A Systematic Review of the Effects and Side Effects of Treatment with Oral Tetracycline in Acne Vulgaris

Master Thesis in Medicine

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Abstract

Background: Commonly prescribed therapies in adolescent Acne vulgaris are topical regimens and oral therapy, with first line antibiotic tetracycline. With the evident increasing resistance of Propionibacterium acnes to oral tetracycline, treatment failures are a consequence.

Aim: The aim of this systematic review was to evaluate the currently published data investigating the efficacy and side effects of treatment with oral tetracycline versus other commonly prescribed acne therapies. The secondary aim was to evaluate the treatment outcome related to propionibacterium acnes resistance to tetracycline.

Methods: An electronic hand search was done in PubMed, Scopus and Cochrane Library databases. Selected studies were limited to those in the English language with a 10-year span ranging from 2002-2012. Search terms conducted for all 3 databases were “Acne vulgaris” and “Tetracycline”. Only Randomized controlled trials (RCT) were included to provide the highest evidence. For each study the risk of bias were assessed and a summary evaluation of the level of evidence (GRADE) were conducted.

Findings: The search provided a total of 95 studies in which they were analysed meeting the predetermined eligibility criteria. In the final qualitative analysis 3 RCT studies were included. There were no significant differences in efficacy comparing topical regimens with antimicrobials versus oral tetracycline. Oral isotretinoin proved to have a superior efficacy compared to oral tetracycline. Adverse effects were predictable for each therapy and oral isotretinoin gave the most severe events.

Interpretation: Oral tetracycline is an effective therapy in moderate to severe inflammatory acne. Even in the presence of resistant Propionibacterium acnes it is a successful treatment, most possibly relating to its anti-inflammatory fashion. Topical therapy with antimicrobials have similar efficacy as oral tetracycline. Although oral isotretinoin provides the best efficacy compared to oral tetracycline it is well known that it gives more adverse effects.

Keywords: Acne vulgaris, tetracycline, systematic review

Contents

Introduction

Background

The prevalence of acne in young adults is about 80 % and not only affecting one persons skin, additionally it may have an impact of the person's social life in a negative way. It has been shown that the assessed quality of life correlates with the extent of the acne lesions(1). While acne is most commonly occurring in adolescents it may persist to adulthood in 50 % of individuals, especially in women(2, 3). One fact that is emphasized by an assembly of researchers and physicians with special interest in acne known as the Global Alliance to Improve Outcomes in Acne group is the often chronicity of acne and stated by Thiboutot et al (2009)"not just a self-limiting disorder of teenagers"(3).

The pathogenesis of acne is multifactorial involving the pilosebacceous unit. Other factors that plays a part in the ensuing breakout is damp climate, psychological stress and chemicals in cosmetic products containing for example propylene glycol, leading to blockage of the comedones(4, 5).

The most commonly used treatment for moderate to severe inflammatory acne is oral antibiotics, which is indicated when topical regimens do not clear the acne or when there is a risk of scarring and pigmentation(6, 7). According to Tan, A. W. et al (2005) the consequence of the prolonged overuse of antibiotics, the increase of resistant strains of Propionibacterium acnes (PA) leads to treatment failure(8). Important reason for this is the prescription of antibiotic therapy for too long periods, combining local and systemic antibiotics and lack of compliance(6, 7). Deciding what treatment one should prescribe in each case depends on the severity of the acne. There is no golden standard scale for acne grading. Now there is over 20 something severity grading scales used worldwide, not contributing to the consistency, making it difficult in the research to evaluate study outcomes(9).

The known effects of tetracycline belonging to the cycline group antibiotics, which is the first line antibiotic used in Sweden for moderate to severe inflammatory acne, are both antimicrobial and anti-inflammatory.

According to Chiou et al (2012) a hypothesis was proposed suggesting that the effectiveness of oral tetracyclines could be questioned though their study indicated a

superiority effect of the placebo therapy, being as effective as treatment with oral minocycline or other commonly prescribed tetracyclines(10).

Aside from the PA increasing resistance to tetracycline, common adverse effects are gastrointestinal dyspepsia, photosensibility and yellowish discoloration and hypoplasia of the teeth enamel in children younger than 10 years of age(11, 12).

In this systematic review the evaluation whether oral tetracycline is an effective treatment in acne, considering the PA resistance versus other available regimens generally used for the same indication is examined. Are there any trends of acne treatment prescription? And if, has it changed over the last years? Which regimen used globally is stated to have the best efficacy?

Acne Vulgaris and its pathophysiology

Acne is a skin disorder, which starts in adolescence with chronic inflammation involving the sebaceous glands in the epidermis. Sites of lesions occur most commonly on the face, neck and upper chest where hair follicles are dens(6). There are several components that together lead to manifestations of acne, such as papules, pustules and occasionally deep pustules and cysts that may lead to scarring of the skin(4). There is a proven genetic component for acne. A strong family history of acne is a predisposition, although there is little known about its mechanisms contributing to the pathogenesis of acne(13). There are four main factors that will come to discussion when enlightening the fundamentals to acne lesion formation:

- Increased sebum production
- Hypercornification of the pilosebacceous duct
- Colonization of PA
- Inflammation(12)

Androgen receptors located on keratinocytes and the cells of the sebaceous gland are regulated by androgens from the gonads and adrenals, which in turn stimulates to increased sebum production and hypercornification(14). Notice there is no overproduction of androgens, instead there’s an amplified sensitivity for the androgens in the follicles of acne patients(12). There is a correlation between increased sebum production and the severity of acne. Microcomedones is the precursor of the acne lesion. Blackheads and whiteheads come from the description

of the comedones, indicating whether it is open and black allowing passage of sebum or closed and white, not permitting sebum outflow leading to inflammatory lesions. A follicle becomes plugged when there is an abnormal differentiation and proliferation of the ductal keratinocytes(4). These keratinocytes are normally shed from the follicular canal; instead they become lodged in the upper part forming the microcomedone(7). After removal of blackheads they restock after 2-6 weeks, denoting the cyclic growth of the comedone. There has been shown that there is a reduction of linoleic acid in sebum of acne patients compared to non-acne cases. Interestingly the level of linoleic acid came back to normal after therapy with oral isotretinoin (an derivative of vitamin-A) and antiandrogens. Low levels of linoleic acid are connected to hypercornification. Pathological production of the cytokine IL-1-alfa by keratinocytes contributes to comedonogenesis. Other factors that regulate the follicle is the Epidermal Growth Factor and Tumor Growth Factor -Alfa, which inhibit sebum production(4). Normally living on the skin after puberty is the anaerobic organism propionibacteria. Propionibacterium acnes are the organisms stated contributing to acne pathogenesis. The bacterium resides in the lipid rich follicle and starts to multiply when the cornified plug blocks the outflow of sebum. PA triggers an adaptive immune response through its production of lipases and hydrolases and activation of toll-like receptors (TLR) on inflammatory cells. The ensuing inflammation is due to the rest products of the enzymatic pathways in the follicle, giving rise to proinflammatory and comedogenic substances. Chemotactic factors and activation of complement makes the wall of the follicle prone to rupture. When the inflammation engages the dermis a papule or pustule is formed(3, 4, 7).

Acne and associated factors

Interestingly there has been shown some correlation between diet and acne. Inuit's diet rich in fish that was substituted for western foods comprising much saturated fat, had a rise in the incidence of acne. Indicated by Kwon, Yoon et al (2012) reduction in glycaemic load seemed to have a linear correlation to improvement of acne(15). A prospective cohort study conducted in Singapore of 94 school students with acne denoted on to what has been speculated by many researchers. Investigating sebum production and stress load, an increased stress load revealed to have a positive correlation with the severity of the acne, although it is not associated to sebum production(16).

