

Investigation of the antibacterial activity of pioglitazone

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Purpose: To evaluate the antibacterial potential of pioglitazone, a member of the thiazolidinediones class of drugs, against Gram-positive (*Streptococcus pneumoniae*) and Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacteria.

Methods: Susceptibility testing was done using the antibiotic disk diffusion method and the minimal inhibitory concentration (MIC) of pioglitazone was measured according to the broth micro incubation standard method.

Results: Pioglitazone induced a dose-dependent antibacterial activity in which the optimal concentration was 80 μ M. Furthermore, results indicated that while *E. coli* was sensitive (MIC = 31.25 ± 3.87 mg/L) to pioglitazone-induced cytotoxicity, *S. pneumoniae* and *K. pneumoniae* were resistant (MIC = 62.5 ± 3.77 mg/L and MIC = 62.5 ± 4.14 mg/L, respectively). Moreover, pretreatment of bacteria with a suboptimal concentration of pioglitazone (40 μ M) before adding amoxicillin, cephalexin, co-trimoxazole, or ciprofloxacin enhanced the antibacterial activity of all agents except co-trimoxazole. This enhancing effect was particularly seen against *K. pneumoniae*.

Conclusion: These results indicate the possibility of a new and potentially important pioglitazone effect and the authors' ongoing studies aim to illustrate the mechanism(s) by which this antibacterial effect is induced.

Keywords: pioglitazone, susceptibility testing, antibiotics, diabetes

Introduction

Thiazolidinediones (TZDs), also known as glitazones, are a relatively new class of oral hypoglycemic, insulin-sensitizing drugs that are used clinically as third-line agents in the management of diabetes mellitus type 2 as they help restore peripheral insulin sensitivity.¹ TZDs activate peroxisome proliferator-activated receptor gamma (PPAR- γ), a nuclear receptor that regulates the production of proteins involved in glucose and lipid homeostasis, and, to a lesser extent, PPAR- γ .¹⁻²

Pioglitazone is one member of the thiazolidinedione family. It is an oral hypoglycemic agent dosed at 15–30 mg/day. The maximum recommended dose for pioglitazone is 45 mg/day, which is associated with peak plasma drug concentrations of pioglitazone in the range of 1050–1329 μ g/L.³⁻⁵ In addition to its hypoglycemic effect, pioglitazone has many other effects.⁶⁻⁸ For example, like other TZDs, pioglitazone has been shown to reduce plasma levels of several inflammatory mediators and tissue-remodeling enzymes. The anti-inflammatory effects of TZDs are also evident in patients with classical metabolic syndrome since they reduce the white blood cell count, P-selectin-positive platelets, acute-phase inflammatory proteins, C-reactive protein, serum amyloid A, and fibrinogen.

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Thus, TZDs have shown efficacy in inflammatory diseases such as psoriasis, ulcerative colitis, and nonalcoholic steatohepatitis.⁹

Previous attempts have been made to investigate the potential antibacterial effect of TZDs and it has been shown that some novel thiazolidine-2,4-dione derivatives possess antibacterial and antifungal activities.^{10,11} However, no study has tested whether the widely used antidiabetic TZDs have antibacterial activity. Therefore, in this study, the authors evaluated the possibility that pioglitazone, the most widely used TZD, possesses antibacterial activity against important Gram-positive microorganisms such as *Streptococcus pneumoniae*, and Gram-negative microorganisms such as *Escherichia coli* and *Klebsiella pneumoniae*.

Material and methods

Microbial culture and growth conditions

The antibacterial activities of pioglitazone and pioglitazone at a suboptimal concentration of 40 μM in combination with other agents (amoxicillin, cephalexin, co-trimoxazole, and ciprofloxacin) were compared against the reference bacteria, which included *E. coli* ATCC 25922 and clinical isolates *S. pneumoniae* and *K. pneumoniae* obtained from the microbiology laboratory at King Abdullah University Hospital, Ar Ramtha, Jordan.

