Bactericidal Antibiotics Promote Reactive Oxygen Species Formation and Inflammation in Human Sinonasal Epithelial Cells

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Abstract

Background—Bactericidal antibiotics have been shown to stimulate reactive oxygen species
(ROS) formation in mammalian cells through mitochondrial dysfunction. This results in oxidative
tissue damage that may have negative consequences for long-term antibiotic use. Antibiotics are
widely and heavily used in the treatment of acute and chronic sinusitis, however the relationship
between antibiotics and ROS formation in sinonasal epithelial cells (SNECs) has not yet been
demonstrated.

Methods—Human SNECs were collected from patients during endoscopic sinus surgery and
grown in culture at the air-liquid interface. Differentiated SNECs were stimulated with the
bactericidal antibiotics amoxicillin and levofloxacin and the bacteriostatic antibiotic
clarithromycin for 24-hours. Reactive oxygen species were quantified via fluorescence. Cell death
was quantified by LDH secretion. Expression of inflammatory markers such as TNF-α and Nrf2
mediated antioxidant genes were measured by RT-PCR.

Results—Cultured SNECs treated with the bactericidal antibiotics amoxicillin and levofloxacin
resulted in a significant increase in production of ROS ($p<0.05$) and secretion of LDH ($p<0.05$).
The increase in ROS formation correlated with an increase in expression of Nrf-2 mediated
antioxidant genes as well as the expression and production of pro-inflammatory cytokine TNF- α,
and IL-1β ($p<0.05$). SNECs treated with clarithromycin did not demonstrate statistically
significant increases in ROS or pro-inflammatory cytokine production.

Discussion—In this study, we demonstrate that treatment of cultured human SNECs with
bactericidal antibiotics leads to formation of ROS with an associated increase in inflammatory and
antioxidant gene expression and cell death. This suggests that long term or inappropriate antibiotic
use in the treatment of sinusitis, may result in oxidative tissue damage to the sinonasal epithelium.
Future studies will explore the clinical implications of such damage to the sinonasal epithelium.

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Introduction

Antibiotics are widely used in the treatment of both acute and chronic rhinosinusitis[1] (CRS). The relationship between the sinonasal bacterial flora and CRS remains unclear. The bacterial infection or colonization that is often seen in CRS may be the primary inflammatory stimulus or could instead occur secondarily as a result of the pre-existing inflammatory state, perhaps acting only as a disease modifier. Irrespective of the role of bacteria as a causal factor for CRS, antibiotics continue to have a central role in the treatment of active CRS[2–4]. While the primary role of antibiotics is to reduce the burden of a bacterial infection, some classes of antibiotics have been shown to have non-microbial functions.

Macrolides, quinolones and penicillins are the most common antibiotics used in the treatment of sinusitis[5] and a diagnosis of acute or chronic sinusitis accounts for 11% of all antibiotics prescribed in an ambulatory setting[6]. The macrolide class of antibiotics targets translation in bacteria resulting in a cessation of growth (bacteriostatic). Macrolides also have anti-inflammatory properties thought to be related to the ring structure of this class of drugs that results in the reduction of pro-inflammatory cytokines[7]. These anti-inflammatory properties have been utilized to guide treatment of lung disease[7] and may also be useful in the treatment of recalcitrant CRS[8].

Quinolones and penicillins are bactericidal antibiotics – those which kill greater than 99.9% of bacteria. In addition to their classic mechanisms of action, bactericidal antibiotics also promote altered bacterial metabolism, respiration and iron homeostasis that results in reactive oxygen species (ROS) formation that contributes to a portion of the cell death[9–16]. Expanding upon this common mechanism of cell death, bactericidal antibiotics were also shown to induce reactive oxygen species formation in mammalian cells through mitochondrial dysfunction which resulted in accumulation of damaged DNA, proteins, and lipids that may have long term consequences to human systems[17].

In this study, we examined the ability of bactericidal antibiotics to induce ROS formation in cultured human sinonasal epithelial cells (SNECs) from subjects without CRS. In addition, we also examined the ability of antibiotics to stimulate antioxidant production and inflammatory cytokines in SNECs. We also demonstrate that over time, continued treatment with antibiotics may also result in increased epithelial cell death.

Materials and Methods

Human Subjects

Thirteen control subjects were enrolled in the study. The research protocol was approved through our Institutional Review process, and all subjects gave signed informed consent. Mucosal tissue was collected from the ethmoid sinuses during endoscopic sinus surgery and

grown in culture at the air-liquid interface (ALI). All control patients were defined as those without CRS who were undergoing endoscopic sinonasal surgery for dacrocystorhinostomy, cerebrospinal fluid leak repair or endoscopic skull base surgery.

The mucosal tissue was transferred to phosphate buffered saline (PBS) supplemented by penicillin (100 μg/mL, Gibco, Gaithersburg, MD), streptomycin (100 μg/mL, Gibco), amphotericin B (2.5 μg/mL, Gibco), and gentamicin (50μg/mL, Gibco). Samples were collected through a cell strainer (BD Falcon) and digested in 4° C overnight in Ham's F12 media containing 0.01% protease Sigma Type XIV (Sigma, St. Louis, MO) supplemented with antibiotics as above. The cells were separated by straining into a conical tube to which fetal bovine serum (FBS, Sigma) was added to a final concentration of 10% to inactivate the protease. Cells were centrifuged at 1200 rpm for 10 minutes in 4°C, after which the supernatant was aspirated. The washed SNEC were re-suspended in Bronchial Epithelium Growth Medium (BEGM) and seeded at a density 1.5×10^4 cells/cm^2 in collagen coated 100-mm culture dishes. The media was changed initially 24 hours after the cells were grown, and then every 48 hours until cells reached confluence.

