International Journal of Pharmacology and Toxicology, 8 (1) (2020) 60-64

International Journal of Pharmacology and Toxicology

Website: www.sciencepubco.com/index.php/IJPT

Research paper

Bioequivalence study of two oral lincomycin formulations (lincopharm 800® and lincoyosr®) in broiler chickens

Ashraf Elkomy [1] *, Mohamed Aboubakr [2]

[1] Professor and Head of Pharmacology, Department, Faculty of Veterinary Medicine, Benha University, Egypt
[2] Assistant Professor of Pharmacology, Faculty of Veterinary Medicine, Benha University, Egypt
**Corresponding author E-mail: ashraf.komy@hotmail.com*

Abstract

The present study was designed to assess the comparative bio-equivalence of Lincopharm 800® and Lincoyosr® in healthy broiler chicken after oral administration of both products in a dose of 20 mg lincomycin base/kg b.wt. Twenty four broiler chickens were divided into two groups. The first group was designed to study the pharmacokinetics of Lincopharm 800®, while the 2nd group was designed to study the pharmacokinetics of Lincoyosr®. Each broiler chicken in both groups was orally administered with 20 mg lincomycin base/kg b.wt. Blood samples were obtained from the wing vein and collected immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 hours after a single oral administration. The disposition kinetics of Lincopharm 800® and Lincoyosr® following oral administration of 20 mg lincomycin base /kg b.wt, revealed that the maximum blood concentration of lincomycin [C_{max}] were 4.81 and 4.62 µg/ml and attained at [t_{max}] of 1.36 and 1.35 hours, respectively. In conclusion: Lincoyosr® is bioequivalent to Lincopharm 800® since the ratios of Cmax, AUC_{0-24} and $AUC_{0-\infty}$ (T/R) was 0.96, 0.92 and 0.91 respectively. These are within the bioequivalence acceptance range. Lincoyosr® and Lincopharm 800® are therefore bioequivalent and interchangeable.

Keywords: *Bioequivalence, Chickens; Lincomycin; oral; Pharmacokinetics*.

1. Introduction

Lincosamides is a group of monoglycoside antibiotics containing amino-acid like side chain. It is a miscellaneous group of protein inhibiting antimicrobials with activities similar to members of the macrolide group of antibiotics. Lincomycin is a member of the lincosamide antibiotics, mainly active against Staphylococci, Streptococci and anaerobic bacteria including Bacteroides fragilis (Giguère, 2006). It is used alone or in combination with other drugs in poultry for oral treatment of bacterial enteric infections, control of respiratory infections and growth enhancement.

The pharmacokinetics of lincomycin have been determined for a variety of animals including sheep (Ziv and Sulman, 1973), dogs (Brown et al., 1975), calves (Burrows et al., 1983; Gouri et al., 2014), pigs (Chaleva and Nguyen, 1987), chickens (Amer, 1987; Soback et al., 1987), and cats (Albarellos et al., 2012).

Lincomycin is used in chickens either alone or in combination with other antibiotics (e.g. lincomycin-spectinomycin) for the treatment of air-sacculitis caused by either M. synoviae or M. gallisepticum and complicated chronic respiratory diseasecaused by E. coli and M. gallisepticum (Abu Basha et al., 2007).

The bioavailability and bioequivalence studies play an important role in determining therapeutic efficacy to register the generic drug products according to the Food and Drug Administration (FDA) regulations (Chen et al., 2001). Bioavailability is defined as the rate and extent to which an active drug ingredient is absorbed and becomes available at the site of drug action. In case of bioequivalence it is defined as statistically equivalent bioavailability between two products at the same molar dose of the therapeutic moiety under similar experimental conditions (Chen et al., 2001; Toutain and Bousquet-Melou, 2004). The drug products are said to be bioequivalent if they are pharmaceutical equivalents or pharmaceutical alternatives and if their rate and extent of absorption do not show a significant differences statistically according to the FDA regulations (Chen et al., 2001).

The aim of this study is to evaluate bioequivalence of two oral lincomycine water soluble powders (Lincopharm 800® and Lincoyosr®) after oral administration of a single dose in broiler chickens.

