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Full Length Research Paper

In vitro effect of tigecycline against *Mycobacterium tuberculosis* and a review of the available drugs for tuberculosis

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The study aimed to investigate the effectiveness of a novel antibiotic drug tigecycline on clinical isolates of *Mycobacterium tuberculosis* and, reviewing defined anti-tuberculosis effects of available agents. Minimal inhibitory concentration (MIC) of tigecycline for 50 *M. tuberculosis* including multidrug-resistant (MDR) clinical isolates (20 MDR isolates) was determined by broth microdilution method in the study. Tigecycline MIC values were ranging between 8 and 64 µg/ml. However, there is not any defined break point for *M. tuberculosis* resistance. In conclusion, it seems that the *in vitro* effectiveness of tigecycline to *M. tuberculosis* is not good but further *in vivo* studies are needed.

Key words: *Mycobacterium tuberculosis,* tigecycline.

INTRODUCTION

One-third of world population is infected with *Mycobacterium tuberculosis* therefore; they have risk for development of active tuberculosis. Approximately 8.8 million people are diagnosed with active tuberculosis that causes 1.6 million deaths per year. Isoniazid, rifampicin, ethambutol and pyrazinamide are primary drugs used in tuberculosis. Treatment period requires at least six months for complete cure (Van den Boogard et al., 2009). Tuberculosis can be treated successfully by primary drugs when drug resistance is not defined. Nevertheless, there is high treatment failure rate in multi-drug resistant tuberculosis (MDR-TB; at least rifampicin and isoniazid resistance) and extensively drug resistant tuberculosis (XDR-TB; resistance to any one of fluoroquinolones and any one of aminoglycoside in addition to MDR-TB). Second-line drugs are used in the treatment of MDR-TB, have many side effects, and require long treatment period that limit their usage (Jacobson et al., 2010).

Therefore, there has recently been an effort for exploring new anti-tuberculosis drugs. While efforts have been exerted in developing new drugs, anti-tuberculosis effects of antimicrobials developed for treatment of other infectious diseases have been investigated. One of the best examples is linezolid. Following the demonstration of its anti-tuberculosis effect, linezolid took place among second-line drugs in a short period (Pholwat et al., 2010). Tigecycline is a novel drug that is bacteriostatic agent with broad antibacterial spectrum. It exhibits antimicrobial effect by blocking of protein synthesis; it binds reversibly to 30S sub-unit of ribosome, blocking the binding of acyl-tRNA to region A (Courvalin et al., 2010). Although tigecycline has demonstrated antibacterial activity against several bacteria, there has not been any data regarding anti-tuberculosis effect.

For this purpose the study aimed to investigate *in vitro* tigecycline effect against clinical isolates of *M. tuberculosis* (including MDR-TB), and review new

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Table 1. Antibiotic susceptibility/resistance profile of tested *M. tuberculosis* clinical isolates.

Resistance profile	Number of isolates
INH	7
STR	2
ETM	2
INH+RIF	8 (MDR)
INH+STR	1
INH+RIF+ETM	7 (MDR)
INH+RIF+STR	2 (MDR)
INH+RIF+STR+ETM	3 (MDR)
Susceptible to all drugs	18
Total	50 (20 MDR)

INH: isoniazid, RIF: rifampicin, STR: streptomycin, ETM: ethambutol, MDR: multidrug resistant.

developed anti-tuberculosis and other available drugs regarding effectiveness on tuberculosis bacilli.

MATERIALS AND METHODS

Mycobacterial isolates

Fifty *M. tuberculosis* clinical isolates and four standard strains, that is H37Rv (susceptible to all drugs), ATCC 35822 (isoniazid resistant), ATCC 35820 (streptomycin resistant) and ATCC 38838 (rifampicin resistant), were tested in the study. Antibiotics susceptibility profile of tested bacteria is summarized in Table 1. Twenty of tested clinical isolates were MDR-TB.

Antibacterial agent

Standard powder of tigecycline was obtained from Wyeth Research. Stock solution of tigecycline was prepared and stored at -80°C until it was used.