SNEC Culture at the Air-Liquid Interface (ALI)

Confluent cells were washed with HBSS prior to trypsinization, then treated at 37°C for 2 minutes with a solution containing 0.2% Trypsin (Sigma), 1% polyvinylpyrrolidone (Sigma), and 0.02% EGTA (Sigma) in HBSS. The trypsin was then neutralized by the addition of an equal volume of cold soybean trypsin inhibitor at a concentration of 1 mg/mL in Ham's F12 media. Dissociated cells were washed and re-suspended into BEGM media and plated into human type IV placental collagen (Sigma, Type VI) coated 12-well Falcon filter inserts (0.4-μm pore size; Becton Dickinson, Franklin Lakes, NJ). When confluent, media was removed from above the cultures and the media below the inserts was changed to LHC Basal Medium:DMEM-H (Gibco) (50:50) containing the same concentrations of additives as BEGM with the exception that the concentration of epidermal growth factor was reduced to 0.63 ng/mL, and penicillin, gentamicin, streptomycin and amphotericin B were omitted (ALI media). Each set of SNEC cultures came from a separate patient source and was maintained at the air-liquid interface with the apical surfaces remaining free of medium for at least 3 weeks prior to study. This differentiated cell culture model, with media in the basolateral compartment and air at the apical surface, is an established model for studying sinonasal epithelial cells that closely resembles nasal cavity mucosa[18,19].

Treatment of SNECs with Antibiotics and Measurement of Reactive Oxygen Species Formation

SNECs were left untreated (control), or treated with 20 μg/ml clarithromycin, 80 μg/ml levofloxacin, 80 μg/ml amoxicillin, or 0.2% acetone (vehicle control - used to dissolve clarithromycin), respectively for 24 hours. RNA was extracted for qPCR analysis as described below. To measure ROS formation, SNECs at the ALI interface from thirteen patients were trypsinized and transferred to a 96 well plate in antibiotic-free ALI media and allowed to adhere overnight. These cells were then stained with 20uM DCFDA (Abcam, Cambridge, MA) for 45 minutes, washed with 1× PBS, and either left untreated (control) or stimulated with 20 μg/ml clarithromycin, 80 μg/ml levofloxacin, 80 μg/ml amoxicillin, or

0.2% acetone. Fluorescence readings were recorded in triplicate for each patient sample at 24 hours using an excitation wavelength of 485 nm and emission wavelength of 528 nm using a microplate reader.

Real Time Polymerase Chain Reaction (PCR)

Total RNA was extracted from the SNECs cultured from thirteen patients following 24 hours of treatment in the presence or absence of antibiotics using the RNeasy Kit by Qiagen according to the manufacturer's directions. DNAse I (Qiagen) was used to treat RNA to remove contaminating genomic DNA. RNA concentration was determined by measuring the OD values at 260 nm. cDNA was synthesized from isolated mRNA by reverse transcribing 500 ng of RNA in a reaction volume of 20 μL using random hexamer primers (Invitrogen) and reagents from the Omniscript RT kit (Qiagen).

Real time PCR analysis was performed for each sample in duplicate using the Applied Biosystems StepOnePlus machine (Foster City, CA) under standard cycling parameters for Taqman per the manufacturer recommendations. For all genes of interest, the reaction mixture consisted of 100ng total RNA, Taqman primers (Life Technologies - see Table 1), and Taqman Fast Universal PCR Master Mix (Applied Biosystems) according to manufacturer recommendations. A Corresponding 18S Taqman control was also run using 5ng total RNA according to the manufacturer's recommendations. Amplicon expression in each sample was normalized to its 18S RNA content, and the level of expression of target mRNA was determined as the delta CT (CT), the difference in threshold cycles for each target and housekeeping gene. All primers were commercially synthesized by Life Technologies (Table 1).

Cytokine Quantification

TNF-α and IL-1β were quantified in basal media and from SNEC lysates by ELISA using commercially available kits (Abcam, Cambridge MA). Lysates and basal media were collected 48 hours after treatment with antibiotics. Antibiotics were re-dosed in fresh media applied to the basal compartment every 24 hours until samples were collected. Basal media was centrifuged for 1 minute at 15,000 rpm and supernatants were collected and frozen at −80°C. Lysates were incubated on ice with shaking for 15 minutes, then centrifuged for 10 minutes at 13,000 rpm. Supernatants were collected and stored at −80°C. The assay was performed according to manufacturer's instructions using the provided pre-coated 96-well plates with samples from five patients run in triplicate.

Cell Death (LDH Assay)

$1 \times$ PBS was incubated and then collected from the apical surface of SNECs from five patients both with and without antibiotic treatment at both 48 and 96-hour time points. Antibiotics were re-dosed in fresh media applied to the basal compartment every 24 hours until samples were collected. Lactate Dehydrogenase (LDH) release was quantified by using a commercially available kit (Life Technologies) with 50 μL of sample in triplicate in 96-well plates (Corning, New York). Absorbance from each well was read using a microplate reader at 450 nm (signal) and 570 nm (background). Negative control ($1 \times$ PBS) and positive control (cell lysates) samples were performed in triplicate for each run.

Statistical Analysis

Raw data from real-time PCR, CM-H2DCFDA assays were entered into a spreadsheet (Excel; Microsoft Corp, Redmond, WA). Statistical analysis was performed using a software program (Excel; Microsoft Corp, Redmond, WA). Data are expressed as mean ± SEM. Statistical significance of differences between the same populations with and without antibiotic treatment was determined using the Student's paired t-test. Differences between different populations were evaluated by employing the two-tailed t-test for sample means with unequal variance. Differences were considered statistically significant at p<0.05.

Results

Bactericidal Antibiotics Stimulate Reactive Oxygen Species Formation

Cultured SNECs from control subjects were left untreated or were treated with 20μg/ml clarithromycin, 80μg/ml levofloxacin or 80μg/ml amoxicillin for 24-hours and ROS formation was quantified using the ROS-sensitive dye DCFDA. There was a significant increase in ROS formation following treatment with either levofloxacin (p = 0.013) or amoxicillin (p = 0.008) whereas treatment with clarithromycin did not lead to a significant increase in ROS formation (Figure 1). These results are consistent with the prior study demonstrating that bactericidal antibiotics stimulate ROS formation in mammalian cells[17] and demonstrates that this phenomena also occurs in human sinonasal epithelial cells.