Grading of acne vulgaris

Acne severity is graded in several acne grading classifications globally. In Sweden most commonly used grading is from mild, moderate to severe acne. The different treatments are based on this classification.

- Grade I/mild acne- Non-inflammatory comedonal acne with open and closed comedones with less than 10 inflammatory papulopustules located to the face.
- Grade II/moderate acne- Comedones accompanied by between 10-40 inflamed papulopustules located to the face.
- Grade III/severe acne- Deep inflamed papulopustules less than 5 mm in diameter and more comedones than in grade II.
- Grade IV/nodulocystic acne- Nodules greater than 5 mm in diameter with pseudo cysts, deep pustules and a large number of comedones. Scarring occur in this form(4, 17).

Propionibacterium acnes resistance

The establishment of which organisms that triggers acne has lead to targeted therapies towards PA. The bacterium is a gram-positive anaerobic organism residing in the hair follicle, harbouring high amount of sebum(18). When trapped in the clogged follicle it multiply and initiate inflammation through mechanisms that up regulates proinflammatory mediators. It is now known that the PA increased resistance since the last 30 years to commonly used antibiotics are leading to treatment failures of acne. Since it was first reported in the 1979, with time the same observations were made globally. Reviewed by Luk et al (2013) the prevalence of PA resistance in Europe is 5- 26.4 % for tetracycline and 45-91 % for erythromycin and clindamycin, not including Italy and Hungary which has a almost non-existent prevalence(19). Resistant strains can be passed to one another through person-to-person contact. The importance of the issue is in concern when selecting an appropriate treatment approach(20, 21). PA is the most resistant to erythromycin, thereafter tetracycline. In Sweden the use of minocycline is obsolete, although it has the least resistance of antibiotics used for acne therapy(18). Reasons for the apparent resistance are long duration of therapy, lack of compliance, over the counter products without clinician's clearance, route of administration and the often prolonged or chronicity of the skin disease(22). The resistance has emerged through point mutation in the 16S rRNA in PA encoding for tetracycline and 23S rRNA encoding for erythromycin. Cross-resistance between erythromycin and clindamycin is also due to point mutation in PA

genome(20). Studies have been made comparing different Minimum Inhibitory Concentrations so called MIC values to determine clinical significant sensitivity of PA to different antibiotics(23). According to Moon et al (2012) increasing MIC values has been undertaken the latest(20). As the most important antibiotic for treatment of acne in Sweden, tetracycline group is our focus in this systematic review. The PA strains investigated in a Korean prospective study involving 100 participants diagnosed with acne showed that the bacterium were susceptible to tetracycline. Even though the common use of tetracycline there is a low prevalence of resistant strains to this systemic antibiotic compared to other antibiotics(20). The question whether the PA resistance to antibiotics used for treating acne has a significant impact on the overall public health may be answered-- that it will in the long run. The reason for this is that other bacteria normally living on the skin also develop resistance for the same antibiotics used for acne. Coagulase-negative staphylococci (CNS) are one of those bacteria that might be a potential disease-causing organism in immune-compromised persons. In turn resistance developed by CNS may transfer by plasmids to Staphylococcus Aureus(19). Ways to restrict the antibiotic resistance of PA conducted by the Global Alliance to Improve Outcomes in Acne group according to Thiboutot et al (2009) by(3):

I. Always treat acne in a combination of antimicrobials and a topical retinoid.
II. Do not precede antibiotic treatment for too long periods and if there is no improvement stop the therapy.
III. Recommend simultaneous usage of products with benzoyl-peroxide (BPO)
IV. Do not prescribe antibiotics in monotherapy (topical and oral)
V. When changing antibiotic therapy it should be reasonable.
VI. Oral and topical antibiotics are not to be used together.
VII. Topical retinoid, preferably in combination with BPO could be used as maintenance therapy.
VIII. Antibiotics should not be prescribed as maintenance therapy.

Treatment of acne vulgaris

When evaluating which therapy to prescribe, the severity and the extent of the lesions should be emphasized. Other factors to be determined are the duration of the acne and

previous treatments and the effects of these. If the patients skin is prone to scarring and heals with pigmentation is also weighed(4). The objectives for the therapy are to target the four pillars (increased sebum production, inflammation, hyperkeratinisation in the hair follicle and colonization of PA) of the pathogenesis to acne and to reduce the emergence of microcomedones and visible acne lesions(3). First-line treatment for grade I mild comedonal acne is firstly monotherapy with topical retinoids and if there is additionally inflammatory lesions, a topical antimicrobial could be added such as benzoyl peroxide (BPO). For grade II moderate inflammatory acne, therapy with topical retinoids and BPO are often used in fixed combination preparations. Those who do not have any effect by topical preparations alone, oral antibiotics are the first line treatment in moderate to severe papulopustular acne(4). The advantage of oral antibiotics is the anti-inflammatory- and bacteriostatic effect on PA(6). It's favourable to use oral or topical antibiotics in combination therapy with retinoids or BPO, decreasing the risk of generating resistance(3). For females who also wish to have a contraceptive effect apart from the suppressive effect on acne, a hormone therapy would be an option. Hormone therapy is frequently prescribed together with a topical retinoid for maintenance therapy(24). For the most severe cases of acne, which is the nodulo-cystic form and acne conglobata, oral isotretinoin is the treatment that would be prescribed, only by specialist in dermatology in Sweden(12). Acne that do not improve with oral antibiotics and heals with scarring is likewise an indication for oral isotretinoin(24). Alternative treatment in this case is high-dose oral antibiotics together with topical retinoid and BPO(4).

Topical therapy

When starting with topical regimens the mainstay is to begin with non-antibiotic medication(6).

Retinoids

Retinoids are derivatives of vitamin A and is the group of topical therapy considered to be a cornerstone in acne treatment. Retinoids are functioning in a comedolytic fashion through regulation and counteraction of the keratinocyte proliferation and comedone formation(12, 24). Advantages of retinoids are the anti-inflammatory effect. Another effect is the increase in skin permeability, enhancing the effect of other topical medications such as antibiotics. Tretinoin and Adapalene are retinoids that are most commonly used for acne stated by an up to date study by Tirado-

Sanchez et al (2013)(25). In the same study the efficacy rate for 90 days of treatment with tretinoin 0.05 % gel was 80 % and 70 % for Adapalene 0.3 % concentration. While the efficacy of Adapalene in the preparation 0.1 % concentration was the least it also had the least adverse reactions. Adverse reactions to topical retinoids are dryness of the skin, scaling, skin irritation, burning and postinflammatory hyperpigmentation. Tretinoin appeared to cause most adverse reactions(25). Tretinoin should not be used in combination with oral isotretinoin or BPO(12). The only form of a topical retinoid combined with BPO is the retinoid derivative adapalene(3).

Antimicrobials

Most commonly used is the bactericidal preparation benzoyl peroxide. The scaling of the superficial epidermis gives the keratolytic effect of this medication(26). Adverse reactions to BPO are skin irritation and it may bleach textiles and hair(12). Although topical antibiotics are not preferable there is a combination preparation named Duac, with BPO and clindamycin. This combination is anti-inflammatory and targets PA. With the so to say protection by BPO, antibiotic resistance is less likely to occur using this preparation(3). For maintenance therapy the medication Epiduo containing a fixed combination of BPO 2.5 % and adapalene 0.1 % is a good alternative to topical antibiotics. This therapy could be used for years having an effect on antibiotic sensitive and resistant strains of PA, the inflammation and hyperkeratinisation(6, 27, 28). The importance of maintenance therapy is that microcomedones decrease while the active preparation is applied to the skin, discontinuation will often lead to recurrence of microcoemdones and acne(3).