Investigation of tigecycline susceptibility by broth microdilution method

Effect of tigecycline against *M. tuberculosis* clinical isolates was investigated by broth microdilution method which was previously described for investigation of susceptibility of anti- tuberculosis agents against tuberculosis bacilli (Coban et al., 2004; Leite et al., 2000). Clinical and Laboratory Standards Institute (CLSI) stated that tigecycline should be tested against bacteria in fresh medium (12 h). It is also stated that plates prepared by fresh medium can be stored in - 80°C until they will be tested (CLSI, 2008). Middlebrook 7H9 liquid medium contains OADC (oleic acid, albumin, dextrose and catalase) was prepared freshly in the study. Tigecycline solutions with concentration of 128 to 0.125 µg/ml were prepared by serial two-fold dilutions in 96 well U-shaped based plates. Antibiotic was not added into one well for growth control. Prepared plates were stored at -80°C until they were tested.

M. tuberculosis colonies were transferred from fresh cultures of Löwenstein-Jensen medium into serum physiologic and 5 to 10 glass beads containing tubes. After they were vortexed for 3 to 5 min, they kept in vertical position for sedimentation of large particles and aerosols for 30 min. Supernatant was transferred into another tube and adjusted to Mc Farland no 0.5 standard. Five microlitres of every specimen, adjusted to Mc Farland no 0.5, was inoculated into plate wells. Plates were inserted into plastic bags and incubated at 37°C. Plates were evaluated on the 14 and 21th days of incubation and, minimum inhibitory concentration (MIC) values were reported on the 21th day. MIC value was determined as the last well of no growth seen by naked eye.

RESULTS

MIC values of tested bacteria evaluated on the 14 and 21th days in the study. MIC values, obtained on the 14th day, usually showed two- fold rising on the 21th day. Results were interpreted according to MIC values obtained on the 21th day. MIC values of tested bacteria were determined between 8 and 64 µg/ml and presented in Table 2. MIC values of H37Rv, ATCC 35822, ATCC 35820 and ATCC 38838 were 32, 32, 64 and 16 µg/ml, respectively. MIC value of three isolates was 8 µg/ml, of 11 isolates was 16 µg/ml, of 30 isolates was 32 µg/ml and of six isolates was 64 µg/ml. Although there is no defined breakpoint value in the study, none of the isolates has been observed susceptible to tigecycline when resistance break point value of 8 µg/ml for non-tuberculosis mycobacteria is considered.

DISCUSSION

It is not found in any study that investigates *in vitro* or *in vivo* effects of tigecycline against clinical isolates of *M. tuberculosis* in literature. Nevertheless, there are studies that show *in vitro* and *in vivo* effectiveness of tigecycline against non *M. tuberculosis* mycobacteria. The *in vitro* studies are reviewed chronologically. Rhomberg and Jones (2002) reported that MIC values of tigecycline (GAR936) against *Mycobacterium marinum* were ranging between 24 and 0.19 µg/ml. Wallace et al. (2002) tested rapidly growing mycobacteria (*Mycobacterium abscessus, Mycobacterium chelonae* and *Mycobacterium fortuitum*) and slowly growing mycobacteria (*Mycobacterium avium* complex, *Mycobacterium lentiflavum, M. marinum* and *Mycobacterium kansasii*), and determined that tigecycline has no effect on slow growing non-tuberculous mycobacteria. In our study, tigecycline MIC values of *M. tuberculosis* isolates had been found 8 to 64 µg/ml. Martin-de-When Hijas et al. (2008) tested 169 clinical isolates of non-pigmented rapidly growing mycobacteria by E-test and microdilution methods, and they had not determined any resistant isolate by any of the methods. Fernandez-Roblas et al. (2008) tested 50 non- pigmented rapidly growing collection strains and 165 clinical isolates, and showed that all isolates and strains had been inhibited by 1 µg/ml concentration of tigecycline. Garcia- Agudo et al. (2009) had also reported that all isolates of rapidly growing mycobacteria had been susceptible to tigecycline.