Bactericidal Antibiotics Stimulate Expression of Nrf-2-Regulated Antioxidant Genes

Left unchecked, ROS formation results in oxidative stress and accumulation of DNA, protein and lipid damage that can lead to a breakdown in normal cellular processes[17,20]. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that binds to antioxidant responsive elements and activates expression of systems that can mitigate the damage caused by ROS[20,21]. We examined expression of Nrf-2-regulated genes *GCLC* (glutamate-cysteine ligase catalytic), *GCLM* (glutamate-cysteine ligase modifier), *HOX1* (heme oxygenase-1), *SOD1* (superoxide dismutase-1), *NQO1* (NAD(P)H quinone oxidoreductase-1), and *p62* (nucleoporin 62) in SNECs following 24-hours of treatment with no antibiotics, clarithromycin, levofloxacin or amoxicillin (Figure 2).

Treatment with clarithromycin did not result in a significant increase in Nrf-2-regulated gene expression compared to vehicle control (Table 2, Figure 2). Treatment with amoxicillin resulted in a significant increase in expression for 2 of the 6 tested genes – *GCLC* (p= 0.024) and *GCLM* (p= 0.024) (Figure 2, Table 2). Treatment with levofloxacin resulted in a significant increase in expression relative to untreated SNECs for 4 of the 6 tested genes - Glutamate-Cysteine ligase genes [*GCLC* (p = 0.001), *GCLM* (p = 0.005)], *NQO1* (p = 0.004) and *p62* (p = 0.005) (Table 2, Figure 2). It is possible that the increased production of ROS following levofloxacin treatment compared to amoxicillin treatment (Figure 1), may account for the increased expression of Nrf-2-regulated genes with levofloxacin compared to amoxicillin. These data suggest that bacteriostatic antibiotics do not stimulate antioxidant gene expression and that bactericidal antibiotics which stimulate ROS production can stimulate expression of some Nrf-2-regulated antioxidant genes.

Bactericidal Antibiotics Stimulate expression of Pro-inflammatory Genes in SNECs

Tumor Necrosis Factor (TNF-α) and Interleukin-8 (IL-8) are important mediators of inflammation and play a role in the early recruitment of neutrophils and other lymphocytes in response to bacterial infection of mucosal surfaces[22,23], and varying levels of these cytokines has been demonstrated in tissue and mucus from those with CRS[24]. Previous work demonstrated an increase in expression of these mediators as a result of infection of cultured SNECs with *S. aureus*[25]. To explore if antibiotic-mediated ROS formation promotes a pro-inflammatory state, we looked at the expression of *TNF-α* and *IL-8* in SNECs following 24-hours of treatment with no antibiotics, clarithromycin, levofloxacin or amoxicillin (Figure 3A). Neither clarithromycin nor the bactericidal antibiotics tested resulted in a significant increase in *IL-8* expression (Figure 3A, Table 2, Table 3). There was a significant increase in *TNF-α* expression following treatment with the bactericidal antibiotics, levofloxacin ($p = 0.013$) and amoxicillin ($p = 0.004$), but not with the bacteriostatic antibiotic, clarithromycin (Figure 3A, Table 2).

Inflammasomes are a part of the innate immune response and consist of protein complexes containing a sensor protein, an adaptor protein (ASC) and caspase 1. These complexes can sense cellular stress from internal and external sources and activate a pro-inflammatory state through activation of IL-1β and IL-18[26]. We measured expression of *IL-1β*, *IL-18* and *caspase*-1 (*CASP1*) in SNECs following 24-hours of treatment with no antibiotics, clarithromycin, levofloxacin or amoxicillin (Figure 3B). Treatment with clarithromycin did not result in a significant increase in expression of *IL-1β*, *IL-18* or *caspase*-1 (Figure 3B, Table 3). Treatment of SNECs with levofloxacin or amoxicillin resulted in a significant increase in expression of *IL-1β* ($p= 0.0002$, $p = 0.0002$), *IL-18* ($p= 0.013$, $p = 0.004$) and *caspase*-1 ($p= 0.006$, $p = 0.01$) (Figure 3B, Table 2). These results suggest that treatment of SNECs with bactericidal antibiotics promotes a pro-inflammatory state.

We next measured basal secretion of TNF-α as well as intracellular levels of the mature form of IL-1β after 48-hours of treatment with levofloxacin or amoxicillin to determine if treatment with bactericidal antibiotics triggers an increase in production of these inflammatory proteins. We found that there was a significant increase in basal secretion of TNF-α by SNECs following treatment with either levofloxacin (115 pg/mL, $p=0.01$) or amoxicillin (99.5 pg/mL, $p = 0.01$) compared to untreated (0.9 pg/mL) SNECs (Figure 4A). We also found a significant increase in intracellular levels of IL-1β in SNEC following treatment with levofloxacin (102 pg/mL, $p = 0.03$) or amoxicillin (67 pg/mL, $p = 0.04$) compared to untreated (18 pg/mL) SNECs (Figure 4B).

SNEC cell death following treatment with Bactericidal Antibiotics

In addition to their pro-inflammatory properties, both TNF-α and Il-1β are associated with cell death. TNF-α can activate pro-apoptotic pathways[27] and production and release of mature Il-1β may occur when the permeability of the cell membrane is increased, potentially triggering cell necrosis[28]. To determine if bactericidal antibiotic treatment is also associated with cell death, we measured excretion of LDH by SNECs - LDH is released by dying mammalian cells and has been used as a marker of cytotoxicity. We measured apical excretion of LDH in SNECs that were untreated or treated with 80μg/ml levofloxacin, or

80µg/ml amoxicillin for 48 and 96-hours, respectively (Figure 5). We found a significant increase in LDH secretion for both levofloxacin and amoxicillin-treated SNEC compared to untreated SNEC at both 48-hours and 96-hours (Figure 5). These data suggest that bactericidal antibiotic treatment are associated with SNEC cell death.