Antibiotics used in a topical preparation are clindamycin, erythromycin and tetracycline. Clindamycin is the only topical antibiotic in use in Sweden and treatment recommendations states it could be used for not longer than 3 months(6, 11). Indicated earlier, topical antibiotics should not be used in monotherapy. Clinicians should prescribe topical antibiotics in a regimen together with BPO(11). Azelaic acid is also said to belong to the antimicrobial group since it inhibits the proliferation of PA and is comedolytic(29). Side effects of topical antibiotics are similar to retinoids producing skin irritation, dryness, scaling and burning. There is no evidence of emergence of resistance for therapy with BPO or azelaic acid according to Haider et al (2004)(11).

Oral therapy

Oral Tetracycline

If topical therapy have no effect in moderate to severe acne after 2 to 3 months of treatment, tetracycline group antibiotics is the first line treatment. These antibiotics are bacteriostatic and anti-inflammatory affecting chemotaxis(4, 6, 30). The severity of acne and the association between the numbers of PA is sparse. The reduction in number of PA by oral antibiotics do not correlate with the clinical efficacy stated by Burns et al. (2010)(4). In Sweden antibiotics from the cycline group indicated for prescription for acne therapy is only tetracycline; others in use globally are lymecycline, oxytetracycline and doxycycline. In other countries minocycline and erythromycin is also used(11, 31). Therapy duration for tetracycline is for 3 months at a dosage of 500 mg x 2 daily or tetralysal 300 mg x 2 with concomitant topical tretinoin or adapalene(12, 31). When cessation of antibiotic therapy, as maintenance therapy a topical retinoid may be considered(6). The spectra of effects and side effects of different tetracyclines are wide. Due to high efficacy of doxycycline and minocycline they are the antibiotics most predominantly prescribed in other countries than Sweden. When the patient cannot tolerate first line antibiotics and when in the second and third trimester of pregnancy or when breastfeeding, second line treatment is the macrolide erythromycin(12, 32). However erythromycin should not be used in the first trimester of pregnancy due to heart complications and it is also rather not used because of its high resistance of PA, but it is a choice if there has been treatment failure(30, 33). According to Leyden et al (2011) concerning the pharmacokinetics of tetracycline, the poor permeability results in 77-88 % absorption when taken orally(30). Tetracycline is not to be taken together with food, especially containing milk and other foods with iron, calcium and magnesium, though it leads to a 50 % reduction of the absorption. Even though the high solubility of tetracycline it is not really lipophilic and it cannot easily penetrate the lipid rich hair follicle. Minocycline and doxycycline have a good absorption and therefor is to a lesser extent affected by food. Because of its very high permeability and excellent uptake in lipid-rich sites, minocycline is very effective but may also give more adverse reactions. It crosses the blood brain barrier and may cause acute vestibular adverse events, intracranial hypertension and Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS syndrome)(30, 34). However, stated by Burns et al (2010) the supposed higher efficacy of minocycline and doxycycline due to higher lipophilicity are not wholly

true as there is no good evidence for this(4). Side effects caused by tetracycline are gastrointestinal irritation with diarrhea, vomiting and dyspepsia. Vaginal candidiasis may occur in women and the influence when on the pill is not certain, giving recommendations to be extra cautious with contraceptives during therapy(4, 12). One particularly evident side effect in children is the potential yellowish discoloration and enamel hypoplasia of the developing teeth's(4, 7). For this reason tetracycline should not be prescribed to children and pregnant women. Rare adverse effects are benign intracranial hypertension and photosensitivity(7). Considering a good treatment outcome, the number of PA is reduced(4).

Oral Isotretinoin

Since it was introduced on the market in the early 1980's, oral isotretinoin is the number one treatment with the potential to completely cure acne. Isotretinoin is a synthetic derivative of vitamin A, targeting all four causative factors in the pathogenesis of acne(4, 12, 27). It is not perfectly defined how isotretinoin exerts its effects, however the effect on sebaceous glands is remarkable having the potential to reduce sebum excretion by 90 % in 6 weeks in administered dosage of 0.5-1.0 mg/kg/day(3, 4). With the decrease in sebum production the decline in colonizing PA is evident thereby indirectly having an antimicrobial and anti-inflammatory action. The prescription of the medication is reserved for specialist in dermatology for severe acne that is reluctant to antibiotics(4, 12). Due to adverse effect such as teratogenicity, female patients must persist on oral contraceptives before, during and 2 months after the therapy(24, 31). Mandatory pregnancy testing before and every 4-week under therapy is the routine. Even patients with moderate acne that have no effect of oral antibiotics could be considered for treatment with oral isotretinoin(4). Indications for oral isotretinoin are patients with moderate to severe acne with no improvement with oral antibiotics and topical therapy after 3 months, acne with post inflammatory hyperpigmentation or scarring, relapsing acne and those with systemic reaction(31). Recommended dosage to start with is 0.5 mg/kg/day, regulating the dosage following the result and tolerability of the patient(27). The absorption is doubled when taken together with food and is therefor recommended. Therapy is usually until a total dosage of 120 mg/kg of body weight is achieved(4, 12, 31). In the previously stated dosage about 85 % of patients gained remission after 16 weeks of therapy(4). Commonly experienced adverse effects (AE) are chelitis, conjunctivitis, dermatitis, facial erythema, dryness of lips and skin and mucositis(24). Not as common but

important to mention is AE giving headaches, benign intracranial hypertension, mood changes and depression(11). In a retrospective cohort study conducted in Sweden by Sundström et al (2010) they found data suggesting an increased risk of suicide attempts 6 months after therapy with oral isotretinoin. Notably the evident risk was already present before starting on therapy(35). If adverse reaction appears such as depression, cessation of the therapy is crucial. Typically liver enzymes and triglycerides may rise during isotretinoin therapy. Therefor these values should be monitored during therapy(4). Important actualities are that tetracyclines should not be prescribed to patients on oral isotretinoin, though both have the potential of causing benign intracranial hypertension(24, 31).

Objectives

In this systematic review we would like to assess the effect and side effects in patients diagnosed with mild, moderate to severe acne in ages 11 to 42 who received different treatment in acne. Treatment with tetracycline alone or in combination therapy compared to control groups allocated to other regimen with antibiotics, topical regimen, oral isotretionin or placebo. Primary objectives are to evaluate the efficacy of treatment with tetracycline comparative to other therapies. Furthermore to evaluate side effects in commonly used therapies in relationship to oral tetracycline. Secondary objectives are to determine outcomes in therapy related to PA resistance. Moreover reviewing trends in prescription of different treatment for acne relating to PA emerging resistance is of interest, reserved for the discussion part. Preferably randomized control studies (RCT) are selected because they provide the highest evidence.

