PHARMACODYNAMICS

Clarithromycin, trimethoprim, and penicillin and oxidative nucleic acid modifications in humans: randomised, controlled trials

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AIMS

In vitro studies have demonstrated that formation of reactive oxygen species (ROS) contributes to the effect of bactericidal antibiotics. The formation of ROS is not restricted to bacteria, but also occurs in mammalian cells. Oxidative stress is linked to several diseases. This study investigates whether antibiotic drugs induce oxidative stress in healthy humans as a possible mechanism for adverse reactions to the antibiotic drugs.

METHODS

This study contains information from two randomised, controlled trials. Participants underwent 1 week treatment with clarithromycin, trimethoprim, phenoxymethylpenicillin (penicillin V), or placebo. Oxidative modifications were measured as 24-h urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo), and plasma levels of malondialdehyde before and after treatment as a measurement of DNA oxidation, RNA oxidation, and lipid peroxidation, respectively.

RESULTS

Clarithromycin significantly increased urinary excretion of 8-oxodG by 22.0% (95% confidence interval (CI): 3.6–40.4%) and 8-oxoGuo by 14.9% (95% CI: 3.7–26.1%). Further, we demonstrated that trimethoprim significantly lowered urinary excretion of 8-oxodG by 21.7% (95% CI: 5.8–37.6%), but did not influence urinary excretion of 8-oxoGuo. Penicillin V did not influence urinary excretion of 8-oxodG or 8-oxoGuo. None of the antibiotic drugs influenced plasma levels of malondialdehyde.

CONCLUSION

Clarithromycin significantly increases oxidative nucleic acid modifications. Increased oxidative modifications might explain some of clarithromycin's known adverse reactions. Trimethoprim significantly lowers DNA oxidation but not RNA oxidation. Penicillin V had no effect on oxidative nucleic acid modifications.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Formation of reactive oxygen species contributes to the effect of bactericidal antibiotics. The formation of reactive oxygen species is not restricted to bacteria, but also occurs in mammalian cells.
- Oxidative stress is associated with several diseases e.g. type 2 diabetes, neurodegenerative-, and cardiovascular diseases.

WHAT THIS STUDY ADDS

- Clarithromycin significantly increases oxidative nucleic acid modifications in humans. Clinical studies are needed to establish whether clarithromycin has safety issues in specific patient populations.
- Trimethoprim significantly lowers DNA oxidation but not RNA oxidation.
- Penicillin V has no effect on oxidative nucleic acid modifications.

Introduction

Antibiotic drugs are widely used against various infections, are classified according to their accepted mechanism of action, and are traditionally grouped in bacteriostatic or bactericidal antibiotics. Bacteriostatic antibiotics prevent growth of bacteria while bactericidal antibiotics kill bacteria [1].

In addition to the accepted mechanisms of bactericidal antibiotic drugs, it has been demonstrated that formation of reactive oxygen species (ROS) contributes to the bactericidal effect, irrespective of their targets [2–9]. The production of ROS is thought to be from activation of the tricarboxylic acid cycle and thereby stimulation of the electron chain [2]. However, there is still no agreement on the clinical relevance of the ROS production [10, 11]. The formation of ROS implies a possible target effect of antibiotic drugs, and furthermore raises the question of a possible mechanism for adverse reactions (AR). It has been demonstrated that bactericidal antibiotics induce ROS formation not only in bacteria but also in mammalian cells [12, 13]. Oxidative modifications are linked to ageing [14] and several diseases e.g. type 2 diabetes (T2D) [15], neurodegenerative diseases [16–18], and cardiovascular diseases [19]. The elevated levels of oxidative modifications are considered prognostic for mortality in patients with T2D [20, 21] and also associated with development of lung cancer in non-smokers [22] and oestrogen receptor positive breast cancer [23]. However, it remains a subject of great interest whether the elevated levels of oxidative modifications are prognostic in neurodegenerative- and cardiovascular diseases [24]. It is possible that elevated levels of oxidative modifications may contribute to drug AR; for example, the finding that 2 weeks' treatment with clarithromycin significantly increases cardiovascular disease three years after treatment in patients with stable coronary heart diseases [25].

Several studies have found an association between bactericidal antibiotic drugs and the production of ROS [2–9, 12, 13], but this has not been found for bacteriostatic antibiotic drugs [2, 3, 12, 13].

The aim of this study is to evaluate the effect of clarithromycin, trimethoprim, and phenoxymethylpenicillin (penicillin V) on oxidative modifications in healthy humans. Clarithromycin and trimethoprim are both classified as bacteriostatic antibiotic drugs, whereas penicillin V is a bactericidal antibiotic drug [1].