Discussion

The use have antibiotics in sinus disease has been steadily increasing with a recent study showing that antibiotics are prescribed in over 80% of visits for acute sinusitis[5] and a diagnosis of acute or chronic sinusitis accounts for 11% of all antibiotics prescribed in an ambulatory setting[6]. Since most acute sinus infections tend to be viral, widespread antibiotic use early in the course of sinusitis may lead to microbial resistance and put patients at risk for adverse effects of antibiotics such as colitis, nephrotoxicity, and tendinopathies in the case of fluoroquinolones[29]. Recent studies by Kalghatgi, *et al*. have demonstrated that bactericidal antibiotics can induce mitochondrial dysfunction and oxidative tissue damage in various mammalian cell types[17]. Previous studies, however, have not explored whether bactericidal antibiotics may cause oxidative tissue damage to the sinonasal epithelium.

In this study, we demonstrate for the first time that treatment of cultured human SNECs with bactericidal antibiotics leads to formation of ROS with an associated increase in inflammatory and antioxidant gene expression and protein production. Treatment with the bacteriostatic antibiotic, clarithromycin did not result in ROS formation or up-regulation of pro-inflammatory or antioxidant gene expression. The resultant exposure of mammalian cells to bactericidal antibiotics can lead to the accumulation of damaged DNA, proteins and lipids which may have deleterious consequences[17].

In this manuscript, we used an epithelial culture-based system to test that treatment with bactericidal antibiotics stimulates human SNECs to generate ROS, upregulate antioxidant defense systems, increase production of inflammatory cytokines and increase cellular cytotoxicity.

Mitochondria are the major source of ROS formation in mammalian cells[30] and are the source of ROS formation following treatment with bactericidal antibiotics[17]. Mitochondria-derived ROS can activate the NLRP-3 inflammasome[31] thereby stimulating an inflammatory response through cleavage of pro-IL-1β and pro-IL-18 by Caspase 1[26]. In the lower respiratory system, activation of NLRP-3 has been associated with both the ability to mount a strong host response against viral lung infection as well as chronic inflammation of the lung such as seen in pulmonary fibrosis[32]. The increased expression of *TNF*-α, *IL-1*β, *IL-18* and *caspase 1* and the increased secretion of TNF-α and production of mature Il-1β following treatment of SNECs with bactericidal antibiotics together with the increase in ROS formation also suggests that the sinonasal epithelial cells may be primed by bactericidal antibiotics toward a pro-inflammatory state.

In addition to their roles in inflammation, both TNF-α and IL-1β are also associated with activation of pro-apoptotic or necrosis death pathways[27,28]. In this study, we also demonstrate that treatment with levofloxacin and amoxicillin for 48 and 96-hours is

cytotoxic (as measured by LDH secretion) to human SNECs compared to untreated SNECs (Figure 5). There is no causal link at this time between antibiotic-driven ROS formation and cell death in cultured human SNECs, and future studies will explore this relationship in terms of varying antibiotic concentrations, duration of treatment as well as working to further elucidate the inflammatory/death pathways stimulated by bactericidal antibiotics. The clinical relevance of this model remains un-explored at this time, and further efforts to understand bactericidal antibiotic-mediated ROS formation may give us insight into the potential clinical relevance of this model, including possible deleterious side effects to sinonasal epithelial tissue from the long-term or inappropriate use of antibiotics in the treatment of sinusitis.

ROS formation by bactericidal antibiotics also resulted in increased expression of some antioxidant genes regulated by Nrf2 (Figure 2). It is possible that pre-treatment with compounds that activate the Nrf2 system prior to treatment with bactericidal antibiotics could protect against the deleterious properties of ROS while maintaining antimicrobial efficacy.

In future studies, it will be interesting to explore how the basal inflammatory differences among SNECs from control, CRS without polyps, and CRS with polyp patients may influence the formation of ROS and the activation of inflammatory pathways following stimulation with bactericidal antibiotics.

While the potential consequences of bactericidal antibiotic-mediated ROS accumulation have yet to be demonstrated in SNECs from those with CRS, the use of repetitive and extended courses of bactericidal antibiotics as part of maximal medical therapy may lead to long-term damage to the sinonasal mucosa in these patients. In particular, this should be considered when treating CRS with nasal polyp patients where the underlying disease state is believed to be propagated by a Th2-biased cytokine milieu[33,34].

Conclusions

Treatment of sinonasal epithelial cells with bactericidal antibiotics results in ROS formation, cell death, and up-regulation of antioxidant defense systems as well as pro-inflammatory mediators. These changes are not seen with the bacteriostatic antibiotic clarithromycin. The results from this tissue culture system suggests a potentially clinically relevant model whereby long-term or inappropriate antibiotic use in the treatment of sinusitis, may result in oxidative tissue damage to the sinonasal epithelium. Future studies will explore the clinical implications of such damage to the sinonasal epithelium.