The organisms were stored at -70°C in BBL™ Trypticase™ Soy Broth with 20% glycerol (Becton Dickinson and Company, Cockeysville, MD). Once ready for batch susceptibility testing, organisms were thawed and passed three times to assure purity and viability. Using the agar plate dilution method, the minimum inhibitory concentrations (MICs) were determined in accordance with the Clinical and Laboratory Standards Institute (CLSI).¹²

Antibiotic solutions were prepared on the day of use according to manufacturer recommendations. Concentrations that ranged from 31.25 to 500 $\mu\text{g/mL}$ were tested. Serial dilutions of two folds were added to molten BBL™ Muller Hinton Gold II agar (Becton Dickinson and Company). After that, the agar plates were slightly cooled and dried. Thereafter, a steer replicator was used to place aliquots containing approximately 5×10^4 colony-forming units per drop for each of the three test strains of *E. coli*, *S. pneumoniae*, and *K. pneumoniae*. Plates were read in duplicate after incubation for 18 hours at 35°C . MIC was defined as the lowest concentration at which there was no growth, or a faint haze or fewer than three discrete colonies. The breakpoints indicated in the last edition of tables of the National Committee for CLSI¹³ were used to determine susceptibility and resistance.

Table 1 Dose titration of pioglitazone against *Escherichia coli*, *Streptococcus pneumoniae* and *Klebsiella pneumoniae*

Pioglitazone conc (μM)	Diameter zone of inhibition (mm)		
	<i>E. coli</i>	<i>S. pneumoniae</i>	<i>K. pneumoniae</i>
5	0	0	0
10	0	0	0
20	0	0	0
40	0	0	0
50	17	0	0
60	18	10	9
70	19	12	17
80	26	20	19
100	21	19	18
150	19	14	12
200	19	14	12

Abbreviation: conc, concentration.

Antimicrobial susceptibility test

Sterile 5 mm diameter filter paper discs that were impregnated with different concentrations of amoxicillin, cephalexin, co-trimoxazole, and ciprofloxacin, were placed in duplicate on Mueller Hinton agar. In some experiments, pioglitazone at a suboptimal concentration of 40 μM was added to the media. The surface was then spread with 0.2 mL of microorganism culture (about 10^8 cells/mL) and the plates were incubated for 24 hours at 37°C . The experiments were carried out in duplicate. The results (mean of three independent experiments) were recorded by measuring the zones of growth inhibition surrounding the discs.

Determination of MIC

The MIC was determined using serial dilution method according to the procedures of the National Committee for Clinical Laboratory Standards.¹³ Briefly, stock solutions of amoxicillin, cephalexin, co-trimoxazole, and ciprofloxacin were sterilized by passing them through a pyrogenic filter. Solutions were then serially diluted to a range of concentrations. Each well of a 96-well plate was prepared by dispensing 100 μL of an appropriate medium, an antibiotic (amoxicillin, cephalexin, co-trimoxazole, or ciprofloxacin), and 20 μL of the inoculum. A standard nutrient broth (Muller Hinton [Sigma-Aldrich, MI, USA]) was used. Turbidity was the sign that indicated the growth of microorganisms, whereas clear wells indicated absence of bacterial growth.

Table 2 Minimal inhibitory concentration (MIC) of pioglitazone

MIC (mg/L)		
<i>E. coli</i>	<i>S. pneumoniae</i>	<i>K. pneumoniae</i>
31.25 (\pm 3.87 mg/L)	62.5 (\pm 3.77 mg/L)	62.5 (\pm 4.14 mg/L)

Abbreviations: *E. coli*, *Escherichia coli*; *S. pneumoniae*, *Streptococcus pneumoniae*; *K. pneumoniae*, *Klebsiella pneumoniae*.