Methods

A handmade protocol for the search was conducted together with the project supervisor and project colleague. Inclusion criteria and outcomes to be measured were specified prior to the search. Information was collected from textbooks in dermatology and a systematic database search. Certain information was hand searched on the web, providing the quantitative information needed to the review as a whole. Inclusion criteria are studies that were assessing acne vulgaris, tetracycline and treatment in acne. Those criteria were measured in a wide array of interventions

in studies we sought. Studies selected were those with our predetermined Participants, Intervention, Comparator group, Outcome and Study design (PICOS) with the outcomes stated in the objectives. Keywords such as treatment, therapy, regimen, effect, side effect, efficacy and adverse effect in context with acne vulgaris and tetracycline were to be evaluated, meeting inclusion criteria in the title or in the searched abstracts. Outcomes to be examined such as effect and efficacy of tetracycline in treatment in acne vulgaris was decided in advance. Other outcomes were side effects of tetracycline in treatment of acne patients. Along the process we discovered that numerous studies were assessing PA resistance related to different acne regimen. This was also selected as a secondary objective bringing an important research question into light of this review.

Eligibility criteria

Study characteristics composed participants that had been diagnosed with acne vulgaris. Graded form mild-moderate to severe acne, including as many studies as possible. Although acne is most prevalent in adolescence, included participants had to be in the age range between 11 to 42 years to widen the selection. The intervention was treatment with oral tetracycline with no specified dosage or timespan and assessment of the efficacy and side effects of the therapy in comparison to other regimens. Because both first and second-generation tetracyclines are used globally for acne, studies assessing comparative intervention with those antibiotics in relation to tetracycline are included. Microbiological examination of resistant PA in clinical trials is also of interest, possibly evaluating indirectly the effectiveness of tetracycline and other commonly used acne therapies.

- **Primary outcome:** to evaluate the effect or the efficacy and side effects/adverse effects of therapy with oral tetracycline in acne vulgaris.
- **Secondary outcome:** PA resistance to tetracycline and what impact it may have on acne therapy outcome.

Studies selected were limited to those in the English language with a 10-year span ranging from 2002-2012. Study designs preferred are those with a clinical focus, randomized with a control group receiving intervention or placebo. Cohort studies with a follow up for at least one year are to be included. No imposition was made about payment. Reports only with full-text and all data published were selected. Excluded studies were in-vitro studies, single case reports, review articles and studies

not concurrent with any of the measures in the specified PICO. Additionally observational studies and studies with less than 30 participants was excluded. Criteria for inclusion and exclusion are displayed in table 1.

Table 1 – Inclusion and exclusionlist table

Inclusion and Eligibility Criteria	Exclusion Criteria
English language	In-vitro studies
Published studies, year span 2002-2012	Case- reports
Full-texts	Review articles
Studies with control group, not only placebo	Studies not concurrent with PICO
Ages 11-42 years	Expert opinion based on theory studies
Mild to severe acne vulgaris	Observational studies
Tetracycline compared to other acne therapies	Studies with less than 30 participants

Inclusion and eligibility criteria for the study featured in the left column and exclusion criteria in the right column.

Information sources

Databases electronically searched were PubMed, Scopus and Cochrane library. The primary search was made in September 2012, although it had to be modified because the inclusion criteria in the primary protocol were too wide generating over 147 studies with about 65 studies assessed for eligibility. Modifications made were limiting the studies selected to a 10-year span instead of a 20-year span that were selected at first. This limiting factor filtered out studies that did not meet the inclusion criteria. Moreover suggestions in the search menu bar for example selecting search area; “medicine” and “pharmacology, toxicology and pharmaceutics” were chosen. The latest updated search was run the 16 of may 2013 searching all three databases and ended the 22 of may 2013. In spite of 2012 have already past, the decision to still follow a 10-year span starting and ending according to the first set inclusion criteria was agreed upon. Additional studies were hand searched from the included studies reference list. However, this hand search did not contribute to any qualitative additional studies that could be included. Reference lists were checked for all studies selected to full-text assessment for eligibility, providing up to date information about different aspects in acne and acne treatment brought up in the final review.

Search

Search terms conducted for PubMed, Scopus and Cochrane library was “Acne vulgaris” and “Tetracycline”. For all three databases, limitations to the “English

language", year range, from 2002 to 2012 were selected. For PubMed the search term was sought in "title and abstract" and for Scopus database in "abstract" thought there was no comparable choice to be selected. In Cochrane library search terms were sought in "trial, Cochrane review". Additional selections in Scopus had to be made because of the large sample size, limiting to "medicine" and "pharmacology, toxicology and pharmaceutics" matching our focus. Because of many hits of different kinds of document types, limiting to "review" and "article" was necessary in Scopus.

Study selection

Selection of studies was made according to the predetermined protocol with inclusion and exclusion criteria. One person conducted the search process. Inclusion criteria were modified along the way when doubts occurred and when studies appeared that was not discussed for eligibility in advance. Such complications were solved together with the project colleague and the supervisor. For the assortment of studies at the end of the search the selection was carried out together with the supervisor. A flow diagram was used to overview the search designed by "Preferred Reporting Items for Systematic Reviews and Meta-Analyses" (PRISMA)(35).

Data extraction

Methods for data extraction were made by presenting studies in a manner similar to a template designed by the Cochrane Collaboration Qualitative Methods Group, 2011(25). One person extracted all data in a systematic manner to reduce risk of bias. For each study PICOS were brought up and reported in the results.

Data items

Outcome measures were presented in tables in the appendix for each outcome identified in the studies. The template was conducted by Health Technology Assessment-Centrum(31). In the table headlines is author/year, country, study design, number of patients, withdrawals/drop-outs, result- intervention/control, comments and risk of bias. Information about the specific study designs was furthermore presented in text form in relation to PICOS reviewed by one person. The report structure is based on the PRISMA-statement design(35).

Risk of bias in individual studies

When assessing risk of bias for each study a modified checklist by Swedish Council on Health Technology Assessment(28) was used screened by one person. Bias to be examined is external validity, internal validity, study limitations and precision.

Additionally an overview of the risk of bias in the individual studies were tabulated according to Cochrane Collaboration(25).

Summary measures

In summary measures, efficacy of treatment with tetracycline was presented in what form studies presented their data including for example relative risk with confidence intervals or odds ratios. If it proved that studies included in the qualitative analysis had a wide variety of methods for conducting studies and did not have a coherent effect measure, the results were not added to a meta-analysis with the enlarged risk of producing bias. Meta-analysis was conducted if it was appropriate.

Risk of bias across studies

For evaluating the risk of bias across studies and quality such as allocation concealment, blinding, selective reporting the GRADE work sheet was assessed for this(26).

Ethics

Establishment of an ethical dilemma did not prove to be necessary for this systematic review though ethical approval and appraisal have already been weighed in the studies within this exploration. Although the studies included did not present any ethical issue it is essential for further researches to take notice that these kinds of studies may be redone in the same fashion with no reservations.

Results

Study selection

The complete number of records identified through the primary database search in PubMed, Scopus and Cochrane Library generated 95 studies. To be more precisely 40 studies were obtained from PubMed, 44 from Scopus and 11 from Cochrane library. The search conducted in each database was screened and compared to each other and duplicates were removed. A number of 36 studies were excluded because of duplicates. A total of 59 studies were left for the assessment of title and abstract for inclusion in coherence with the protocol. Several articles were eliminated at this point, not meeting the criteria for inclusion. An exclusion list/table was piloted with comments provided alongside reason for exclusion, see appendix. After 48 articles

were excluded there were 11 studies left in full-text for assessment of eligibility. Of these studies, 9 were extracted from PubMed and 2 from Scopus. However, after discussion with my supervisor Jan Faergemann about the eligibility criteria with focus on the primary outcome, it was evident that an extra exclusion criteria hade to be set up. Not all of these studies were of interest when assed in full-text because 4 of them were in-vitro studies and 1 were measuring the wrong outcome. Then another 5 articles were excluded. Temporary 6 studies were selected for the final study, out of these, 3 studies had to be excluded because of low evidence entailing to observational studies. Out of the 3 remaining RCT studies that maid it to the final qualitative study, all was extracted from PubMed database. The complete search is displayed in a flow diagram in the appendix.