Acknowledgments

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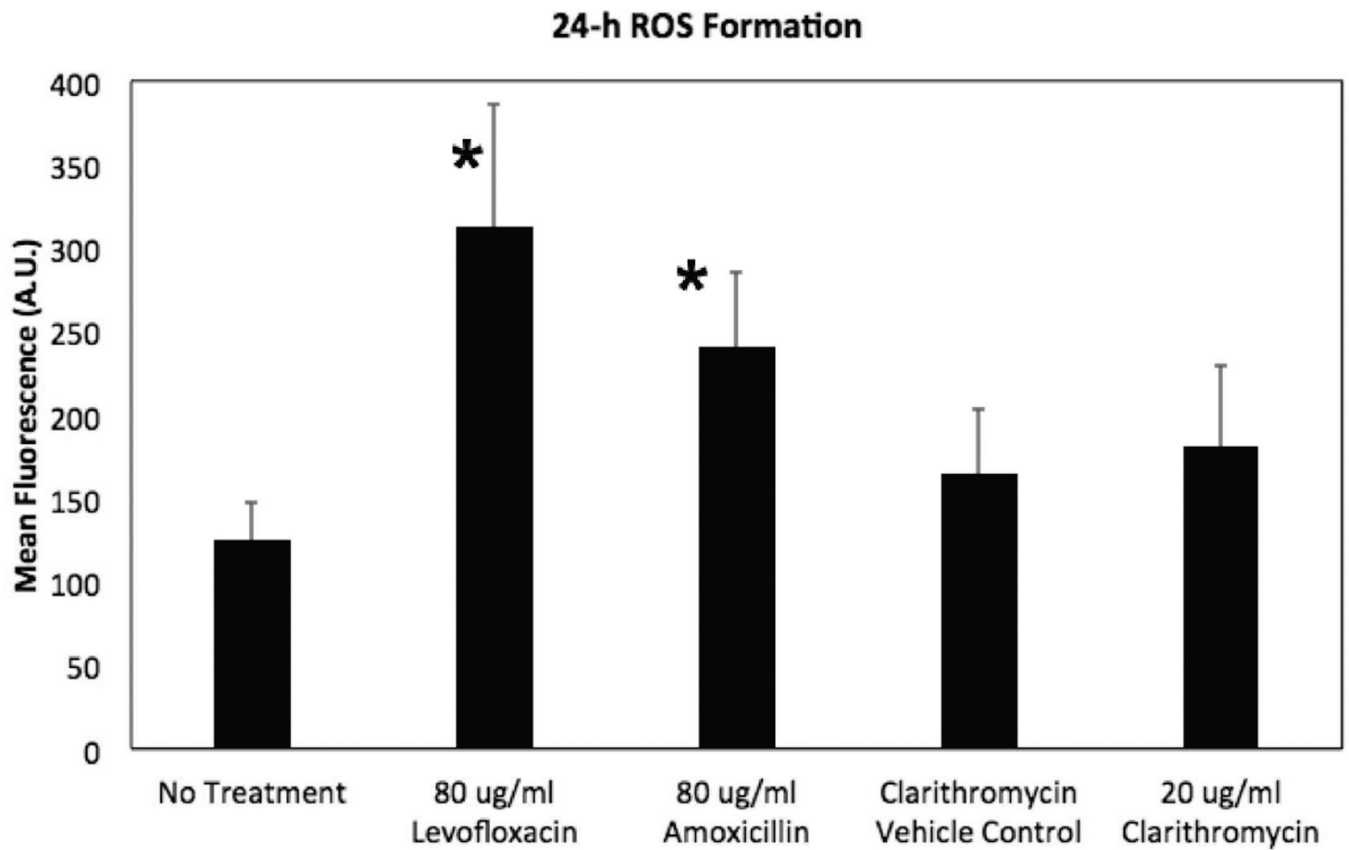
Figure 1.
Bactericidal Antibiotics Stimulate ROS Formation in SNECs. ROS formation was detected using the fluorescent probe CM-H2DCFDA in SNEC after treatment for 24 hours with no antibiotic (No treatment), 0.2% acetone (clarithromycin vehicle Control), 20μg/mL clarithromycin, 80μg/mL amoxicillin, and 80μg/mL levofloxacin, respectively. Shown are mean fluorescence +/− standard error of the mean (s.e.m.). * indicates p <0.05 relative to untreated samples.
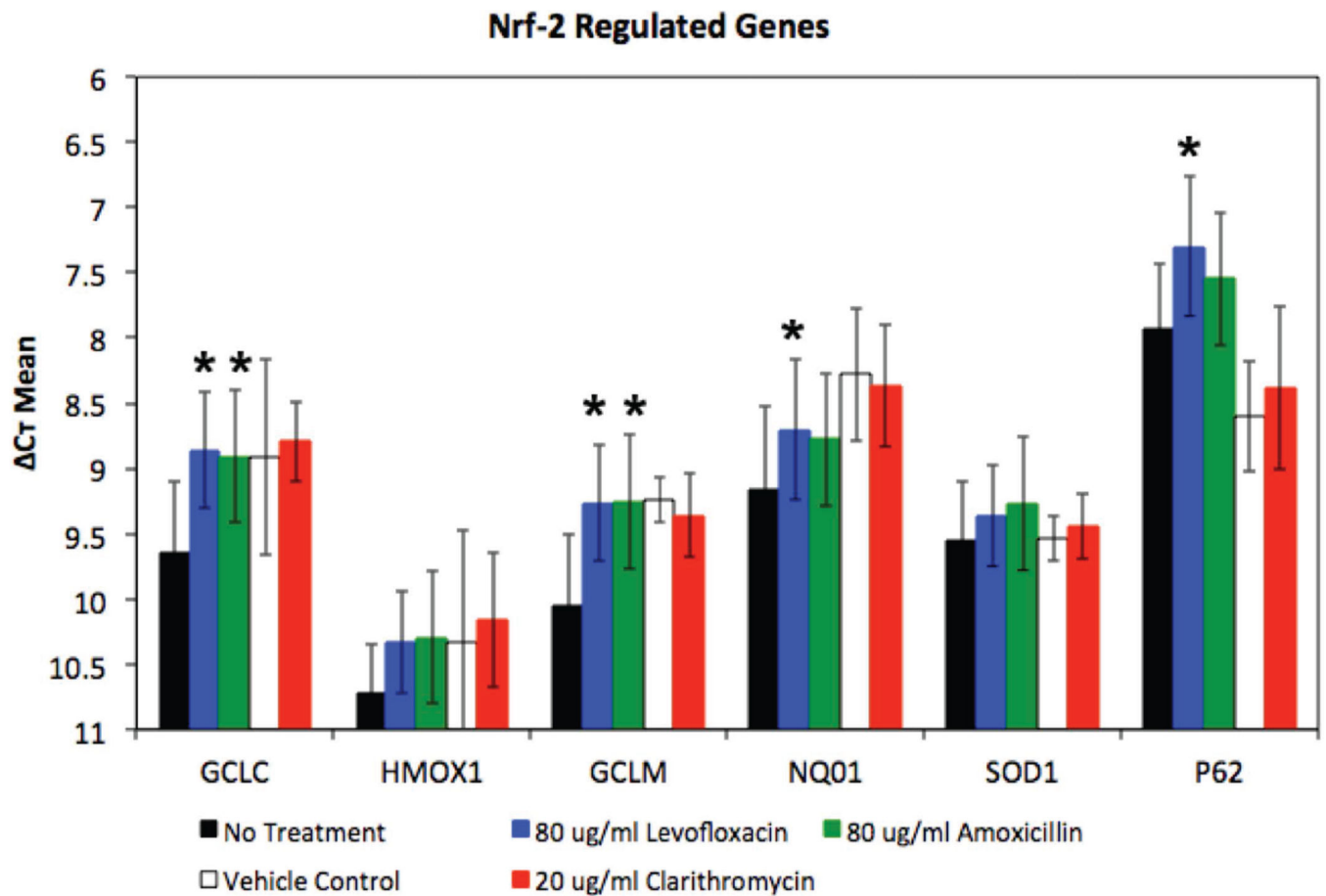
Figure 2.
Bactericidal Antibiotics Stimulate Expression of Nrf-2-Regulated Genes in SNECs.
Expression of Nrf-2-regulated genes, *GCLC, GCLM, HMOX1, NQO1, SOD1* and *p62* were
measured by RT-PCR in SNEC after treatment for 24 hours with no antibiotic (No
treatment, black fill rectangles), 0.2% acetone (clarithromycin vehicle Control, No fill
rectangles), 20μg/mL clarithromycin (red rectangles), 80μg/mL levofloxacin (blue
rectangles), and 80μg/mL amoxicillin (red rectangles), respectively. Shown are mean ΔCt +/
− s.e.m. * indicates $p < 0.05$ relative to untreated samples.