We measured three markers of oxidative modifications in order to differentiate the effects in various cellular compartments. We used 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), 8-oxo-7,8-dihydroguanosine (8-oxoGuo), and malondialdehyde (MDA) as measurement of intranuclear- (DNA oxidation), cytosolic- (RNA oxidation), and plasma oxidative modifications (lipid peroxidation), respectively [26, 27]. The measurements were conducted before and after exposure to the three antibiotics and placebo.

Methods

The present study provides information from two trials; CLAROX (EudraCT: 2008-001299-61; ClinicalTrials.gov: NCT00707330) and PENTRIOX (EudraCT: 2010-022762-27; ClinicalTrials.gov: NCT02188472). Both trials have the same inclusion criteria, study outcome, and sample size. The trials are designed as randomised, controlled with cross-over or parallel groups, respectively.

Trial design

CLAROX. CLAROX is a randomised, controlled, cross-over study, where participants in random order received no treatment or active treatment for 1 week separated by a 2-week washout period.

The study has an open-label design to diminish logistic and cost. The laboratory analyses were performed blinded to treatment information.

Trial medicine was obtained from the Pharmacy of the Capital Region of Denmark.

The trial design is illustrated in supplementary information. Fasting participants underwent five visits at the trial site after informed consent. (i) A screening visit to ensure the participants eligibility to the trial. A blood sample and basic

clinical tests including measurement of blood pressure, pulse, height, weight and an electrocardiogram (ECG) were recorded. (ii) At the second visit, trial medicine was dispensed to the participants. A 24-h urine sample and a blood sample were collected. (iii) After the first treatment schedule, the third visit took place. Participants delivered a 24-h urine sample, and a blood sample was collected. (iv) Before the second treatment schedule, participants underwent a fourth visit. Blood pressure and an ECG were recorded, and a blood sample was collected. (v) At the fifth and final visit, participants delivered a 24-h urine sample, excess trial medicine, and a blood sample was collected.

The trial was approved by the Regional Committee on Biomedical Research Ethics (H-D-2008-026) and the Danish Health and Medicines Authority. The trial was conducted in accordance with the Declaration of Helsinki.

PENTRIOX. PENTRIOX is a double-blinded, randomised, placebo-controlled study with three parallel groups. The treatment arms consisted of treatment with trimethoprim, penicillin V, and placebo, each for 1 week.

Trial medicine was randomised and labelled by the Pharmacy of the Capital Region of Denmark.

The trial design is illustrated in supplementary information. Non-fasting participants underwent three visits at the trial site after informed consent. (i) A screening visit to ensure the participants eligibility to the trial. A blood sample and basic clinical tests including measurements of blood pressure, pulse, height, weight and an ECG were recorded. (ii) At the start visit, trial medicine was dispensed to participants. A 24-h urine sample and a blood sample were collected. (iii) At the third and final visit, participants delivered a 24-h urine sample, excess trial medicine and a blood sample was collected.

The trial was approved by the Regional Committee on Biomedical Research Ethics (H-1-2010-099) and the Danish Health and Medicines Authority. The trial was conducted in accordance with the Declaration of Helsinki.

Participants

In both CLAROX and PENTRIOX, the trial population consisted of healthy, Caucasian males who were non-smokers with a body mass index >18 kg m^2 and <30 kg m^2. The exclusion criteria are available in supplementary information, and were in general selected to ensure the safety of participants as well as avoid possible influence on the primary outcome, oxidative nucleic acid modifications.

Participants were recruited through approved advertisement in local media, online, and in public places. Potential participants who met the inclusion criteria received written information in the form of the approved informed consent.

To ensure adherence, participants received a text message as a reminder of the medicine intake. The participants answered the text message after medicine intake, hereby confirming adherence. Satisfactory adherence level of 65% was predefined in the protocol. Returned medication was destroyed at the trial site.

Participants were informed prior to the trial about possible side effects. They were told to contact an investigator if they experienced any side effect. Adverse events were registered after each treatment schedule. All adverse events were reported at the end of the trials to the Danish Health and Medicines Authority and the Danish National Committee on Biomedical Research Ethics.

Intervention

CLAROX participants received 1 tablet clarithromycin (Klacid Uno; BGP Products, Maidenhead, UK) 500 mg once daily for 1 week. The trial medicine was administrated with a meal in the morning. Participants in the control schedule received no treatment.

PENTRIOX participants received two tablets: 330 mg penicillin V (Pancillin; Sandoz, Holzkirchen, Germany), 100 mg trimethoprim (Trimopan; Orion Pharma, Espoo, Finland), or placebo twice daily for 1 week. The trial medicine was administrated with a meal in the morning and in the evening.

All trial medicine was administered in clinically relevant dosages [28–30], and complied with GMP [31] and GCP regulations [32].