2. Materials and methods

2.1. Drugs

Lincopharm 800® was obtained from Bayer Australia (it was used as reference product) and Lincoyosr® was obtained from Boston Company, Elyoser Division, Egypt (it was used as test product). Both are formulated as water soluble powders for use in drinking water and each one gram contains 800 mg Lincomycin base (as lincomycin hydrochloride).

2.2. Broiler chickens and experimental design

Twenty four healthy broiler chickens (30 days old and weighing 1.60 – 1.85 kg) were obtained from Benha private poultry farm, Egypt. They were kept individually in cages, within a ventilated, heated room (20˚C), and 14 hours of day light. They received a standard commercial ration free from any antibiotics before starting the experiment to insure complete clearance of any anti-bacterial substances from their bodies. Water was offered ad-libitum.

2.3. Bioequivalence study

Broiler chickens were used to study the bio-equivalence of Lincopharm 800® and Lincoyosr® after oral administration. Broiler chickens were divided into two groups. The 1st group (12 broiler chickens) was used to study the pharmacokinetics of Lincopharm 800®. The 2nd group (12 broiler chickens) was used to study the pharmacokinetics of Lincoyosr®. Broiler chickens in the 1st group were administered orally (in drinking water) with Lincopharm 800® in a dose of 20 mg lincomycin base/kg b.wt, while broiler chickens in the 2nd group were administered orally with Lincoyosr® in a dose of 20 mg lincomycin base.

2.4. Blood samples

Blood samples were obtained from the wing vein (1 ml) and collected in test tubes immediately before and at 0.08, 0.16, 0.25, 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after a single oral administration (groups 1 and 2). Samples were centrifuged at 3000 rpm for 10 minutes and the obtained sera were used for the estimation of lincomycin concentration. The serum samples were stored at −20˚C until drug assay.

2.5. Analytical procedure

Lincomycin was assayed in serum of chickens by modified spectrophotometric method (Rajeevkumar and Subramanian, 2010) by using a double beam UV- visible spectrophotometer (T60U, United Kingdom). A stock solution (100 µg / ml) of lincomycin in distilled water or serum (antibiotic free) of normal chickens were prepared. Standard concentrations were obtained by further dilution to obtain concentrations varying from 1.25, 2.5, 5, 10, 25, 50 to 100 µg lincomycin per milliliter-distilled water or serum. Optical densities of the drug molecule of different concentrations were read at 196 nm, using a quartz cuvette by a double beam UV- visible spectrophotometer. Concentrations of the drug at different time intervals were obtained and then plotted against optical densities on a graph paper to obtain standard curves.

2.6. Pharmacokinetics analysis

Serum concentrations of lincomycin versus time data obtained during the study were utilized for calculating various pharmacokinetic variables using a compartmental and non-compartmental analysis using computerized program, WinNonline 4.1 (Pharsight, USA).
The peak concentrations, C_{max} and time to peak, T_{max} were obtained from the serum concentration-time data directly. The areas under the serum concentration of lincomycin time curves from time 0 to the last sample collected (AUC_{0-24}) were calculated using linear trapezoidal method (Baggot, 2001). While $AUC_{0-\infty}$ was derived from $AUC_{0-24} + AUC_{24-\infty}$, where $AUC_{24-\infty} = C24/ß$. For bioequivalence evaluation, the ratios of C_{max} (T/R), AUC_{0-24} (T/R) and $AUC_{0-\infty}$ (T/R) were calculated. Values within the bioequivalence acceptable range at 90% confidence interval, 0.80 – 1.25 were considered for accepting the null hypothesis of bioequivalence between the reference and the test brands (EMEA, 2002, 2006).

3. Results

The mean serum concentrations of lincomycin in Lincopharm 800® and Lincoyosr® following oral administration of 20 mg lincomycin base/kg b.wt, in broiler chickens are shown in (Table 1 and Figure 1).