Inflammatory Cytokines

A

Inflammasome-Related Genes

B

■ No Treatment ■ 80 ug/ml Levofloxacin ■ 80 ug/ml Amoxicillin
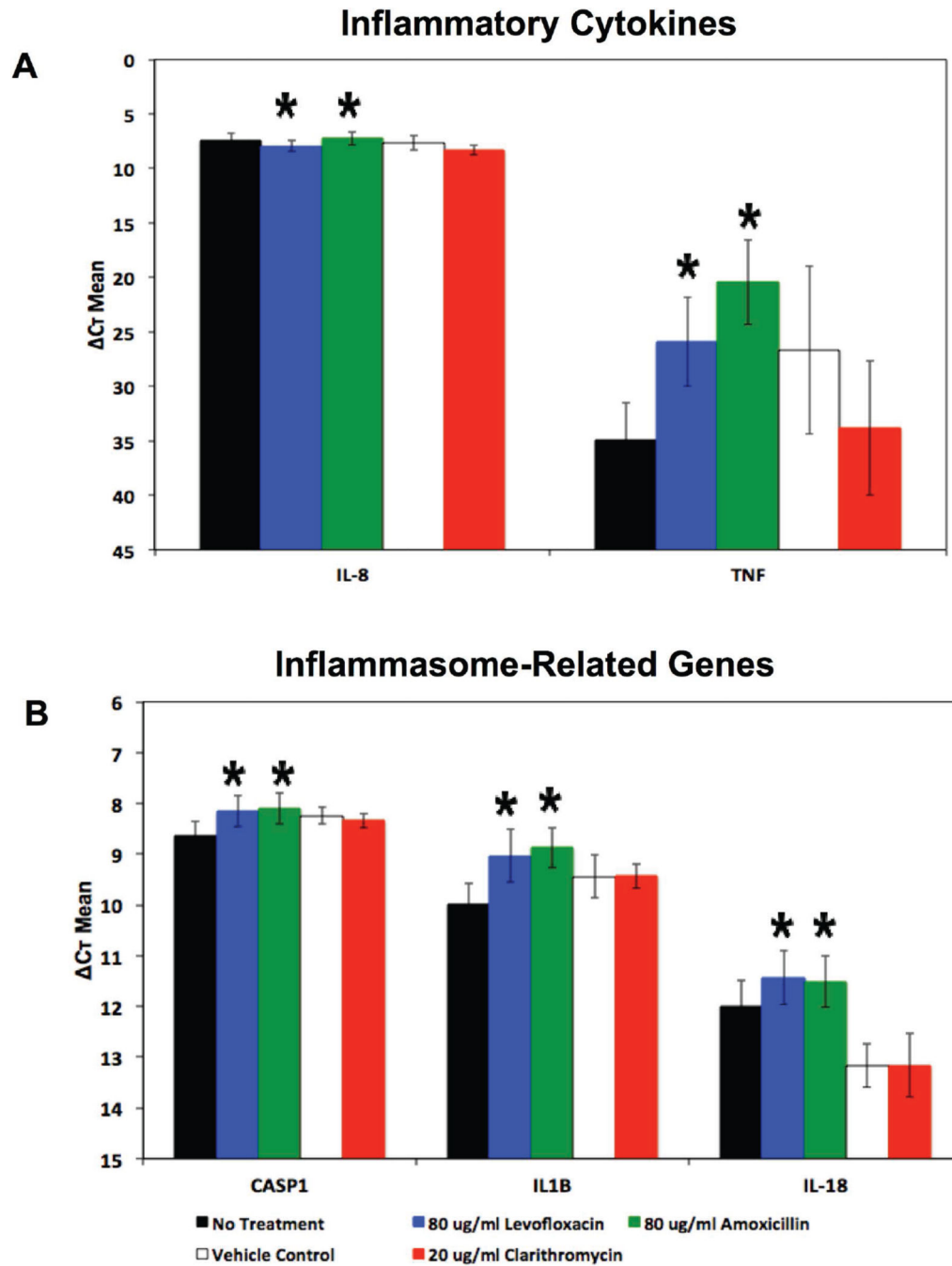□ Vehicle Control ■ 20 ug/ml Clarithromycin

Figure 3.
Bactericidal Antibiotics Stimulate Expression of Pro-Inflammatory Genes in SNECs.
Expression of inflammatory markers (A) *TNF*-α, *IL*-8, and (B) *IL-1*β, *IL-18 and CASP1*
were measured by RT-PCR in SNEC after treatment for 24 hours with no antibiotic (No
treatment, black fill rectangles), 0.2% acetone (clarithromycin vehicle Control, No fill
rectangles), 20μg/mL clarithromycin (red rectangles), 80μg/mL levofloxacin (blue
rectangles), and 80μg/mL amoxicillin (red rectangles), respectively. Shown are mean   Ct +/
− s.e.m. * indicates p <0.05 relative to untreated samples.