Study outcomes

Primary outcomes. The primary outcomes were urinary excretion of 8-oxodG and 8-oxoGuo as a measurement of whole body DNA- and RNA oxidation, respectively [26].

The 24-h urine collection was sampled from 24 h prior to the second, third and fifth visit in CLAROX and prior to the second and third visit in PENTRIOX. The volume urine collected in each container was recorded, well mixed and an aliquot was stored at –20°C until analysis.

If participants recorded loss of a urine void, an estimated value was added to the recorded diuresis. Satisfactory adherence level of 75% was predefined in the protocol.

The analysis was done simultaneous by tandem mass spectrometry with isotope dilution, details and quality control are reported elsewhere [33]. As mentioned by Henriksen *et al.* [33], the lower limit of quantification for both 8-oxodG and 8-oxoGuo was 1.0 nmol l^{-1}. The average within-day precision (percentage of relative standard deviation) was 3.7 and 4.4%, and the average between-day precision was 2.3 and 4.0% for 8-oxodG and 8-oxoGuo, respectively. The accuracy (percentage recovery) was 106.9 and 106.2% for 8-oxodG and 8-oxoGuo, respectively [33].

Secondary outcome. The secondary outcome was plasma levels of MDA as a measurement of lipid peroxidation [27].

Blood samples were collected in 10-mL heparin prepared utensils. The samples were centrifuged for 10 min and plasma was transferred to three sample vials and stored at –80°C until analysis. Details of sample preparation, analysis and quality control are reported elsewhere [34]. In brief, plasma samples were treated with phosphotungstic acid to remove other compounds that may interfere with the assay. Subsequently, butylated hydroxytoluene, a chain-breaking antioxidant that prevent peroxidation during the assay itself, was added, and MDA reacted with thiobarbituric acid (TBA 2.3 μmol l^{-1}) in the presence of acetic acid (1.75 mol l^{-1}) for 1 h at 95°C to form the MDA(TBA)$_2$ fluorescent adduct. Following extraction with butanol, the genuine MDA(TBA)$_2$ adduct was selectively quantified by high-performance liquid chromatography (HPLC) with fluorescence detection

(excitation, 515 nm; emission, 553 nm). The limit of quantification was 0.1 μmol l^{-1} MDA and the within- and between-day coefficients of variation for the entire assay were 8.2% and 14.1%, respectively [34]. The analyses were performed at Department of Veterinary Pathobiology, University of Copenhagen.

Pretrial power calculation

With 10% coefficient of variation a sample size of 25 participants was estimated to provide 80% power, and to detect 8% increase in oxidative nucleic acid modifications at 5% significance.

Due to possible dropout, it was decided to include 30 participants in each trial.

Statistics

Data management and analysis were performed using R version 3.2.2 [35].

Data was analysed per-protocol. Clinical and demographic characteristics were analysed using a Student t test in CLAROX and one-way ANOVA test in PENTRIOX.

Primary and secondary outcome were analysed using a paired t test in CLAROX and Student t test in PENTRIOX. Data were investigated for normal distribution and verified using a non-parametric test.

A two-sided P-value < 0.05 was considered statistically significant.

Results

Study population and flow

CLAROX.   Participant flow is illustrated in Figure 1. Twenty-eight potential participants were assessed for eligibility. Two potential participants were excluded by investigators due to

health issue: chronic inflammatory disease (one participant) and cardiac disease (one participant). Twenty-six participants were randomised. One participant was withdrawn from treatment due to penicillin treatment of a skin infection. Twenty-five participants successfully completed the trial and their urine- and blood samples were analysed.

Baseline clinical and biochemistry characteristics of the participants are presented in Table 1. The characteristics were within normal range and did not differ between groups, except diastolic blood pressure which was significantly lower in the group that received clarithromycin treatment followed by control treatment ($P = 0.02$).

There was no significant difference in baseline urinary excretion of 8-oxodG ($P = 0.10$) or 8-oxoGuo ($P = 0.44$) between the groups.

According to registration by trial participants, the overall adherence of urine samples was 93%. According to a control system based on phone text messages from participants, adherence of medicine intake was 99%.

Twelve participants reported a total of 13 adverse events (AE). Nine were evaluated to be related to the medicine (AR), none were serious AE. Gastrointestinal reactions and nausea were the most frequently observed events.