Study characteristics

For the remaining 3 studies all of them were randomized controlled trials. They were all accessed in full-text and in the English language meeting eligibility criteria. The studies were carried out in Sweden, Iran and the United States. The duration of intervention was 6 months with a 2-month follow-up in “Clinical and Microbiological Comparisons of isotretinoin vs. tetracycline in Acne Vulgaris” by Oprica et al (2007)(36), 3 months in “azitromycin versus tetracycline in the treatment of acne vulgaris” by Rafiei et al (2006)(29) and 18 weeks in “Comparison of five antimicrobial regimens for treatment of mild to moderate inflammatory facial acne vulgaris in the community: randomised controlled trial” by Ozolins et al (2004)(34). A total of 991 participants were enrolled to the included studies. Similarities in the inclusion and exclusion criteria were found for the studies. Participants were in the age range 11- 42 and were diagnosed with mild- moderate or severe acne vulgaris. Exclusion criteria for enrolment were in brief summary, participants that recently had been on systemic treatment for acne, with systemic disease or other dermatological disease, with a hormonal disorder or with a drug induced acne. Pregnant women, participants planning to get pregnant or women who were breastfeeding were also excluded. Those who were taking medication that could interfere with the outcome and if any experienced hypersensitivity reaction or allergic reaction to the drugs tested were excluded. Those participating in other clinical trials were excluded in two studies. The intervention in all three studies was oral tetracycline, alone or together with a topical regimen compared to other common acne therapies. In two studies skin samples were taken for bacterial culture, most often form the face. They were

incubated on blood agar plates to determine the density and frequency of PA resistance. Studies were performed to determine the MIC of tetracycline and other antibiotics or regimen for acne treatment to PA.

In one study comparison was made between tetracycline and oral isotretinoin. In another study oral tetracycline was compared to azithromycin, a macrolide antibiotic similar to erythromycin. One study compared five regimens in acne that all displayed antimicrobial properties. Two studies combined topical retinoids in their intervention in some way. Outcome measures that are shared in the studies were the efficacy of the treatment, adverse effects connected to different regimens and evaluation of PA resistance to tetracycline compared to other acne treatment. One study assessed the quality of life in relation to the influence of the skin disease. Tables for the different shared outcomes are provided in the appendix.

For simplification, the studies will hereafter be named Study I to III. Study I is Oprica et al (2007), study II is Rafiei et al (2006) and study III is Ozolins et al (2004).

STUDY I

The aim of the study conducted by Oprica et al (2007)(36) was to determine clinical efficacy and microbiological efficacy on PA in treatment with oral tetracycline plus topical adapalene compared to oral isotretinoin.

Participants were enrolled from patients at Karolinska University Hospital Huddinge, Stockholm at the Dermatology department. Diagnostic criteria were moderate to severe inflammatory acne vulgaris graded in line with “Leeds technique”. In this meaning, moderate acne was a manifestation with papulo-pustular and nodular acne lesions. Severe acne diagnosis was given to those with the nodular form adjoining the conglobated form. The age group assessed were 15-35 years and 52 participants were enrolled with 26 participants in each group. Reasons for exclusion were participants who had used acne therapy in the previous 8 weeks or who had taken oral isotretinoin within 12 months. Pregnant women, those with intention of getting pregnant and breastfeeding women were excluded. Participants that were under medications that could interfere with tetracycline for example; antacids, iron supplements, anticoagulants and retinoids, were excluded. Those with other skin diseases; patients who were participating in other clinical trials or who had experienced hypersensitive reaction to the drugs to be allocated in the study were also excluded. Patients were

randomly allocated to either oral tetracycline 500mg twice a day + topical adapalene 0.1 % (TET/ADA group) once a day or oral isotretinoin(ISO group) in two divided doses of 1mg/kg/day. Samples for skin bacterial cultures were taken to assess clinical outcome relative the density and antimicrobial susceptibility of PA. The samples were taken at baseline and at 2, 4 and 6 months of treatment and at the 2 months of follow up. One person, not participating in the study with a code generated by a computer, knew the randomization process. Both groups were treated for 6 months with a follow-up at 2, 4 and 6 months and thereafter 2 months after termination of therapy. Only the TET/ADA group received maintenance therapy with topical adapalene after 6 months of oral therapy. The ISO group were informed not to take other medications containing vitamin A, tetracycline and aspirin due to potential adverse effects. The women in the ISO group received oral contraceptives before, during and after the treatment. The TET/ADA group was informed about possible adverse effects on foetuses, not to become pregnant during therapy. Antiseptic cosmetics were not allowed due to the risk of interfering with the bacterial cultures performed. Information about limitation of sun exposure was given. Methods to examine the clinical efficacy were determined in advance. Two dermatologists assessed this independently. For baseline and all follow up visits, lesions were counted and graded according to Leeds technique. Assessment of the face, chest and the back was done based on the type of lesion: non-inflammatory, superficial and deep inflammatory. Another assessment of the effect of treatment was the patient's experience of the treatment and the impact of skin disease in relationship to quality of life. Participants filled out a self-administered questionnaire before and after treatment. Microbial samples were taken from the forehead, left and right cheek, back and chest. The samples were incubated on blood agar with antibiotics at breakpoint values. MIC values were measured for each antibiotic tested and resistance to any antibiotics was stated if the bacteria were growing in spite of breakpoint values or over the breakpoint value. Breakpoint values were concurrent with the European Committee on Antimicrobial Susceptibility Testing recommendations. Tested antibiotics were tetracycline, clindamycin, erythromycin and linezolid. At the follow up visits participants were asked about experience of any side effects. The ISO group was monitored with blood count, liver enzymes, cholesterol and lipids. A complete blood count was taken of the TET group. Statistical analyses used were specified in advance in an intention-to-treat population.

STUDY II

The aim of the study conducted by Rafiei et al (2006)(29) was to compare the efficacy and safety of treatment with azithromycin versus oral tetracycline in acne vulgaris.

Participants were enrolled from the outpatient clinic of Emam Khomeini University Hospital, Ahwaz, Iran. A number of 290 patients met their inclusion criteria. Those with moderate to severe papulopustular acne vulgaris were selected. Additional inclusion criteria were acne that appeared for the first time or relapsed. Excluded were participants with systemic disease, hormonal disorders and those with drug-induced acne. Pregnant and breastfeeding mothers were not included. Patients that hade received systemic acne therapy during the past 3 months were excluded. If previous drug reactions had appeared for the medications to be allocated they were excluded. Two groups of participants were randomly allocated to either a therapy with oral azithromycin or oral tetracycline for 3 months, with a number of 148 participants in the group receiving azithromycin and 142 in the group allocated to tetracycline. The dosage for azithromycin was in pulse dosage, starting the therapy with 500mg/day for 3 consecutive days in a week for a month. Subsequently a decreased azithromycin dose of 250mg/day every other day was given for the last 2 months. The other group received 1g/day of oral tetracycline for one month, thereafter 500mg/day for 2months. For the last 2 months of therapy, topical tretinoin 0.05 % was added to the regimen in both groups. Classification of acne severity was based on number of lesions and the location. The study was randomized in that sense that every other patient was allocated to one specific treatment by the clinician. A blinded investigator measuring the outcome held the follow-up visits. The follow-up was conducted after 1 and 2 months. In the last visit patients were asked about adverse effects and compliance. Further exclusion of participants was due to lost of follow up, a total of 54 patients left the study for this reason. Statistical analysis was chosen in advance and the study was done in a per-protocol analysis.