Basal TNF Secretion
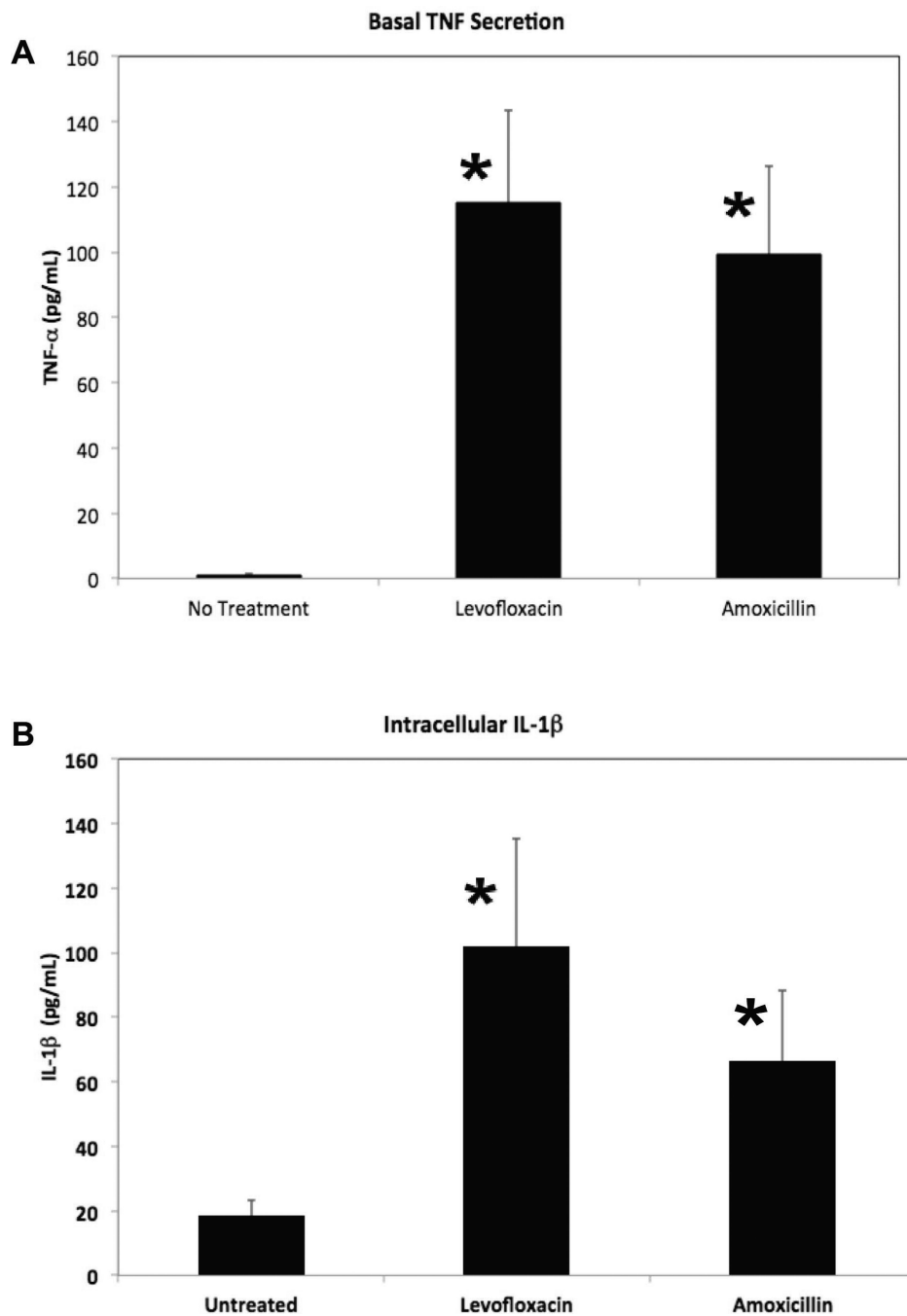
A

Intracellular IL-1β

B

Figure 4.
Bactericidal Antibiotics Stimulate Production of Pro-Inflammatory Proteins in SNECs. (**A**) Basal secretion of TNF-α was quantified by ELISA in SNEC after 48 hours of treatment with no antibiotic (No treatment), 80μg/mL levofloxacin, and 80μg/mL amoxicillin, respectively. (**B**) Intracellular concentration of IL-1β was quantified by ELISA in SNEC after 48 hours of treatment with no antibiotic (No treatment), 80μg/mL levofloxacin, and 80μg/mL amoxicillin, respectively. Shown are mean concentration (pg/mL) +/− s.e.m. of TNF-α and IL-1β, respectively. *indicates $p < 0.05$ relative to untreated samples.
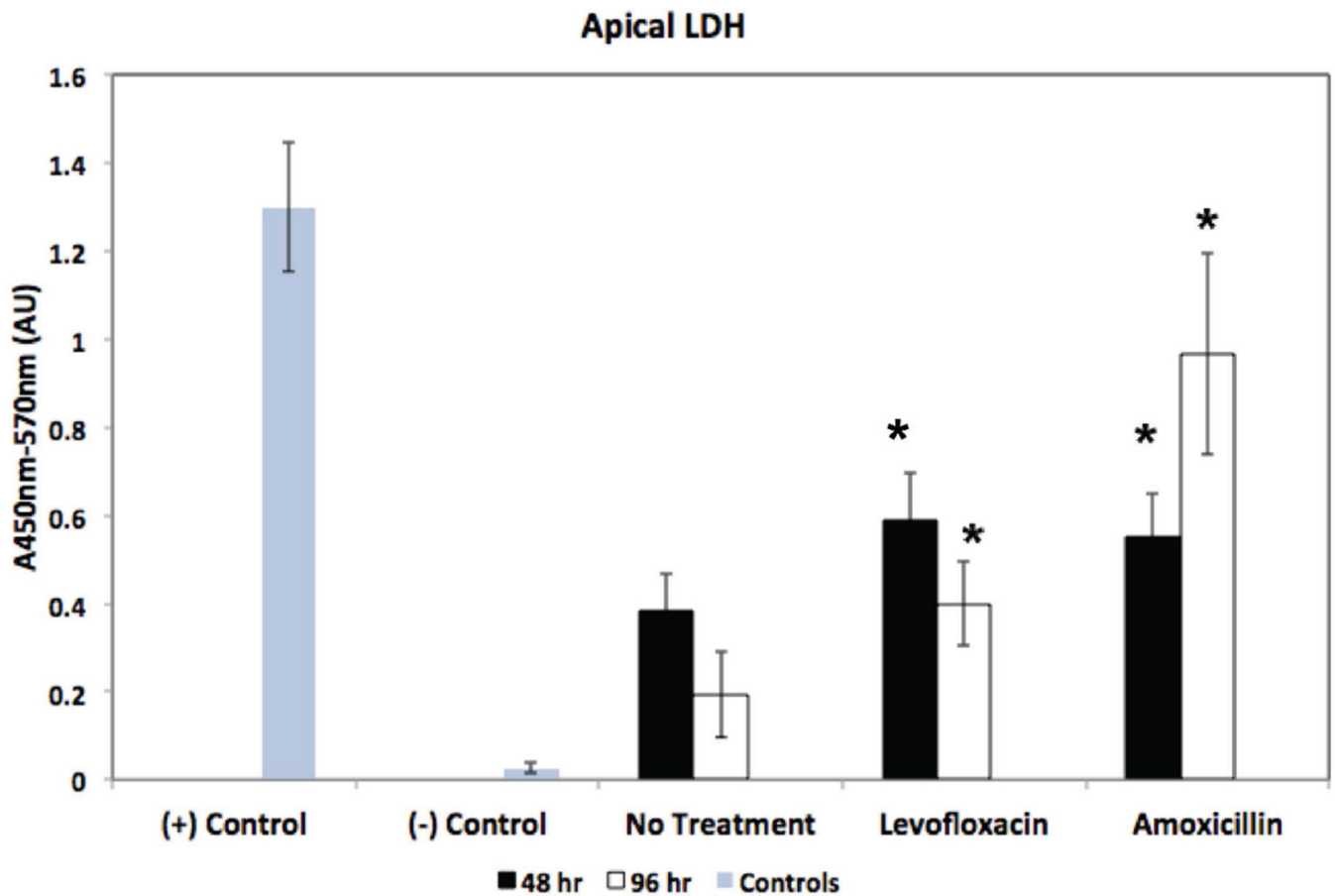
Figure 5.
Bactericidal Antibiotic treatment decreases viability of human SNECs. SNEC viability as measured by apical LDH secretion (Absorbance 450nm–570nm) after 48-hours (black bars) and 96-hours (white bars), respectively, of treatment with no antibiotic (no treatment), 80μg/ml levofloxacin or 80μg/ml amoxicillin. Gray bars indicate LDH measured from lysed cells [positive control, (+) control], and LDH measured in 1× PBS [negative control, (−) control]. Shown are mean values +/− s.e.m. *indicates p<0.05 relative to untreated samples at respective time-point.

Table 1

RT-PCR Taqman primers

Gene	Probe
GCLC	Hs00155249_m1
GCLM	Hs00157694_m1
HMOX1	Hs01110250_m1
NQO1	Hs00168547_m1
SOD1	Hs00533490_m1
IL8	Hs00174103_m1
TNF-α	Hs01113624_g1
p62	Hs01061917_g1
CASP1	Hs00354836_m1
IL-1β	Hs01555410_m1
IL18	Hs01038788_m1
18S	Hs99999901_s1

Table 2