PENTRIOX.   Participant flow is illustrated in Figure 2. One hundred and twelve potential participants were assessed for eligibility. Twenty-two of the potential participants were excluded at screening visit due to: hypercholesterolaemia (11 participants); elevated blood pressure (two participants); elevated ALAT (two participants); isolated hypertriglyceridaemia (one participant); hypopotassaemia (one participant); previous heart arrhythmia (one participant); murmur of the heart (one participant); use of illegal drugs (one participant); and withdrawal for no given reason (two participants).
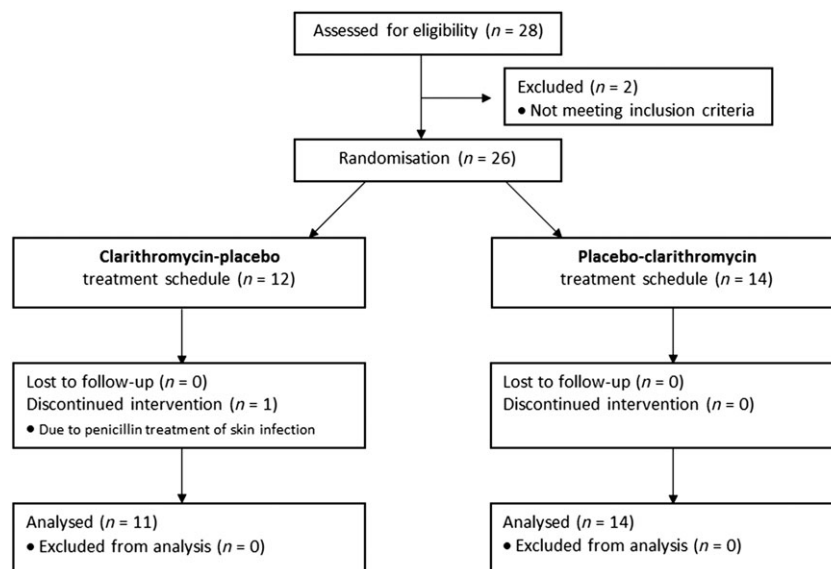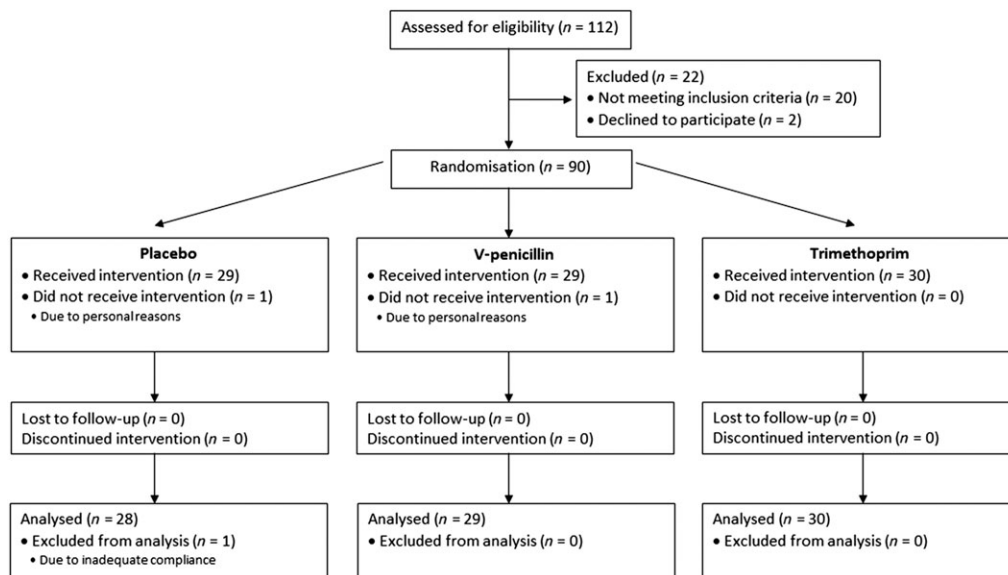

Figure 1

Participants flow for the CLAROX trial

Table 1

Baseline clinical and biochemistry characteristics (CLAROX)

	Clarithromycin–control	Control–clarithromycin	P^a
n	11	14	
Age (years)	24.2 (3.0)	24.3 (2.0)	NS
Weight (kg)	78.8 (5.4)	77.6 (6.2)	NS
Height (m)	1.84 (0.05)	1.84 (0.05)	NS
Body mass index (kg m^{-2})	23.4 (1.7)	22.9 (1.6)	NS
Systolic blood pressure (mmHg)	127.2 (9.2)	126.7 (11.9)	NS
Diastolic blood pressure (mmHg)	73.8 (4.8)	79.3 (5.6)	0.02
Pulse (beats min^{-1})	61.7 (8.8)	62.0 (11.3)	NS
QTc interval (ms)	384.7 (32.3)	393.0 (31.1)	NS
Total cholesterol (mmol l^{-1})	3.8 (0.6)	4.1 (0.7)	NS
HDL cholesterol (mmol l^{-1})	1.5 (0.3)	1.5 (0.3)	NS
LDL cholesterol (mmol l^{-1})	2.2 (0.6)	2.4 (0.5)	NS
Triglycerides (mmol l^{-1})	0.8 (0.4)	0.8 (0.3)	NS
Serum creatinine (mmol l^{-1})	78.8 (8.0)	79.3 (8.4)	NS
Ferritin (μmol l^{-1})	131.5 (53.2)	110.3 (61.6)	NS
Iron (μmol l^{-1})	19.4 (7.6)	22.0 (11.8)	NS
Transferrin saturation	0.29 (0.11)	0.31 (0.17)	NS
Potassium (mmol l^{-1})	4.0 (0.2)	4.0 (0.2)	NS
Magnesium (mmol l^{-1})	0.9 (0.1)	0.9 (0.1)	NS

Values are presented as mean (standard deviation)
[a]Student t test; NS = not statistically significant.