STUDY III

The aim of this study conducted by Ozolins et al (2004)(34) was to examine the efficacy, cost-effectiveness and treatment outcome in relation to PA resistance for five antimicrobial regimens in mild to moderate acne vulgaris.

This was a randomized study with 649 participants with the intention-to-treat. A number of participants were enrolled from the National Health Service network and some additional from colleges. Criteria for inclusion was mild to moderate acne vulgaris. This estimate was based on identification of 15 non- inflammatory and inflammatory lesions located in the face. Exclusion criteria were: acne secondary to other disease or medication, nodular, truncal and comedonal acne was also rejected. Additionally pregnant women or women with intent to get pregnant and breastfeeding mothers were excluded. Acne with late onset (>26 years) was also excluded. Those with previous therapy with oral isotretinoin or other on-going acne therapy treated by a dermatologist had to be excluded, not to interfere with the result. Participants participating in other clinical trial and any with known hypersensitivity to the allocated regimens were excluded. Participants were not allowed to use any acne regimens for 4 weeks before the start of the study. The 5 different interventions that were allocated is visualised in the table 2.

Table 2- Regimen in study III Ozolins et al

	Regimen 1	**Regimen 2**	**Regimen 3**	**Regimen 4**	**Regimen 5**
Oral	500mg oxytetracycline 1x2	100mg minocycline 1x1	Placebo 1x1	Placebo 1x1	Placebo 1x1
Topical	Placebo cream twice a day	Placebo cream twice a day	5% benzoyl peroxide twice a day	5 % benzoyl peroxide + 3% erythromycin twice daily	2% erythromycin in the morning, 5% benzoyl peroxide in the evening

Different regimens that were allocated in study III. Regimen 1-5, oral and topical treatments. Oral placebo in regimen 3-5 and topical placebo in regimen 1-2.

A computer generated code allocated participants to their specific regimen. The coordinator of the trial and the pharmacy staff delivering the regimens at baseline, after 6 weeks and at the 12 week of follow up knew this code. The regimens were delivered in opaque boxes. Reason for placebo treatments was to standardize the trial and minimize bias. Participants were instructed not to discuss their treatment specifics with their assessors who were blinded. Outcomes assessed were self reported moderate improvement on a 6- point likert scale at the follow up visits and number of lesions. Skin swabs were taken at the follow-ups and evaluated for PA resistance to different antibiotics. Adverse effects were monitored at each follow-up visit.

Risk of bias in individual studies and across studies

The risk of bias in individual studies regarding the directness concerning the study population and its external validity was overall good. Only one study, Oprica et al (2007) did not display data with the study population and participants excluded before randomization. The internal validation measuring the process of randomization was done in a fashion minimizing the risk of manipulation using a computer generated code for allocation in Oprica et al (2007) and Ozolins et al (2004). All participants that were selected for the randomization received the intervention. Though all three studies were conducted in a parallel-group design, meaning that all groups received an intervention they were all similar and comparable. To reduce the risk of confounders such as age, gender and different acne severity, those measures were taken in to consideration in the final analysis by stratification. The most concerning factor was the lack of masking participants and clinician in all studies. However, investigators measuring the outcome were blinded in Ozolins et al (2004) and Rafiei et al (2006). The dropout was acceptable through out studies with a dropout of 13 participants in study I, 54 in study II and a drop out rate of 23% in study III. It was measured upon in advance in Ozolins et al (2004) to detect a minimum relative effect of 30 % of one regimen compared to the other 4 regimens with an 80 % power. Adherence was taking in to consideration in all studies, often the participants were asked about it or the clinicians collected unused packages containing the intervention drug. Information on how to provide effect measures in the final synthesis was determined in advance for all studies. The reporting was less satisfactory in Rafiei et al (2006) compared to the other two studies. Likewise the precision relating to the presentation of the results, methods of measuring effect and power-analysis was not as detailed as desired in this study compared to the other 2. Ozolins et al (2004) was the only study bringing up declaration of interest and conflict of interest statement. Study characteristics are presented in table 3. When assessing the overall evidence, a worksheet evaluating quality of evidence was used designed by the GRADE workgroup(26). For all outcome measures the quality was assessed in summary. Outcome for evaluating the side effects of tetracycline compared to other regimen, the quality of evidence was moderate. Outcomes for efficacy of oral tetracycline and the evaluation of PA resistance to tetracyclines, the average quality of evidence was low. See GRADE table in the appendix.

Table 3- Study characteristics table

Trials	Concealment of Randomisation	RCT stopped Early	Patients Blinded	Health Care Providers Blinded	Data Collectors Blinded	Outcome Assessors Blinded
Oprica et al., (2007)	Yes	No	No	No	No	No
Rafiei et al., (2006)	No	No	No	No	Yes	Yes
Ozolins et al., (2004)	Yes	No	No	No	Yes	Yes

Study characteristics are presented for all three studies. Oprica et al and Ozolins et al both provided a satisfactory concealment of randomisation. In none of the studies patients and health care providers were blinded. Only Rafiei et al and Ozolins et al had data collectors and outcome assessors that were blinded about the treatment.

Syntheses of Results

Tables for the three outcome measures are provided in the appendix.

Outcome 1- Efficacy evaluation

In Study I there was a reduction of superficial inflammatory, deep inflammatory and non-inflammatory lesions ($p<0.001$) for both groups after 6 months. A significant difference in reduction in non–inflammatory lesions was seen between the tetracycline plus adapalene group compared to the oral isotretinoin group after 2 months. Likewise in superficial inflammatory lesions a significant reduction was seen after 4 months. Oral isotretinoin had the greatest reduction of lesions, even in the follow up period. The TET/ADA group had an increase of all lesions in the follow up period. The reduction was quite similar in both groups for deep inflammatory lesion. At the end of the 6 months of therapy, 16.6 % in the oral isotretinoin group had no inflammatory lesions compared to 4 % in the TET/ADA group ($p>0.05$). At the end of the follow up period no one in the TET/ADA -group had any inflammatory lesions compared to the oral isotretinoin group where 20.8 % had no inflammatory lesions. When the treatment was discontinued, only the ISO group showed a persistent decrease in acne severity ($p=0.052$). The overall difference during the 6 months of therapy was not significant between the groups, however after the follow up, isotretinoin had an advantage ($p=0.009$).

In Study II, the group receiving tetracycline, 48.3 % had a moderate improvement compared to 40.7 % in the azithromycin group. While measuring good to excellent improvement, azithromycin had a 44 % improvement and was superior to tetracycline by 31.4 % after 3 months of therapy for both measures. Partial or complete resolution of acne was seen in 84.7 % in the azithromycin group compared to 79.7 % in the

tetracycline group. There was no significant difference when measuring clinical response rate between both groups ($p>0.05$).

The efficacy in Study III was compared between regimen 1 and 4 in this systematic review because this comparison was the most interesting relating an oral therapy to a fixed topical drug combination. The study showed similar efficacy between all regimens. The improvement of the acne severity according to participants at 18 weeks in odds ratios (95 % CI) for regimen 4 versus regimen 1 was 1.64 (0.98-2.74). After 12 weeks of therapy regimen 4 had a 61 % improvement according to assessors compared to regimen 1 which had a 47 % improvement. Regimen 4 was significantly better than regimen 1 when assessing reduction in acne severity after 18 weeks with a difference of 0.18 (0.06-0.29) when adjusted for confounders.