Table 3 Cytotoxicity of pioglitazone and other clinically used antibiotics

Bacteria species	Pioglitazone	Amoxicillin	Cephalexin	Co-trimoxazole	Ciprofloxacin
<i>E. coli</i>	0	9	19	22	31
<i>K. pneumoniae</i>	0	0	0	0	32
<i>S. pneumoniae</i>	0	8	24	25	35

Notes: Amoxicillin, cephalexin, co-trimoxazole, and ciprofloxacin were used at concentrations of 10 µg/disc, 30 µg/disc, 30 µg/disc, 20 µg/disc, respectively. Data represent diameters of zone of inhibition (in mm).

Abbreviations: *E. coli*, *Escherichia coli*; *S. pneumoniae*, *Streptococcus pneumoniae*; *K. pneumoniae*, *Klebsiella pneumoniae*.

For each experiment, a sterility check (50% dimethyl sulfoxide [DMSO; Sigma-Aldrich, MI, USA] and medium), negative control (50% DMSO, medium and inoculum), and positive control (50% DMSO, medium, inoculum, and ciprofloxacin) were included. The microtiter plates were incubated at 37°C for 24 hours and were examined for growth in daylight. The MIC of the preparations was the lowest concentration in the medium that completely inhibited the visible growth. The solvent value was deducted accordingly to obtain the final results of activity. All experiments were performed in triplicate.

Chemicals

Amoxicillin, cephalexin, co-trimoxazole, and ciprofloxacin were a generous gift from Al-HIKMA pharmaceuticals (Amman, Jordan). Pioglitazone was a generous gift from Dar Al-Dawa pharmaceuticals (Amman, Jordan). All drugs were used as pure material.

Results

Results (shown in Table 1) revealed that pioglitazone induced a dose-dependent antibacterial activity against the reference bacteria. An inhibition zone of 10 mm was chosen as representative of bacterial susceptibility to the compound. The optimal concentration of pioglitazone was 80 µM. As shown in Table 2, *E. coli*, *S. pneumoniae*, and *K. pneumoniae* had MIC values to pioglitazone of 31.25 ± 3.87 mg/L, 62.5 ± 3.77 mg/L, and 62.5 ± 4.14 mg/L, respectively. These MIC values, although indicating the antibacterial activity of pioglitazone, are considered relatively high compared with those of standard antibiotics. Thus, *E. coli*, *S. pneumoniae*, and *K. pneumoniae* are considered resistant to pioglitazone alone.

The possibility that pioglitazone might enhance the activity of other clinically used antibacterial agents was then considered. Pioglitazone at suboptimal concentra-

tion of 40 µM and other agents (amoxicillin, cephalexin, co-trimoxazole, and ciprofloxacin) were used to assess the antibacterial activity against the reference bacteria. As shown in Table 3, pioglitazone alone, at suboptimal concentration, did not induce antibacterial effect against any of the bacteria. While all bacteria were resistant to amoxicillin, they were sensitive to ciprofloxacin-induced cytotoxicity. *E. coli* and *S. pneumoniae* were moderately sensitive to co-trimoxazole and cephalexin. Furthermore, pretreatment of bacteria with suboptimal concentration of pioglitazone (40 µM) enhanced the antibacterial activity of all agents except co-trimoxazole. This enhancing effect of pioglitazone was particularly seen against *K. pneumoniae* (Tables 4 and 5).

Discussion

The emergence of drug resistance along with the poor compliance of patients, undesirable adverse drug effects, and the significant elevation of cost due to combination therapy, exposes the strong demand for new therapeutic regimens having the same or higher beneficial properties of antibiotics but with reduced adverse effects. Pioglitazone, a member of the thiazolidinediones class of medications, has been shown to induce different effects such as hypoglycemic effect in diabetic patients,¹⁴ anti-inflammatory effects,^{7,8} and to improve vascular endothelial function and microalbuminuria.¹⁴ In this study, the authors have shown, for the first time, a dose-dependent antibacterial activity of pioglitazone against reference bacteria. For clinical isolates, only *E. coli* was sensitive to pioglitazone actions, while both Gram-positive *S. pneumoniae* and Gram-negative *K. pneumoniae* were resistant. Furthermore, pretreatment of bacterial cultures with pioglitazone enhanced the antibacterial activity of amoxicillin, cephalexin, and ciprofloxacin but not that of co-trimoxazole.