Figure 2

Participants flow for the PENTRIOX trial

Ninety participants were randomised to treatment. Two participants withdrew due to personal reasons, and one participant was excluded due to inadequate adherence.

Baseline clinical and biochemistry characteristics of the participants are presented in Table 2. The characteristics were within normal range and did not differ between groups.

There was no significant difference in baseline urinary excretion of 8-oxodG ($P = 0.61$) or 8-oxoGuo ($P = 0.93$) between the groups.

According to trial participants, the overall adherence of urine samples was 94%. According to a control system based on phone text message from participants, adherence of medicine intake was 99%.

Seventeen participants reported a total of 23 AE. Ten were judged to be related to the medicine (AR), none were serious AE. Gastrointestinal discomfort was the most frequently observed event.

Primary endpoints

Data for primary endpoints (8-oxodG and 8-oxoGuo) are presented in Figure 3.

Clarithromycin significantly induced DNA oxidation by increasing urinary excretion of 8-oxodG with 22.0% (95% CI: 3.6–40.4%) compared to control ($P = 0.02$). Trimethoprim significantly lowered urinary excretion of 8-oxodG by 21.7% (95% CI: 5.8–37.6%) compared to placebo ($P < 0.01$). There was no significant difference in urinary excretion of 8-oxodG following penicillin V treatment compared to placebo ($P = 0.63$).

Clarithromycin significantly induced RNA oxidation by increasing urinary excretion of 8-oxoGuo by 14.9%

(95% CI: 3.7–26.1%) compared to control ($P = 0.01$). There was no statistically significant difference in urinary excretion of 8-oxoGuo between placebo treatment and trimethoprim treatment ($P = 0.71$) or penicillin V treatment ($P = 0.90$).

Secondary endpoints

Data for secondary endpoint (MDA) are presented in Figure 4.

There was no statistically significance difference in MDA, when treatment with clarithromycin, penicillin V, and trimethoprim was compared to control/placebo ($P > 0.05$).

Discussion

This study is the first to demonstrate that 1 week treatment with clarithromycin increases oxidative nucleic acid modifications and 1 week treatment with trimethoprim lowers DNA oxidation in non-infected humans. The bactericidal antibiotic penicillin V does not influence oxidative nucleic acid modifications.

Our study supports the finding that ROS production from antibiotic drugs is not restricted to bacteria but also takes place in mammalian cells [12, 13]. This is not an intended effect when administering the drugs. Increased levels of oxidative modifications is linked to a number of different diseases [16–19], and therefore increased oxidative modifications in relation to antibiotic treatment need confirmation and further exploration.

The biomarkers used to evaluate oxidative nucleic acid modifications are 8-oxodG and 8-oxoGuo. The biomarkers

Table 2

Baseline clinical and biochemistry characteristics (PENTRIOX)

	Placebo	Penicillin V	Trimethoprim	P [a]
n	28	29	30	
Age (years)	24.4 (2.9)	23.8 (3.0)	25.2 (2.9)	NS
Weight (kg)	78.5 (8.9)	79.7 (8.0)	81.3 (9.2)	NS
Height (m)	1.84 (0.05)	1.84 (0.05)	1.87 (0.07)	NS
Body mass index (kg m^2)	23.2 (2.1)	23.6 (1.9)	23.4 (2.3)	NS
Systolic blood pressure (mmHg)	128.8 (7.0)	129.7 (6.7)	130.1 (8.2)	NS
Diastolic blood pressure (mmHg)	77.0 (7.5)	77.5 (7.5)	77.0 (7.1)	NS
Pulse (beats min^{-1})	66.2 (9.1)	67.2 (12.8)	68.2 (12.7)	NS
Total cholesterol (mmol l^{-1})	4.4 (0.7)	4.2 (0.6)	4.2 (0.6)	NS
Serum creatinine (mmol l^{-1})	78.7 (11.8)	78.1 (12.3)	79.3 (8.7)	NS
Ferritin (μmol l^{-1})	126.1 (80.9)	110.3 (74.9)	120.9 (71.6)	NS
Iron (μmol l^{-1})	18.8 (6.9)	21.4 (8.8)	21.7 (5.4)	NS
Transferrin saturation	0.28 (0.12)	0.32 (0.13)	0.34 (0.09)	NS
Haemoglobin (mmol l^{-1})	9.5 (0.5)	9.4 (0.6)	9.4 (0.5)	NS
Folate (mmol l^{-1})	14.7 (7.4)	13.9 (6.4)	14.4 (5.8)	NS