Outcome 2- Side effects evaluation

In Study I, side effects were monitored through out the treatment. In the tetracycline plus topical adapalene group, 10 % experienced side effects such as abdominal pain and transitory nausea. 15 % reported dry skin, redness and itching of the skin once during the treatment. The other group allocated to oral isotretionin had a higher percentage of adverse effects after 2 months of therapy, relating to dryness of the skin 91.4 %, inflammation of the lips 95.8 %, dry eyes 75 % and nose bleed 54 %. Some of the side effects improved after 6 months and after stopping treatment 83 % hade no adverse effects. One patient experienced an acne flare and had to discontinue the treatment. Dryness of the skin made 2 participants discontinue the treatment. Three participants complained of tiredness and fatigue. One patient had a transitory increase in liver enzymes. Although within normal range, many patients in this group had an increase in triglycerides.

Similar to Study I, 11 % in the tetracycline group reported gastrointestinal side effects in Study II. 6.8 % experienced epigastric pain, 4.2 % complained of diarrhea and 2.5 % experienced vulvovaginal pruritus. In comparison to the tetracycline group, 10.9 % experienced gastrointestinal side effects in the azithromycin group. More specifically 5% complained of heartburn and epigastric pain and 5.9% complained of diarrhea. When comparing for example regimen 1: oxytetracycline plus topical placebo to regimen 4: Oral placebo plus topical erythromycin in combination with benzoyl peroxide in Study III, regimen 1 gave more side effects compared to regimen 4. In regimen 1, 22 participants complained of gastrointestinal upset, 11 of central nervous

system (CNS) symptoms relating to headache and 5 experienced skin irritations out of 131 participants after 6 weeks. In relation to this numbers, regimen 4 consisting of 127 participants, 8 participants experienced gastrointestinal upset, 4 had CNS symptoms and 11 had skin irritation. Both groups had similar mean patient-assessed –irritation score.

Outcome 3- P.acnes resistance evaluation

In Study I both groups gave an overall reduction of colonization with PA. However in none of the groups there was a significant reduction in resistant PA. At baseline the TET/ADA group had more resistant strains of PA than the ISO group, but after adjusting for confounders there was no difference. After the 2 months of follow up there was a higher probability for the TET/ADA group to have a higher quantity of resistant PA to clindamycin and tetracycline compared to the ISO group with odd ratios of 0.06, 95 % CI (0.013-0.37), $p<0.01$ for TET/ADA and 0.05, 95 % CI (0.006-0.49), $p<0.001$for the ISO group. After 6 months of therapy, the TET/ADA group gained resistant strains of PA whereas in the ISO group it remained constant or was lost. There was no statistically significant association between the occurrence of tetracycline resistant strains of PA and clinical response in the TET/ADA group or the ISO group.

In study III the impact of PA resistance was measured as the effect of colonization with tetracycline-resistant PA on treatment outcome. Of patients that were colonized with resistant strains, 47 % reported moderately improvement at 18 weeks compared to 56 % in patientes that had no resistant strains to tetracycline. In the same measurement for regimen 4 there was a reported improvement of 65 % for participants with tetracycline-resistant strains compared to 67 % in those that had no resistant strains. The reduction of mean skin lesion count after 18 weeks was significantly less effective for regimen 1 versus regimen 4 in those with resistant strains 23.1 (11.8-34.5). 48 % of participants in regimen 4 were colonized with erythromycin-resistant PA at baseline. After 18 weeks of treatment there was a reduction of 9 % in the participants. The number of erythromycin-resistant PA had no effect on participants rating themselves as moderately improved compared to regimen 1 with tetracycline resistant strains of PA. Topical erythromycin gave the largest reduction (16 %) in population of all PA strains after 18 weeks.

Discussion

Summary of evidence

In spite of the relatively diverse quality of the three RCT studies assessed in this review, oral tetracycline showed to have no superior efficacy in relation to oral isotretinoin, azithromycin and the combination therapy benzoyl peroxide plus topical erythromycin. Our conclusion is that treatment with topical antimicrobials have similar or equal efficacy as oral tetracyclines. It seems that the key component to establish a good response on acne lesions is by the anti-inflammatory effect. Topical antimicrobials such as erythromycin act in an anti-inflammatory fashion, as does BPO indirectly by reducing the number of PA's. The main action of oral antibiotics is by the anti-inflammatory effect and it could explain why PA's resistance to tetracyclines did have no influence on treatment outcome in Oprica et al (2007).

There was no robust evidence to certainly appraise the efficacy of treatment with oral tetracycline throughout studies. Factors that were not always comparable were different inclusion criteria for enrolment, grading of acne severity and the study designs conducted. In Study I and II, tetracycline was combined with topical therapy which makes it difficult to distinguish what effects was produced by the respective drugs alone. It is most likely that synergistic effects affected the outcome. However tetracycline is a tolerable and effective alternative treatment to oral isotretinoin in acne vulgaris, according to Oprica et al (2007)(36). Also suggesting that topical therapy antimicrobials could be a good alternative treatment according to the results of this review.

Evidence grade was generally moderate for evaluation of side effects, while they were assessed in the same manner and was similar in all 3 studies. The most common side effects complained about in those who received oral tetracycline were gastrointestinal upset with epigastric pain and diarrhea, which is according to the other literature(24, 37). Adverse effects such as photosensibility were not assessed in any of the studies, maybe because of the lower prevalence or because information about limiting sun exposure was informed in advance. Women in the tetracycline group complained about vulvovaginal pruritus, a side effect not apparent in the other control groups receiving other regimens. Side effects with dry skin, redness and irritation of the skin was produced by the topical therapy when this side effects was experienced in the tetracycline plus topical therapy group. Dryness of the skin is an

attribute of oral isotretinoin therapy, therefor making it problematic to blind the clinicians assessing the outcome for this group during the study, though this adverse effect is evident. Side effects generated by tetracyclines were interpreted to be more tolerable in comparison to oral isotretionin. Adverse effects produced by topical therapy were not assed in comparison to oral tetracyclines. This comparison is difficult to make because of different routes of administration and ways of exerting its effects.

When assessing dropout rates due to adverse effects, it was higher in the group receiving only topical therapy compared to the groups receiving systemic therapy in Study III. This association was also made in Nast A Fau et al (2012) where no conclusion could be made with sufficient evidence to determine whether topical or systemic therapy is superior to one another in acne(38).

To generate a more definite comparison and evaluation of the efficacy of oral tetracycline, a study conducted comparing oral tetracycline alone versus another therapy in monotherapy is of interest. This could for example comprise topical adapalene, oral isotretionin or topical erythromycin. However with the increase of resistant PA, monotherapy with antibiotics is not preferred(38). Acceptable evidence particularly for Study I, which was conducted in Sweden, stated that there was no impact on treatment outcome in relation to the number of resistant PA colonizing the skin. However in Study III, the presence of tetracycline resistant PA's had a significantly impact on treatment outcome when treated with oxytetracycline and minocycline. The trend is towards favouring the conclusion made in study III. Several studies have likewise suggested that PA resistance have a negative influence on treatment outcome(23, 39, 40). Important to take notice is that the difference between these two studies previously mentioned was that the latter did not use topical retinoids during and as maintenance therapy to the oral therapy. It is now well known that combination therapy with a topical retinoid is much more effective than oral therapy alone(38).