Table 2. Tigecycline MIC values and susceptibility to anti-tuberculosis drugs

	MIC of TGC	INH	RIF	STR	ETM
H37Rv	32	S	S	S	S
ATCC 35822	32	R	S	S	S
ATCC 35820	64	S	S	R	S
ATCC 38838	16	S	R	S	S
1	64	R	S	S	S
2	64	R	R	S	S
3	64	S	S	S	S
4	32	R	R	S	S
5	32	R	R	R	R
6	8	R	R	S	S
7	64	R	R	R	R
8	32	R	R	S	R
9	32	R	S	R	S
10	32	R	R	S	S
11	32	R	R	S	R
12	32	R	R	S	S
13	32	R	R	S	S
14	32	R	R	S	R
15	64	R	R	R	R
16	32	R	R	S	S
17	32	R	S	S	S
18	32	S	S	R	S
19	32	R	R	R	S
20	16	S	S	R	S
21	16	S	S	S	R
22	16	S	S	S	S
23	16	S	S	S	S
24	32	R	S	S	S
25	64	R	S	S	S
26	32	S	S	S	S
27	32	S	S	S	S
28	16	S	S	S	R
29	32	S	S	S	S
30	32	R	R	S	S
31	32	S	S	S	S
32	32	S	S	S	S
33	16	R	S	S	S
34	32	S	S	S	S
35	32	S	S	S	S
36	16	S	S	S	S
37	32	S	S	S	S
38	32	R	R	S	R
39	32	S	S	S	S
40	32	R	S	S	S
41	32	R	R	S	R
42	32	R	R	S	R
43	16	S	S	S	S
44	16	R	S	S	S
45	32	S	S	S	S
46	16	S	S	S	S
47	32	R	R	S	R
48	16	S	S	S	S
49	8	R	R	R	S
50	8	S	S	S	S

Table 2. Contd.

INH: isoniazid, RIF: rifampicin, STR: streptomycin, ETM: ethambutol, TGC: tigecycline, S: susceptible, R: resistance.

In vivo studies usually appear as case reports in literature. Peres et al. (2009) had reported that tigecycline had been clinically useful for pulmonary infection developed due to *Mycobacterium chelonae,* one of rapidly growing mycobacteria, and unresponsive to treatment in stem cell transplant recipient patient. Garrison et al. (2009) had reported that *M. abscessus* infections had been treated by tigecycline in solid organ recipients.

It is understood that tigecycline is effective against rapidly growing mycobacteria, but it is not effective against slowly growing (including *M. tuberculosis*) mycobacteria under the view of above summarized data. However, combination effects of tigecycline and other anti-tuberculosis drugs should be known for infections, thus, combination treatment is required.

Recently, studies have been carried out for exploring new drugs for treatment of tuberculosis in consequence of increasing MDR- TB and especially defined XDR-TB cases. Members of fluoroquinolones, moxifloxacin and gatifloxacin had been shown among new drugs for the treatment of tuberculosis. They exhibit their effects by blocking DNA gyrase enzyme resulting in inhibition of chromosomal replication (Van den Boogard et al., 2009).

TMC207 is diarilquinoline that acts by inhibiting of mycobacterial ATP synthesis. It is equally effective against both susceptible and resistant isolates. It had been shown that it has higher bactericidal potency than first line regime in mouse (Van den Boogard et al., 2009; Dhillon et al., 2010).

Although nitroimidazopyrans are developed for cancer chemotherapy, they are effective against both of active growing and dormant bacilli of *M. tuberculosis.* Nitroimidazopyran PA-824 is a prodrug and it should be converted to active form by mycobacterial glucose-6-phosphate dehydrogenase or its co-enzyme, co-enzyme P_{420}. Active form of PA-824 inhibits synthesis of proteins and cell wall lipids. It is only effective against susceptible and resistant members of *M. tuberculosis* complex (Stover et al., 2000; Manjunatha et al., 2006; Van den Boogard et al., 2009). Another member of nitroimidazopyrans, OPC-67683 is inhibitor of biosynthesis of mycolic acid. While isoniazid inhibits all mycolic acid subgroups, OPC-67683 inhibits synthesis of methoxy and ketomycolic acid (Matsumoto et al., 2006; Sasaki et al., 2006; Van den Boogard et al., 2009).

Diamine SQ109 inhibits mycobacterial cell wall synthesis but it is not known yet what is the definite target. It had been shown that there had been synergistic activity between SQ109, isoniazid and especially rifampicin (Chen et al., 2006; Nikonenko et al., 2007). Pyrrole LL3858 is active against resistant isolates; however, it is not known the mycobacterial target (Van den Boogard et al., 2009). Nikonenko et al. (2009) had shown that SQ641, analogue of capuramycin, had been effective against *M. tuberculosis in vitro*. Pasca et al. (2010) had reported that clinical isolates of *M. tuberculosis* had been susceptible to benzothiazinones (BTZ043).