Table 4 Effect of pioglitazone (P) on other clinically used antibiotics – induced cytotoxicity

Diameter zone of inhibition (mm)				
Bacteria species	Amoxicillin + P	Cephalexin + P	Co-trimoxazole + P	Ciprofloxacin + P
<i>E. coli</i>	15	19	22	37
<i>K. pneumoniae</i>	10	8	7	42
<i>S. pneumoniae</i>	12	28	30	39

Table 5 Minimal inhibitory concentration (MIC) of pioglitazone (P) combined with other antibiotics

Bacteria species	MIC (mg/L)							
	Amox	Amox + P	Cepha	Cepha + P	Co-trimoxazole	Co-trimoxazole + P	Cipro	Cipro + P
<i>E. coli</i>	250	125	250	125	125	125	250	125
<i>K. pneumoniae</i>	500	250	500	250	125	62.5	250	125
<i>S. pneumoniae</i>	500	250	125	31.25	125	62.5	125	62.5

Abbreviations: *E. coli*, *Escherichia coli*; *S. pneumoniae*, *Streptococcus pneumoniae*; *K. pneumoniae*, *Klebsiella pneumoniae*; amox, amoxicillin; cepha, cephalexin; cipro, ciprofloxacin.

Previous studies have shown that thiazolidinediones induce their hypoglycemic effect, anti-inflammatory effects, and improved vascular endothelial function via transcription through their direct activation of the nuclear receptor PPAR- γ .^{9,15} Since this receptor lacks prokaryotes, it is highly unlikely that the antibacterial activity of pioglitazone can be attributed to a known mechanism of action of thiazolidinediones. However, pioglitazone has been shown to promote cytotoxicity, suppress growth, and induce apoptosis through enhancing cell death via the tumor necrosis factor-related mechanism.¹⁶ It has also been shown to induce a rapid production of reactive oxygen species (ROS) unrelated to the PPAR- γ .^{17,18} This production of ROS was associated with the cytotoxic effect of pioglitazone.^{17,18} It is possible that the currently reported enhanced antibacterial activity of common antibiotics is related to the generation of ROS induced by pioglitazone. However, further work is needed to characterize such possible mechanisms.

In concordance with the authors' results, novel thiazolidine-2, 4-dione derivatives have been synthesized and their antimicrobial activity has been examined in a number of studies. In one study, phenylethylsulfanyl-1,3-thiazolo-thiazolidine-2,4-dione derivatives (VII a-f, VIII a-f) and 5-methyl-[1,2,4]triazolyl-sulfanyl-1,3-thiazolo-thiazolidine-2,4-dione derivatives (IX a-f, X a-f) were synthesized and investigated for their antibacterial and antifungal activities against *S. aureus*, methicillin-resistant *Staphylococcus aureus*, *Bacillus subtilis*, *E. coli*, and *Candida albicans*. The derived compounds were found to be active against these bacteria.¹⁰ In another study, the antimicrobial activity of these derivatives was evaluated against *B. subtilis* (Gram-positive), *Pseudomonas aeruginosa* (Gram-negative), and *Streptomyces* spp. where they displayed different degrees of antimicrobial activities or inhibitory actions.¹¹ In the present study, it was shown that pioglitazone possesses a dose-dependent antibacterial activity against Gram-positive (*S. pneumoniae*) and Gram-negative (*E. coli* and *K. pneumoniae*) bacteria. This antibacterial activity of pioglitazone was manifested as enhanced antibacterial activity of standard antibiotics including amoxicillin, cephalexin,

and ciprofloxacin against *S. pneumoniae*, *E. coli*, and *K. pneumoniae*.

These results raise the possibility of a new and important potential effect of pioglitazone. The authors' ongoing studies aim to illustrate the mechanism(s) by which pioglitazone induces its antibacterial effect.

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Disclosure

The authors declare no conflicts of interest in relation to this work.

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