Gene expression changes following treatment with no antibiotic (no treatment), 80μg/ml levofloxacin, and 80μg/ml amoxicillin, respectively. Shown are average Ct, standard error (s.e.) Ct, and p-values comparing levofloxacin or amoxicillin treatment to no treatment. Statistically significant p-values highlighted gray.

Gene	No Treatment Ct mean	Ct s.e.m.	80 μg/ml Levofloxacin Ct mean	Ct s.e	p-value	80 μg/ml Amoxicillin Ct mean	Ct s.e.	p-value
GCLC	9.64	0.54	8.86	0.45	0.001	8.91	0.68	0.024
GCLM	10.06	0.55	9.27	0.44	0.005	9.26	0.63	0.024
HMOX1	10.72	0.37	10.34	0.39	0.423	10.30	0.49	0.268
NQO1	9.16	0.63	8.70	0.54	0.004	8.78	0.76	0.125
SOD1	9.55	0.45	9.36	0.39	0.596	9.27	0.49	0.405
p62	7.93	0.50	7.30	0.53	0.005	7.55	0.51	0.275
IL-8	7.42	0.62	7.91	0.51	0.235	7.23	0.60	0.258
TNF	34.94	3.39	25.90	4.12	0.037	20.45	3.86	0.002
CASP1	8.64	0.29	8.16	0.30	0.006	8.10	0.31	0.019
IL1B	9.99	0.42	9.04	0.53	0.0002	8.87	0.40	0.0002
IL18	11.99	0.74	11.43	0.89	0.013	11.50	0.75	0.004

Table 3

Gene expression changes following treatment with 0.2% acetone (Clarithromycin Vehicle Control), and 20μg/ml clarithromycin, respectively. Shown are average ΔCt, standard error (s.e.) ΔCt, and p-values comparing clarithromycin treatment to clarithromycin vehicle control treatment. Statistically significant p-values highlighted gray.

Gene	Vehicle Control		20 μg/ml Clarithromycin		
	Ct mean	Ct s.e.	Ct mean	Ct s.e.	p-value
GCLC	8.91	0.75	8.80	0.30	0.81
GCLM	9.24	0.17	9.36	0.32	0.30
HMOX1	10.34	0.86	10.16	0.51	0.64
NQO1	8.28	0.51	8.37	0.47	0.52
SOD1	9.53	0.17	9.44	0.25	0.44
p62	8.60	0.43	8.39	0.62	0.24
IL-8	7.66	0.65	8.31	0.45	0.32
TNF	26.66	7.71	33.84	6.16	0.28
CASP1	8.24	0.17	8.34	0.14	0.45
IL1B	9.44	0.42	9.43	0.24	0.87
IL18	13.17	0.25	13.17	0.05	0.67