Table 1: Mean (X ± S.E) Serum Concentrations (mg/ml) of Lincomycin in Lincopharm 800® and Lincoyosr® Following Oral Administration of 20 mg Lincomycin Base/kg b.wt In Broiler Chickens (N = 12)

Time post Administration (hour)	Mean serum concentration (µg/ml)	
	Group 1 Lincopharm 800® (Reference)	Group 2 Lincoyosr® (Test)
0.08	0.45±0.04	0.38±0.02
0.16	1.03±0.09	0.94±0.04
0.25	2.44±0.13	2.37±0.11
0.5	3.67±0.14	3.53±0.14
1	5.19±0.23	5.01±0.21
2	4.34±0.21	4.11±0.18
4	3.24±0.12	3.10±0.12
6	2.28±0.03	2.12±0.06
8	1.67±0.02	1.52±0.05
12	1.07±0.01	0.96±0.04
24	0.53±0.01	0.50±0.01

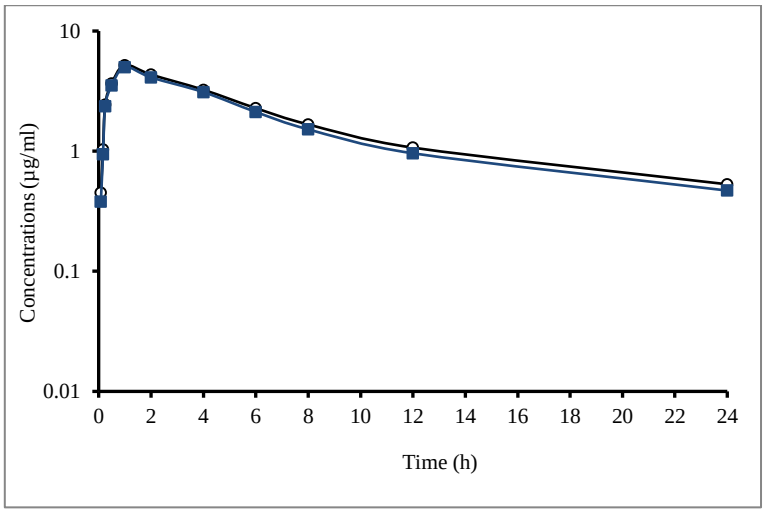

Fig. 1: Semilogarthimic Plot Showing the Serum Concentrations-Time Profile of Lincomycin in Lincopharm 800® (○) and Lincoyosr® (■) Following Oral Administration of 20 mg Lincomycin Base/kg b.wt In Broiler Chickens (N = 12).

The mean pharmacokinetic parameters of lincomycin in Lincopharm 800® and Lincoyosr® after oral administration of 20 mg lincomycin base/kg b.wt, in broiler chickens are shown in (Table 2).

Table 2: Mean (X ± S.E) pharmacokinetic parameters of lincomycin in Lincopharm 800® and Lincoyosr® following oral administration of 20 mg lincomycin base/kg b.wt in broiler chickens (n = 12)

Parameter	Unit	Lincopharm 800® (Reference)	Lincoyosr® (Test)
K_{ab}	h^{-1}	1.95 ± 0.06	1.91 ± 0.04
K_{el}	h^{-1}	0.068 ± 0.001	0.070 ± 0.001
$t_{1/2(ab)}$	h	0.35 ± 0.01	0.36 ± 0.01
$t_{1/2(el)}$	h	10.09 ± 0.34	9.87 ± 0.33
C_{max}	µg ml^{-1}	4.81 ± 0.15	4.62 ± 0.17
t_{max}	h	1.36 ± 0.03	1.35 ± 0.03
AUC	µg $ml^{-1}h^{-1}$	47.82 ± 2.47	43.95 ± 2.11
AUMC	µg $ml^{-1}h^{-2}$	587.63 ± 34.63	519.65 ±33.87
MRT	h	12.28 ± 0.47	11.82 ± 0.43

k_{ab}; K_{el} absorbtion and elimination rate constant after oral administration; $T_{1/2(ab)}$ absorbtion half life after oral administration; $T_{1/2(el)}$ elimination half life after oral administration; C_{max} maximum plasma concentration; T_{max} time to peak plasma concentration; AUC; area under serum concentration-time curve; AUMC area under moment curve; MRT mean residence time.

The disposition kinetics of lincomycin in Lincopharm 800® and Lincoyosr® following oral administration of 20 mg lincomycin base/kg b.wt, revealed that the maximum blood concentration [C_{max}] were 4.81 and 4.62 µg/ml and attained at [T_{max}] of 1.36 and 1.35 hours, respectively. The mean ratio of C_{max} and AUC of the reference and tested formulations were within bioequivalence range and summarized in Table 3. All the experimental chickens remained healthy during and after the study.