Values are presented as mean (standard deviation)
[a]One-way ANOVA; NS = not statistically significant
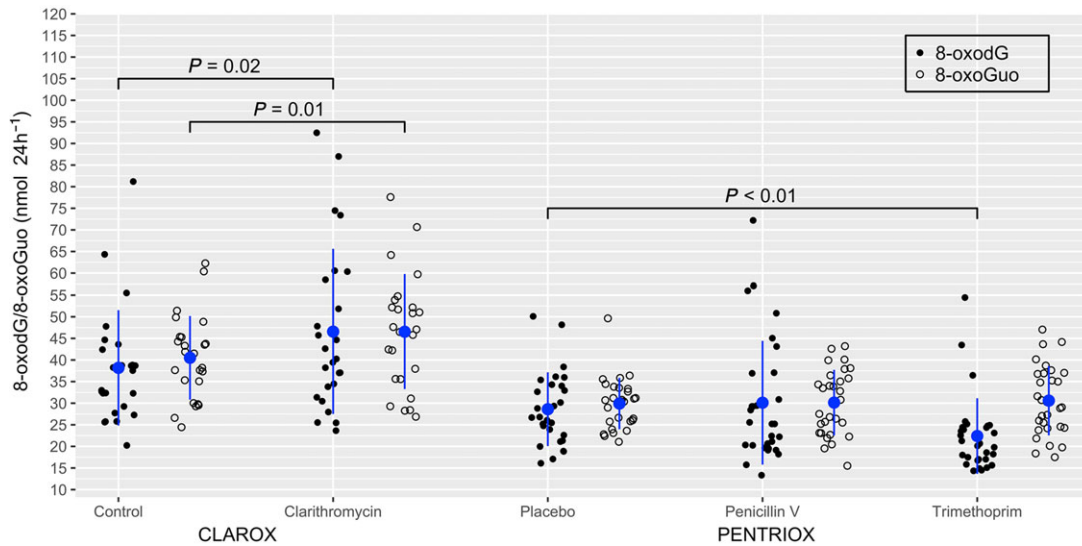
Figure 3

Values of urinary excretion of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG; solid) and 8-oxo-7,8-dihydroguanosine (8-oxoGuo; open) for each participant after treatment. The data are further denoted as mean (blue circle) and standard deviation (blue line). Clarithromycin significantly increased urinary excretion of 8-oxodG and 8-oxoGuo compared to control. Trimethoprim significantly lowered urinary excretion of 8-oxodG but not 8-oxoGuo compared to placebo. Penicillin V had no significant effect on urinary excretion of 8-oxodG or 8-oxoGuo compared to placebo
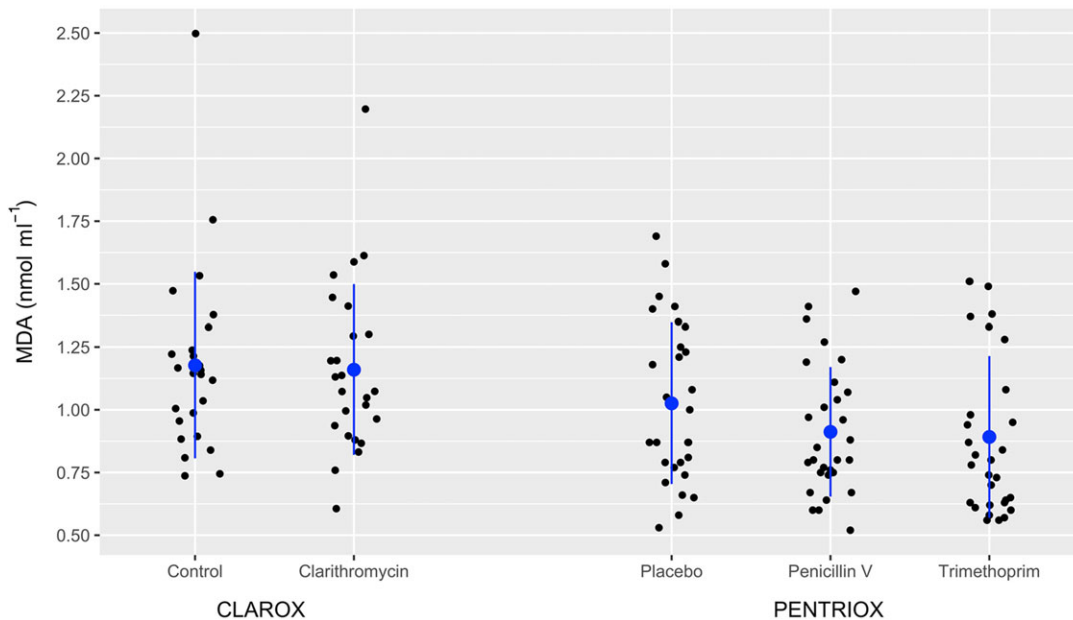