M. tuberculosis has BlaC which is encoded chromosomally and structurally synthesized Ambler class A beta-lactamase. Hugonnet and Blanchard (2007) had shown that BlaC beta-lactamase had been irreversible inhibited by clavulanate. However, they had indicated that clavulanate containing combinations could be used against MDR-TB and XDR-TB isolates. Chambers et al. (1995) had investigated susceptibility of tuberculosis to beta-lactam antibiotics after they had determined the presence of 4 penicillin binding proteins in *M. tuberculosis* H37Ra serotype. They had shown *in vitro* effectiveness of imipenem, amoxicillin-sulbactam, amoxicillin-clavulanate, cefoxitin- clavulanate and ceftriaxon-clavulanate. Dincer et al. (2004) had shown that combination of beta-lactam and beta-lactamase inhibitors had been *in vitro* effective against *M. tuberculosis.* Chambers et al. (1998) evaluate bacilli load in patient's sputum by giving amoxicillin- clavulanate in active tuberculosis patients, and they had reported early bactericidal effect.

There are studies on the effectiveness of beta- lactam drugs, meropenem and imipenem, on tuberculosis bacilli. Hugonnet et al. (2009) had investigated the effectiveness of meropenem and clavulanate combination on 13 XDR-TB isolates and, they had determined that it had shown sterilization effect for aerobic culture condition within 14 days. Chamber et al. (2005) had reported that imipenem had antimicrobial activity in both of human and mice tuberculosis models. Coban et al. (2008) had determined *in vitro* meropenem MIC values against *M. tuberculosis* isolate had been 0.25->32 µg/ml.

Recently, Forgacs et al. (2009) had indicated that 43 of 44 *M. tuberculosis* isolates had been susceptible to trimethoprim/sulphamethoxazole. Furthermore, they had shown that it could be successfully used in one infected patient by susceptible isolate and, they had eventually reported that it could be used in MDR-TB and XDR -TB cases. Similarly, Ong et al. (2010) had also tested 12 isolates and found all isolates susceptible.

Mefloquine is a licensed anti-malarial agent effective to chloroquine resistant serotypes. It had been shown that it is effective to *M. avium intracellulare,* non-tuberculosis mycobacteria and *M. tuberculosis*. In addition to these, chlorpromazine, thioridazine and promethazine, are members of phenothiazine which is an anti-psychotic drug class, had been shown to be *in vitro* effective against *M. tuberculosis* isolates. Nevertheless, it requires higher concentration than clinical accessible dose for that activity. However, this concentration can be 10-fold higher in macrophages which phagocyte tuberculosis bacilli. Phenothiazines had been shown to enhance rifampicin (RIF) and streptomycin (STR) but not isoniazid (INH) activity against *M. tuberculosis* isolates (Viveiros and Amaral, 2001).

In conclusion, it seems that the effectiveness of tigecycline, one of the novel antibiotics with broad antibacterial spectrum, is not good against slowly growing mycobacteria including *M. tuberculosis* as it also showed in our study. Nevertheless, further studies are needed for determination of its combination effects with other anti-tuberculosis agents and effects against intracellular tuberculosis bacilli. When effectiveness of available antibiotics and other drugs against *M. tuberculosis* is considered, it seems to be favorable for future due to such promising agents. As a result of treatment challenges in MDR-TB and especially in XDR-TB isolates, knowing the effectiveness of available antibacterial agents assures a significant contribution to the next moves for the treatment of these isolates.

REFERENCES

Chambers HF, Moreau D, Yajko D, Miick C, Wagner C, Hackbarth C, Kocagoz S, Rosenberg E, Hadley WK, Nikaido H (1995). Can penicillins and other β-lactam antibiotics be used to treat tuberculosis? Antimicrob. Agents Chemother., 39: 2620-2624.

Chambers HF, Kocagoz T, Sipit T, Turner J, Hopewell PC (1998). Activity of amoxicillin/clavulanate in patients with tuberculosis. Clin. Infect. Dis., 26: 874-877.

