. and Parker, K.L. (2008). Goodman & Gilman's The Pharmacological Basis of Therapeutics. 11th Ed. Med. Pub. Div., N.Y., Chic., San Franc., Lisbon, Lond., pp: 1173.

Craig, A.W. and Suh, B. (1991). Protein binding and the antibacterial effects. Method for the determination of protein binding. In *Antibiotics in laboratory Medicine*, 3rdedn. Ed Lorian, V., pp., 367-402. Williams & Wilkins, Baltimore, Maryland, USA.

Clothier, K.A., Leavens, T., Griffith, R.W., Wetzlich, S.E., Baynes, R.E., Riviere, J.E. and Tell, L.A. (2011): Pharmacokinetics of tulathromycin after single and multiple subcutaneous injections in domestic goats (*Capra aegagrus hircus*). *Journal of Veterinary Pharmacology and Therapeutics*, 34, 448-454.

El-Banna, H.A. (1999). Pharmacokinetic interactions between flunixin and sulphadimidine in horses. *DtschTierarztlWochenschr.*;106(9):400-

3. El-Hewaity, M. (2014): Influence of Flunixin on the Disposition Kinetic of Cefepime in Goats. *Advances in Pharmacological Sciences Volume 2014*, Article ID 471517, 5

Elmas, M., Yazar, E., Uney, K., Ays_eEr (Karabacak) and BunyaminTras (2008). Pharmacokinetics of enrofloxacin and flunixinmeoglumine and interactions between both drugs after intravenous co-administration in healthy and endotoxaemic rabbits. *The Veterinary Journal* 177, 418-424.

Evans, N.A. (2005). Tulathromycin: an overview of a new triamilide antibiotic for livestock respiratory disease. *Veterinary Therapeutics: research in applied veterinary medicine*, 6, 83-95.

Gibaldi, M. and Perrier, D. (1982). *Pharmacokinetics*, 2nd edn, pp., 409-424. MarcelDekker, New York.

Godinho, K.S.; Keane, S.G.; Nanjiani, I.A.; Benchaoui, H.A.; Sunderland, S.J.; Jones, M.A.; Weatherley, A.J.; Gootz, T.D.; and Rowan, T.G. (2005). Minimum inhibitory concentrations of tulathromycin against respiratory bacterial pathogens isolated from clinical cases in European cattle and swine and variability arising from changes in in vitro methodology. *Veterinary Therapeutics: research in applied veterinary medicine*, 6, 113-121.

Goudah, A., Sher Shah, S., Shin, H.C., Shim, J.H. and Abd El-Aty, A. M.

(2007). Pharmacokinetics and Mammary Residual Depletion of Erythromycin in Healthy Lactating Ewes. *J. Vet. Med. A* (54) 607-611

Grismer B., Rowe J.D., Carlson J., Wetzlich S.E., and Tell L.A. (2014). Pharmacokinetics of tulathromycin in plasma and milk samples after a single subcutaneous injection in lactating goats (*Capra hircus*). *Journal of veterinary pharmacology and therapeutics*; 37 : (2) 2058.

Iqbal, Z., Abbas Khan, AttiqaNaz, Jamshaid A. Khan and Ghulam S. Khan (2009). Pharmacokinetic Interaction of Ciprofloxacin with Diclofenac A Single-Dose, Two-Period Crossover Study in Healthy Adult Volunteers. *Clin Drug Invest* 29 (4): 275-281

Haddad, N.S., Pedersoli, W.M., Ravis, W.R., Fazeli, M.H. and Carson, R.L. (1985). Combined pharmacokinetics of gentamicin in pony mares after a single intravenous and intramuscular administration. *American Journal of Veterinary Research*, 46: 2004-2007.

Konigsson, K., Torneke, K., Engeland, I.V., Odensvik, K. and Kindahl, H. (2003). Pharmacokinetics and Pharmacodynamic Effects of Flunixin after Intravenous, Intramuscular and Oral Administration to Dairy Goats. *Acta vet. scand.*, 44, 153-159.