Figure 4

Values of plasma malondialdehyde (MDA; solid) for each participant after treatment. The data are further denoted as mean (blue circle) and standard deviation (blue line). There was no significant difference in MDA, when treatment with clarithromycin, penicillin V, and trimethoprim was compared to control/placebo

are both clinically relevant: 8-oxodG has been identified as a biomarker associated with development of lung cancer in non-smokers [22] and development of oestrogen receptor positive breast cancer [23]; and 8-oxoGuo is associated with overall mortality in patients with type 2 diabetes [20, 21].

The clinical importance of RNA oxidation might be explained by the observation that RNA oxidation is able to induce cellular apoptosis in human cell lines [36] and translational errors in animal models [37], even though the qualitative effect remains unknown.

Neurodegenerative and cardiovascular diseases are also associated with elevated levels of oxidative modifications; however, the prognostic value of elevated oxidative modifications remain unknown in these diseases [24].

We did not investigate the effect of clarithromycin on glutathione – an important nonenzymatic antioxidant [14]. The effect of clarithromycin on glutathione is an important subject for further research, since paracetamol depletes liver glutathione [38] and some patients have an inherited glucose-6-phosphate dehydrogenase deficiency that reduces the level of glutathione [39].

The observed effect of clarithromycin on urinary excretion of 8-oxodG and 8-oxoGuo compared to other lifestyle habits and diseases might seem small. Smoking increases urinary excretion of 8-oxodG by 50% [40], untreated patients with hemochromatosis have a 2.5-fold increased urinary excretion of 8-oxoGuo [41], and patients with type 2 diabetes have a 2-fold increased urinary excretion of 8-oxoGuo in highest quartile compared to the lowest quartile associated with increased mortality [21]. However, taking into account the short-term exposure compared to long-term lifestyle habits and chronic diseases the effect of clarithromycin treatment may still be relevant, particularly if certain organs are more affected than others.

There are several adverse reactions related to clarithromycin, where the mechanism is unknown. The CLARICOR trial demonstrated that 2 weeks' treatment with clarithromycin 500 mg/day showed a significant increase in cardiovascular disease (i.e. cardiovascular mortality, myocardial infarction, unstable angina, cerebrovascular attack, or peripheral vascular disease) 3 years after treatment in patients with stable coronary heart diseases [25]. However, the effect of clarithromycin on cardiovascular disease is not present, when participants were treated with statins at entry [42]. The fact that our results illustrate how 1 week of clarithromycin treatment increases oxidative nucleic acid modifications, and that oxidative modifications are linked to cardiovascular diseases [19] make us hypothesize that the CLARICOR results [25] relate to increased oxidative nucleic acid modifications.

Even though statins lower calcium-induced oxidative modifications [43], simvastatin did not reduce oxidative modifications in healthy humans [44]. The limited and inconsistent literature exploring a possible antioxidant effect of statins are not sufficient to rule out whether the reversed effect of statins in the CLARICOR results could be explained by an antioxidant effect by the statins.

In addition, the present study demonstrates that trimethoprim lowers DNA oxidation but not RNA oxidation. To our knowledge, this is the first time a drug treatment has been found to lower the urinary excretion of 8-oxodG. The result was unexpected, because methotrexate with same effect on folate as trimethoprim, although more pronounced, increases oxidative modifications [45]. Further, the result is at variance with a previous *in vitro* study, where trimethoprim increased DNA damage measured by tail percentage in comet assay at dosage of 100 μg/mL in rainbow trout gonad-2 cells, and had no effect on DNA damage in Chinese hamster ovary-K1 cells [46]. Our finding is worth noting, since increased levels of urinary excretion of 8-oxodG have been proposed to be associated with the development of certain lung and breast cancer [22, 23].

Antibiotic drugs cause bacterial death. Therefore, it could be questioned if bacterial death increases the urinary excretion of 8-oxodG and 8-oxoGuo, thus acting as a possible confounder of the results in this study. However, previous studies have demonstrated that increased cell turnover does not increase urinary excretion of 8-oxodG [47–49]. This finding supports the accepted hypotheses that urinary excretion of 8-oxodG is derived from repair of DNA oxidation [50], and we do not expect bacterial death to influence the result. To our knowledge, no studies have explored cell turnover as a possible confounder to urinary excretion of 8-oxoGuo.