Table 3: Bioequivalence between Lincopharm 800® (Reference) and Lincoyosr® (Test) Formulations

Bioequivalence	C_{max}	AUC_{0-24}	$AUC_{0-\infty}$
Lincopharm 800® (Reference)	4.81±0.15	40.10±2.05	47.82±2.47
Lincoyosr® (Test)	4.62±0.17	37.25±1.73	43.95±2.11
Point estimate	0.96	0.92	0.91
Acceptable range	0.80-1.25	0.80-1.25	0.80-1.25
Conclusion	BE	BE	BE

BE-Bioequivalence.

4. Discussion

Antibiotics are widely used as veterinary drugs or as feed additives to promote growth (Yoshida et al., 1971; 1973; Yoshimura et al., 1991).

The effectiveness of a drug is partly dependent on its formulation, route of administration and metabolic pattern. These factors determine the serum concentration-time profile of the drug.

Qualitative and quantitative differences in dosage might be attributed to these differences in results. These variations in pharmacokinetic parameters were relatively common and frequently related to method used, healthy status of animal and specific interspecies variation (El-Sayed et al., 1989).

Following administration of a single oral dose of lincomycin to healthy broiler chickens, therapeutic concentration were achieved 5 minutes post administration in all the chickens. The concentration was detected up to 24 hours in the serum of chickens and exceeds the MIC of lincomycin against Mycoplasma synoviae= 0.50 µg/ml; Kreizinger et al., 2017).

In the present study, Lincomycin reached its maximum plasma concentration (4.81 and 4.62 µg/ml for both Lincopharm 800® and Lincoyosr®, respectively. This C_{max} was higher than recorded in chickens (1.62 µg/ml; Amer, 1987) and nearly similar to that of pigs (5 µg/ml; Nielsen and Gyrd-Hansen, 1998) and (5.15 µg/ml; Fan et al., 2012). On the other hand, it was lower than reported in cat (22.52 µg/ml; Albarellos et al., 2013) and in healthy chickens (10.72 µg/ml; Abo Sreea, 2014).

The present results revealed that lincomycin reached its maximum plasma concentration after maximum time (t_{max}) of 1.36 and 1.35 h for both Lincopharm 800® and Lincoyosr®, respectively. This result was lower than reported in fasted pigs (2.9 h; Nielsen and Gyrd-Hansen,

1998), while it was higher than that recorded in healthy chickens (0.80 h; Amer, 1987) and (0.76 h; Abo Sreea, 2014) and in cats (0.80 h; Albarellos et al., 2012).

Lincomycin was eliminated with the elimination half-life ($t_{1/2el}$) of 10.09 and 9.87 h for both Lincopharm 800® and Lincoyosr®, respectively. This result was higher than that recorded in healthy chickens (3.35 h; Amer, 1987), cats (4.12 h; Albarellos et al., 2013), and in healthy chickens (1.74 h; Abo Sreea, 2014).

Bioequivalence study is a test to assure the clinical efficacy of a generic versus brand drugs (Chen et al., 2001). Bioequivalence refers to a comparison between generic formulations of a drug, or a product in which a change has been made in one or more of the ingredients or in the manufacturing process, and a reference dosage form of the same drug. This study shows that the bioequivalence ratio for mean AUC_{0-24}, $AUC_{0-\infty}$ and C_{max} (T/R) of Lincoyosr® versus the reference products (Lincopharm 800®) were 0.96, 0.92 and 0.91 respectively. These values were within the recommended range at the level of 90% confidence interval, 0.80–1.25 (U.S. Food and Drug Administration, 2003).

5. Conclusions

Based on the above pharmacokinetic and statistical results that calculated in the current study, we concluded that Lincoyosr® which manufactured by Boston Company, Elyoser Division, Egypt was bioequivalent to Lincopharm 800® which manufactured by Bayer, Australia and both products can be used as interchangeable drug in veterinary medicine practice especially in poultry.

References

[1] Abo Sreea, T. (2014) Effect of acidifiers on pharmacokinetic and tissue residues of lincomycin in broiler chickens. Thesis presented to Faculty of Veterinary Medicine, Cairo University.

[2] Abu-Basha EA, Gehring R, Albwa'neh SJ. (2007) Pharmacokinetics and bioavailability of spectinomycin after i.v., i.m., s.c. and oral administrationin broiler chickens. J Vet Pharmacol Ther. 30(2):139-44. https://doi.org/10.1111/j.1365-2885.2007.00825.x.