Nowakowski, M.A., Inskeep, P.B., Risk, J.E., Skogerboe, T.L., Benchaoui, H.A.,

Meinert, T.R., Sherington, J., and Sunderland, S.J. (2004): Pharmacokinetics and lung tissue concentrations of tulathromycin, a new triamilide antibiotic, in cattle. *Veterinary Therapy: research in applied veterinary medicine*, 5, 60-74.

Nutsch, R.G.; Hart F.J; Rooney K.A; Weigel D.J; Kilgore W.R. and Skogerboe T.L. (2005). Efficacy of tulathromycin injectable solution for the treatment of naturally occurring swine respiratory disease. *Vet. Ther.*, 6: 214-224.

Ogino, T., Mizuno, Y., Ogata, T., and Takahashi, Y., (2005). Pharmacokinetic interactions of flunixin meglumine and enrofloxacin in dogs. *American Journal of Veterinary Research* (66) 1209-1213.

Robb, E.J.; Tucker C.M., Corley L., Bryson W.L. and Rogers K.C. (2007). "Efficacy of tulathromycin versus enrofloxacin for initial treatment of naturally occurring bovine respiratory disease in feeder calves". *Vet. Ther.*; 8: 127-135.

Romanet J, Smith GW, Leavens TL, Baynes RE, Wetzlich SE, Riviere JE, and Tell LA (2012). Pharmacokinetics and tissue elimination of tulathromycin following subcutaneous administration in meat goats. *American journal of veterinary research*, 73(10): 1634-1640.

Skogerboe, T.L.; Rooney, K.A.; Nutsch, R.G.; Weigel, D.J.; Gajewski, K. and Kilgore, W.R. (2005). Comparative efficacy of tulathromycin versus florfenicol and tilmicosin against

undifferentiated bovine respiratory disease in feedlot cattle. *Vet. Ther.*, 6: 180-196. Snedecor, G.W. and Cochran, W.G. (1976). *Statistical methods*. 6th Ed. Ames, Iowa, USA, pp.,502-503.

Tohamy, M.A. (2011). pharmacokinetic interactions of flunixin and orbifloxacin in buffalo calves. *Insight pharmaceutical sciences* 1 (3): 2933.

Tohamy, M.A., El-Gendy, A.A..M andTaha, A.Attia (2011). Some pharmacokinetic aspects of tulathromycin in Fresian cattle calves.*Journal of American Science*7(5): 651-655.

Venner, M.; Kerth R. and Klug, E. (2007). Evaluation of tulathromycin in the treatment of pulmonary abscesses in foals. *Vet. J.*, 174: 418- 421. Villarino, N., Brown, S.A. and Martin-Jimenez, T., (2013). The role of the macrolide tulathromycin in veterinary medicine. *The Veterinary Journal*, 198, 352-357.

Wang X., Tao YF, Huang L.L., Chen D.M., Yin S.Z., Ihsan A., Zhou W., Su S.J., Liu Z.L., Pan Y.H., and Yuan Z.H. (2012). Pharmacokinetics of tulathromycin and its metabolite in swine administered with an intravenous bolus injection and a single gavage. *Journal of Veterinary Pharmacology and Therapeutics*,35(3):282-289 Whittam, T., Firth, E.C., Hodge, H., and Turner, K., (1996). Pharmacokinetic interactions between repeated dose phenylbutazone and gentamicin in the horse. *Journal of*

Veterinary Pharmacology and Therapeutics 19, 454-459.

Young, G., Smith, G.W., Leavens, T.L., Wetzlich, S.E., Baynes, R.E., Mason, S.E., Riviere, J.E. and Tell, L.A. (2011). Pharmacokinetics of tulathromycin following subcutaneous administration in meat goats. *Research in Veterinary Science*, 90, 477-479.

Zu-Gong, Y, J. Chun-Ao, G. Yong-Gang, H. Yi-Yi and C. Da-Jianl (2007). pharmacokinetics of flunixinmeglumine after intravenous and intramuscular administration in pigs. *Agric. Sci. Chin.*, 6: 1396- 1401.