Chambers HF, Turner J, Schecter GF, Kawamura M, Hopewell PC (2005). Imipenem for treatment of tuberculosis in mice and humans. Antimicrob. Agents Chemother., 49: 2816-2821.

Chen P, Gearhart J, Protopopova M, Einck L, Nacy CA (2006). Synergistic interactions of SQ109, a new ethylene diamine, with front-line antitubercular drugs in vitro. J. Antimicrob. Chemother., 58: 332-337.

Clinical and Laboratory Standards Institute (2008). Performance standards for antimicrobial susceptibility testing; eighteenth informational supplement. CLSI document M100-S18, Wayne, PA.

Coban AY, Birinci A, Ekinci B, Durupinar B (2004). Drug susceptibility testing of *Mycobacterium tuberculosis* by the broth microdilution method with 7H9 broth. Mem. Inst. Oswaldo Cruz, 99: 111-113.

Coban AY, Bilgin K, Tasdelen Fisgin N, Uzun M, Durupinar B (2008). Effect of meropenem against multidrug-resistant *Mycobacterium tuberculosis.* J. Chemother., 20: 395-396.

Courvalin P, Leclercq R, Rice LB (2010). Antibiogram. In: Tetracyclines, Poyart C. Eska Publishing, ASM Press, p. 335-336.

Dhillon J, Andries K, Phillips PPJ, Mitchison DA (2010). Bactericidal activity of the diarylquinoline TMC207 against *Mycobacterium tuberculosis* outside and within cells. Tuberculosis, 90: 301-305.

Dincer I, Ergin A, Kocagoz T (2004). The *in vitro* efficacy of β -lactam and β-lactamase inhibitors against multidrug resistant clinical strains of *Mycobacterium tuberculosis*. Int. J. Antimicrob. Agents, 23: 408-411.

Fernandez-Roblas R, Martin-de-Hijas NZ, Fernandez-Martinez AI, Garcia-Almeida D, Gadea I, Esteban J (2008). *In vitro* activities of tigecycline and 10 other antimicrobials against nonpigmented rapidly growing mycobacteria. Antimicrob. Agents Chemother., 52: 4184-4186.

Forgacs P, Wengenack NL, Hall L, Zimmerman SK, Silverman ML,

Roberts GD (2009). Tuberculosis and trimethoprim/sulfamethoxazole. Antimicrob. Agents Chemother., 53: 4789-4793.

Garcia-Agudo L, Garcia-Martos P, Jesus I, Rodriguez-Iglesias M (2009). Assessment of *in vitro* susceptibility to antimicrobials of rapidly growing mycobacteria by E-test. Rev. Med. Chil., 137: 912-917.

Garrison AP, Morris MI, Doblecki Lewis SD, Smith L, Cleary TJ, Procop GW, Vincek V, Rosa-Cunha I, Alfonso B, Burke GW, Tzakis A, Hartstein AI (2009). *Mycobacterium abscessus* infection in solid organ transplant recipients: report of three cases and review of the literature. Transpl. Infect. Dis., 11: 541-548.

Hugonnet JE, Blanchard JS (2007). Irreversible inhibition of the *Mycobacterium tuberculosis* β-lactamase by clavulanate. Biochem., 46: 11998-12004.

Hugonnet JE, Tremblay LW, Boshoff HI, Barry CE, Blanchard JS (2009). Meropenem-clavulanate is effective against extensively drug-resistant *Mycobacterium tuberculosis.* Science, 323: 1215-1218.

Jacobson KR, Tierney DB, Jeon CY, Mitnick CD, Murray MB (2010). Treatment outcomes among patients with extensively drug-resistant tuberculosis: systematic review and meta-analysis. Clin. Infect. Dis., 51: 6-14.

Leite CQ, Beretta AL, Anno IS, Telles MA (2000). Standardization of broth microdilution method for *Mycobacterium tuberculosis*. Mem. Inst. Oswaldo Cruz, 95: 127-129.

Manjunatha UH, Boshoff H, Dowd CS, Zhang L, Albert TJ, Norton JE, Daniels L, Dick T, Pang SS, Barry CE (2006). Identification of a nitroimidazo-oxazine-specific protein involved in PA-824 resistance in *Mycobacterium tuberculosis.* Proc. Natl. Acad. Sci. USA, 103: 431-436.