[3] Albarellos GA, Montoya L, Denamiel GA, Velo MC & Landoni MF (2012) Pharmacokinetics and bone tissue concentrations of lincomycin following intravenous and intramuscular administrations to cats. Journal of Veterinary Pharmacology and Therapeutics, 35, 534–540. https://doi.org/10.1111/j.1365-2885.2011.01355.x.

[4] Albarellos, GA, Montoya, L, Denamiel, GA. Velo, M.C. & Landoni, M.F. (2013) Pharmacokinetics and skin concentrations of lincomycin after intravenous and oral administration to cats. J. S. Afr. Vet. Assoc, 84(1), 1-5. https://doi.org/10.4102/jsava.v84i1.968.

[5] Amer Aziza. (1987) Some pharmacokinetic aspects of certain antibiotics in chickens" Ph.D. Thesis presented to Faculty of Veterinary Medicine, Cairo University.

[6] Baggot, J.D. (2001). The physiological Basis of veterinary clinical pharmacology. 1st ed. Blackwell, London. https://doi.org/10.1002/9780470690567.

[7] Bakker, E.P. (1992) Aminoglycoside and aminocyclitol antibiotics: hygromycin B is an atypical bactericidal compound that exerts effects on cells of Escherichia coli characteristics for bacteriostatic aminocyclitols. Journal of General Microbiology, 138, 563–569. https://doi.org/10.1099/00221287-138-3-563.

[8] Brown RB, Barza M, Brusch JL, Hashimoto Y&Weinstein L. (1975) Pharmacokinetics of lincomycin and clindamycin phosphate in a canine model. Journal of Infectious Diseases, 131, 252–260. https://doi.org/10.1093/infdis/131.3.252.

[9] Burrows GE, Barto, PB, Martin B & Tripp ML, (1983) Comparative pharmacokinetics of antibiotics in newborn calves, lincomycin, and tylosin. American Journal of Veterinary Research, 44, 1053–1057.

[10] Burrows, G.E. (1980) Aminocyclitol antibiotics. Journal of American Veterinary Medical Association, 176, 1280–1281.

[11] Burrows, G.E., Morton, R.J. & Fales, W.H. (1993) Microdilution antimicrobial susceptibilities of selected gram-negative veterinary bacterial isolates. Journal of Veterinary Diagnostic Investigation, 5, 541–547. https://doi.org/10.1177/104063879300500407.

[12] Chaleva E and Nguyen DL. (1987) Pharmacokinetic research on Pharmachem's lincomycin hydrochloride in pigs. Veterinarni Medicina Nauki, 24, 47– 51.

[13] Chen, M.L., V. Shah, R. Patnaik, W. Adams, A. Hussain, D. Conner, M. Mehta, H. alinowski, J. Lazor, S.M. Huang, D. Hare, L. Lesko, D. Sporn and R. Williams, (2001). Bioavailability and bioequivalence: An FDA regulatory overview. Pharmaceutical Res., 18: 1645-1650. https://doi.org/10.1023/A:1013319408893.

[14] EL-Sayed, MGA, Hatem, ME and EL-Komy, AAA. (1989) Disposition kinetics of gentamycin in normal and endometric cows using microbiological assay. Deutsche Tierärztliche Wochenschrift, 96, 412-415.

[15] EMEA (2002): Guidelines for the conduct of bioequivalence studies for veterinary medicinal products,1-11[http://www.emea.eu.int/pdfs/vet/ewp/001600en.pdf]2001. Accessed: 30.12.2002.

[16] EMEA. (2006): The European Agency for Evaluation of Medicinal Products. Questions and Answers on Bioavailability and Bioequivalence Guidance.

[17] Fan, H Wang, Z Wang, L Ye, Y and Huang, X. (2012) Pharmacokinetics of lincomycin hydrochloride and spectinomycin sulfate suspension for injection in swine. Journal of South China, 33(2), 244-247.

[18] Giguère S. (2006) Lincosamides, pleuromutilins, and streptogramins. Antimicrobial therapy in veterinary medicine, 4th edn, 179-184.