MDA is a biomarker of lipid peroxidation. ROS react with polyunsaturated acids and forms MDA as an end product [51]. Surprisingly, none of the investigated antibiotics had any significant effect on the level of MDA. From this we conclude that: either the antibiotics do not induce lipid peroxidation measurable as MDA at the concentrations achieved in this study or oxidative modifications may be compartmentalized in the organism and within the cell. Although unbound MDA has been shown to be distributed relatively freely by passive diffusion despite its reactivity, most tissue and plasma MDA is considered to be protein bound [52, 53]. However, in the present study, we measured total MDA.

Previous studies have shown that MDA is a valuable marker on a group base, in particular when measured by specific assays [27]. Thus, the finding underlines that the term oxidative stress is relatively unspecific [54] and the notion that a number of biomarkers are necessary to understand the pathogenesis of different diseases that relates to oxidative modifications [51].

In order to achieve valid results, when measuring urinary excretion of 8-oxodG and 8-oxoGuo, the methodology is essential. Enzyme-linked immunosorbent assay (ELISA) measurements are frequently used, because the method is inexpensive and easy to use. However, the method is limited by the lack of specificity (probably due to unspecific antibodies [55]) compared to chromatography-based techniques such liquid chromatography coupled with electrochemical detection (LC-EC) or coupled with tandem mass-spectrometry (LC-MS/MS) [56]. Thus, the mean values of the ELISA methods have turned out to be higher than the values produced by the chromatographic methods [57, 58] and the association between the methods are weak [59]. The specificity of the ELISA method can be improved by a longer incubation at lower temperature i.e. 4°C, but there is still discrepancy between the methods [55]. Liquid chromatography coupled with tandem mass-spectrometry gives very high specificity and requires little sample preparation and is thus preferred to determine urinary excretion of 8-oxodG and 8-oxoGuo. The specificity of the method may be further improved by using ultra-HLPC and a qualifier ion [56].

The same pattern is found in regards of methods to determine MDA. Colorimetric assays based on the TBA reaction or other conjugation reactions are frequently used because of their simplicity and low cost. However, the TBA and other colorimetric MDA assays lack specificity and has been shown to detect multiple specimens unrelated to MDA. In the literature, this has given rice to reference values that differ up to two orders of magnitude from the more reliable methods based on HPLC [27].

It has been widely accepted, that only bactericidal antibiotic induces ROS formation, hence contributing to the antibiotic effect in bacteria [2–9], and this is also demonstrated in mammalian cells [12, 13]. Our study does not investigate *in vitro* mechanisms, but the effect on whole body level. Therefore, the study might not detect changes in particular cells or a particular organ. However, it is surprisingly that clarithromycin, a bacteriostatic antibiotic, increases oxidative nucleic acid modifications which is not seen, when participants are treated with penicillin V, a bactericidal antibiotic. This finding is at variance with the well-established hypothesis that only bactericidal antibiotic induces ROS formation – at least when the effect is measured on a whole-body level in humans. Our study design does not allow an explanation of the underlying cellular mechanisms. However, we hypothesize that compartmentalization within the cell and organism of oxidative modifications could explain the inconsistence between the markers of lipid peroxidation and oxidative nucleic acid modifications. If this is the case, our results suggest that clarithromycin only increases intracellular oxidative modifications in a compartmentalized fashion.

Strengths and limitations

The strengths of the trials are the controlled and randomised trial designs, and the high level of adherence. There are limitations to our study. The trials have rather small sample sizes, and the open-label design in the CLAROX trial could result in possible placebo effect and observer bias. However, the urine adherence collection is recorded with no difference between the groups, and the outcome of the trial is analysed without knowledge of the treatment.

Conclusion

In conclusion, we found that penicillin V has no effect, but clarithromycin increases whole body RNA and DNA oxidation in healthy humans. This finding raises the question whether increased oxidative modifications could explain some of the adverse reactions seen in relation to clarithromycin treatment.

To our surprise trimethoprim lowers DNA oxidation in healthy participants. To our knowledge, this is the first treatment found to lower the urinary excretion of 8-oxodG, and merits verification and further investigation in future studies.

Competing Interests

There are no competing interests to declare.

Contributors

E.L., H.P., V.C. and L.K.K. wrote the article; H.P., M.T.P, S.P. and L.K.H. designed the research; H.P., M.T.P, S.P., L.K.H., J. A., E.J.S., K.B., M.P., A.W., T.H. and J.L performed the research; E.L., H.P., V.C., L.K.K. and C.T.P. analysed data.

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Information S1 Trial design
Information S2 Exclusion criteria
Information S3 Representative high-performance liquid chromatogram