Martin-de-Hijas NZ, Fernandez-Roblas R, Fernandez-Martinez AI, Gadea I, Esteban J (2008). Usefulness of the Etest for studying tigecycline susceptibility of non-pigmented rapidly growing mycobacteria. Int. J. Antimicrob. Agents, 32: 363-371.

Matsumoto M, Hashizume H, Tomishige T, Kawasaki M, Tsubouchi H, Sasaki H, Shimokawa Y, Komatsu M (2006). OPC-67683, a nitro-dihydro-imidazooxazole derivative with promising action against tuberculosis *in vitro* and in mice. PLoS Med., 3: e466.

Nikonenko BV, Protopopova M, Samala R, Einck L, Nacy CA (2007). Drug therapy of experimental tuberculosis (TB): improved outcome by combining SQ109, a new diamine antibiotic, with existing TB drugs. Antimicrob. Agents Chemother., 51: 1563-1565.

Nikonenko BV, Reddy VM, Protopopova M, Bogatcheva E, Einck L, Nacy CA (2009). Activity of SQ641, a capuramycin analog, in a murine model of tuberculosis. Antimicrob. Agents Chemother., 53: 3138-3139.

Ong W, Sievers A, Leslie DE (2010). *Mycobacterium tuberculosis* and sulfamethoxazole susceptibility. Antimicrob. Agenst Chemother., 54: 2748-2749.

Pasca MR, Degiacomi G, Ribeiro ALJL, Zara F, De Mori P, Heym B, Mirrione M, Brerra R, Pagani L, Pucillo L, Troupioti P, Makarov V, Cole ST, Riccardi G (2010). Clinical isolates of *Mycobacterium tuberculosis* in four European hospitals are uniformly susceptible to benzothiazinones. Antimicrob. Agents Chemother., 54: 1616-1618.

Peres E, Khaled Y, Krijanovski OI, Mineishi S, Levine JE, Kaul DR, Riddell IV J (2009). *Mycobacterium chelonae* necrotizing pneumonia after allogeneic hematopoietic stem cell transplant: report of clinical response to treatment with tigecycline. Transpl. Infect. Dis., 11: 57-63.

Pholwat S, Heysell S, Stroup S, Foongladda S, Houpt E (2010). Rapid first and second line drug susceptibility assay for *M. tuberculosis* isolates using qPCR. J. Clin. Microbiol., 49: 69-75.

Rhomberg PR, Jones RN (2002). *In vitro* activity 11 antimicrobial agents, including gatifloxacin and GAR936, tested against clinical isolates of *Mycobacterium marinum.* Diagn. Microbiol. Infect. Dis., 42: 145-147.

Sasaki H, Haraguchi Y, Itotani M, Kuroda H, Hashizume H, Tomishige T, Kawasaki M, Matsumoto M, Komatsu M, Tsubouchi H (2006). Synthesis and antituberculosis activity of a novel series of optically active 6-nitro-2,3-dihydroimidazol[2,1-b]oxazoles. J. Med. Chem., 49: 7854-7860.

Stover CK, Warrener P, Van Devanter DR, Sherman DR, Arain TM, Langhorne MH, Anderson SW, Towell JA, Yuan Y, McMurray DN, Kreiswirth BN, Barry CE, Baker WR (2000). A small-molecule nitroimidazopyran drug candidate for the treatment of tuberculosis. Nature, 405: 962-966.

van den Boogard J, Kibiki GS, Kisanga ER, Boeree MJ, Aanoutse RE (2009). New drugs against tuberculosis: problems, progress, and evaluation of agents in clinical development. Antimicrob. Agents Chemother., 53: 849-862.

Viveiros M, Amaral L (2001). Enhancement of antibiotic activity against poly-drug resistant *Mycobacterium tuberculosis* by phenothiazines. Int. J. Antimicrob. Agents, 17: 225-228.

Wallace Jr RJ, Brown-Elliott BA, Crist CJ, Mann L, Wilson RW (2002). Comparison of the *in vitro* activity of the glycylcycline tigecycline (formerly GAR-936) with those of tetracycline, minocycline, and doxycycline against isolates of nontuberculous mycobacteria. Antimicrob. Agents Chemother., 46: 3164-3167.