[19] Gouri,S. Venkatachalam,D. and Dumka,VK. (2014) Pharmacokinetics of lincomycin following single intravenous administration in buffalo calves. Trop Anim Health Prod, 46(6), 1099-102. https://doi.org/10.1007/s11250-014-0595-4.

[20] Guerin-Faublee, V., Flandrois, J.P., Broye, E., Tupin, F. & Richard, Y. (1993) Actinomyces pyogenes: susceptibility of 103 clinical animal isolates to 22 antimicrobial agents. Veterinary Reserach, 24, 251–259.

[21] Holloway, W.J. (1982) Spectinomycin. The Medical Clinics of North America, 66, 169–173. https://doi.org/10.1016/S0025-7125(16)31450-X.

[22] Jeff, M., Zimmerman, L. & James, M. (2002) In vivo selection of spectinomycin-binding RNAs. Nucleic Acids Research, 30, 5425–5435. https://doi.org/10.1093/nar/gkf687.

[23] Jordan, F.T., Forrester, C.A., Ripley, P.H. & Burch, D.G. (1998) In vitro and in vivo comparisons of valnemulin, tiamulin, tylosin, enrofloxacin, and lincomycin/spectinomycin against Mycoplasma gallisepticum. Avian Disease, 42, 738–745. https://doi.org/10.2307/1592709.

[24] Kreizinger Z, Grózner D, Sulyok KM, Nilsson K, Hrivnák V, Benčina D, Gyuranecz M. (2017) Antibiotic susceptibility profiles of *Mycoplasma synoviae* strains originating from Central and Eastern Europe. BMC Vet Res. 13(1):342. https://doi.org/10.1186/s12917-017-1266-2.

[25] Nielsen, P and Gyrd-Hansen, N. (1998) Bioavailability of spiramycin and lincomycin after oral administration to fed and fasted pigs. Journal of Veterinary Pharmacology and Therapeutics, 21, 251–256. https://doi.org/10.1046/j.1365-2885.1998.00131.x.

[26] Rajeevkumar, P. and Subramanian N. (2010) Determination of Lincomycin hydrochloride in pure form and pharmaceutical formulations. International Journal of Chem Tech Research, 2(4), 2052-2055.

[27] Schwarz, S., Kehrenberg, C., Salmon, S.A. & Watts, J.L. (2004) In vitro activities of spectinomycin and comparator agents against Pasteurella multocida and Mannheimia haemolytica from respiratory tract infections of cattle. The Journal of Antimicrobial Chemotherapy, 53, 379–382. https://doi.org/10.1093/jac/dkh059.

[28] Soback S, Ziv G, Bogin E, Cohen Z and Earon Y. (1987) Pharmacokinetic changes of several antibiotics in chickens during induced fatty liver. Research in Veterinary Science, 43, 49–54. https://doi.org/10.1016/S0034-5288(18)30740-9.
[29] Toutain, P.L. and A. Bousquet-Melou, (2004). Bioavailability and its assessment. J. Pharmacol. Therap., 27: 455-466. https://doi.org/10.1111/j.1365-2885.2004.00604.x.
[30] U.S. Food and Drug Administration. 2003. Guidance for industry: bioavailability and bioequivalence studies for orally administered drug products.U.S. Food and Drug Administration, Washington, DC.
[31] Yoshida, M., Kubota, D., Yonezawa, S. et al. (1971). Transfer of dietary spiramycin into the eggs and its residue in the liver of laying hen. The Japanese Journal of Antibiotics 8, 103-110. https://doi.org/10.2141/jpsa.8.103.
[32] Yoshida, M., Kubota, d., Yonezawa, S. et al. (1973). Transfer of dietary oxytetracycline into the eggs and its residue in the liver of laying hen. The Japanese Journal of Antibiotics 10, 29-36. https://doi.org/10.2141/jpsa.10.29.
[33] Yoshimura, H., Osawa, N., Rasa, F. et al. (1991). Residues of oxytetracycline and oxytetracycline in eggs after medication via drinking water to laying hens. Food Additives and contaminants 8, 65-69. https://doi.org/10.1080/02652039109373956.
[34] Ziv G and Sulman G. (1973) Penetration of lincomycin and clindamycin into milk in ewes. British Veterinary Journal, 129, 83–91. https://doi.org/10.1016/S0007-1935(17)36593-4.