Although tetracyclines are not as effective as the previously mentioned treatments in acne, it gives few side effects, is easier to prescribe, needs no monitoring of blood chemistry and reduces inflammatory lesions in a satisfactory way. As previously suggested in the background of this review by Chiou et al (2010) was that oral tetracyclines did not have a superior efficacy compared to placebo therapy(10). None

of the 3 studies assessed compared oral tetracycline in monotherapy versus strict placebo therapy. Therefor it is difficult to draw any conclusions about the efficacy of tetracycline alone.

The trend in prescription of acne therapy has changed over the last years. According to Thevarajah et al (2005) there were a significant decline in the 1990-2002 in prescription of antimicrobial therapies, while there was an significant raise in the usage of non-antimicrobial regimens such as topical and oral retinoids(41). This observation was suggested to have a relationship to the awareness of the increase in PA resistance to antibiotics(41). In another study conducted by Kinney et al (2010) assessing the same topic, there was a significant increase in prescription of tetracyclines from 1997-2006 and a drop in prescription of erythromycin and oral isotretinoin(42).

It is evident that oral isotretinoin is more effective in treatment outcome in comparison to oral tetracycline. The effectiveness of oral isotretionin comes from its fast onset of action and the wide target of exerting its effects and the prolonged remission after cessation of therapy. These well known facts concerning oral isotretinoin has been stated in several studies pointing on a 90 % efficacy of reducing sever inflammatory lesions(43).

New questions brought in to light is weather an increased number of resistant PA actually have a significant impact on treatment outcome though the assessed studies proved different answers to this question. While resistant strains of PA may colonize the skin surface (even without causing acne), when skin samples are taken from acne patients, this sample does not measure the portion of resistant strains residing within the hair follicle, where the actual pathogenesis of acne take place. In studies, many times these skin samples are taken with a skin swab of the affected area. Unless the skin sample clearly is reflecting the number of resistant strains of PA residing in the hair follicle, one cannot really state weather the treatment outcome is associated with this number of resistant PA. Though antibiotics and topical retinoids work in an anti-inflammatory fashion they indirectly inhibits PA way of action. This might be the explanation in Study I, were the number of tetracycline-resistant PA’s increased during the therapy as expected but did not affect the treatment outcome, expressed as total number of inflammatory lesions.

According to Tan et al (2003) it makes sense to state that an increased number of resistant strains contribute to more treatment failures. However PA that are resistant in vitro might not be the case in vivo(7, 44). So, does an increased number of resistant PA's contribute to acne, and is this factor significantly affecting and correlating to the treatment outcome? Reflecting the large spectra of studies assessing this outcome, the answer is certainly yes, there is a correlation.

The quality of evidence across studies was diverse for the three assessed outcomes. The main reason for the prevalent low evidence in two of the outcomes was the process of randomization and lack of blinding participants and clinicians. However it is reasonable to think it was difficult to make a proper masking with no ethical conflict. In the study were oral isotretinoin was given, adverse effects are more apparent and females had to take oral contraceptives. Though it is important for those taking oral isotretinoin not to get pregnant during therapy, it is obvious that it was not ethical to blind the patients. There was some inconsistency across studies when assessing the outcome of tetracycline resistant PA to oral tetracycline. Especially two studies had a different way of direction concerning their PA resistance and treatment effect outcome, contributing to the lack of consistency throughout studies.

Limitations

As for limitations in this review there are several aspects to discuss. At the outcome level the main limitations are that in the studies reviewed, the methods of randomization, grading of acne severity and the regimens were different across studies. Only one study compared oral tetracycline alone versus another antibiotic. Monotherapy comparisons were highly preferred but appeared to be a rare study intervention though most of studies compared combination therapies.

There are several weaknesses related to the study and review level. There was a small sample of remaining studies assessed, though the search did not generate numerous high quality studies. Of those remaining, 3 of them were RCT studies, however none of them used placebo control groups. As for the quality of evidence it were in average low to moderate across studies for each outcome, which makes it difficult to make any general definite conclusions more than what is already stated in the present literature. Another limitation is that the studies did not provide all their data with effect measures with confidence intervals. Making it hard to draw conclusions and

compare the results. As for the reviewing it is possible that publication bias might have occurred for one study where the results was very vaguely presented compared to the other studies. Only two studies with the highest evidence stated that their analysis was in an intention-to-treat analysis. It is possible that an overestimation of the result is made in the study, which did not have this as an objective. Only one study provided calculations on number of participants that had to be in each control group to produce a statistical power. Limitations related to the search strategy could be that only studies published in the English language were selected and only those published in full-text. As for the inclusion and eligibility criteria, one might have produced a study selection to review not generally representative to the general population. Strengths of this review are the systematic manner of the reviewing due to the PRISMA statement, Cochrane working group and HTA-centrum(25, 31, 35).

Conclusions and Implications

Oral tetracycline is clearly an effective treatment in moderate to severe acne vulgaris, even more effective in the combination with topical retinoids. Topical therapy with retinoids and antimicrobials have similar efficacy as oral tetracyclines. The reason for this might be originating from the anti-inflammatory effect, which they all provide. There are other treatments that may promise better efficacy, however at the cost of more adverse effects. It is important to stress the fact that resistant strains of PA are increasing and that long term treatment with oral tetracycline's, especially with lacking compliance selects resistant strains, which in turn may transfer resistance to other bacteria for example coagulase negative staphylococci (CNS). In this way, making it difficult to treat simple infections with the most commonly used antibiotics, it is a relevant subject to emphasize. The most important group to acknowledge these facts may be the general practitioners, which are the category of clinicians that most often treat these cases. There is a general misunderstanding that acne is something naturally that you have to accept. However this is not always the case. Patients presenting with acne should be taken seriously though there is an evident correlation between assessed quality of life and the severity of acne. To evaluate the efficacy of oral tetracycline and the impact of the number of tetracycline-resistant PA, further research is recommended comparing oral tetracycline versus other acne therapy in monotherapy.

Populärvetenskaplig sammanfattning

Akne är en mycket vanlig hudsjukdom som drabbar upp emot 80 % av alla ungdomar. Uppkomsten är beroende av flera faktorer och består av en ökad talgproduktion, igenproppning av talgkörtelns utförsgång på huden, ökat antal bakterier(Propionibacterium Acnes) och inflammation. Akne kan ses som svarta pormaskar och varfyllda finnar i ansikte och ibland på bålen som kan bli inflammerade. Akne indelas efter svårighetsgrad och det är också efter denna indelning den behandlas. Från den lättaste behandlingen ofta bestående av lokala krämer som verkar på översta hudlagret eller mot bakterier till tablettbehandling med antibiotika och i de värsta fallen med det mycket potenta läkemedlet Roaccutan. Med den ökade användningen av antibiotika sållas motståndskraftiga bakterier fram mot de vanligaste antibiotikagrupperna som används vid akne.

Förstahandsvalet vid måttlig till svår akne är tablettbehandling med antibiotikagruppen tetracykliner. Antibiotika läker inte ut akne utan verkar antiinflammatoriskt och på bakteriens tillväxt medan Roaccutan har förmågan att helt läka ut akne, dock med en risk för fler och svårare biverkningar.

I denna systematiska litteraturöversikt behandlas ämnet tablettbehandling med antibiotika och dess effekt och biverkningar i behandling av akne. Då vetenskapen ligger till grund för val av behandling är det viktigt att behandlare har tillgång till en överblick av kunskapsläget och kan använda sig av den i sin klinik. Behovet av kritisk granskning av publicerade vetenskapliga artiklar är stort då en behandling kan stå emot en annan där klarheten för ett visst resultat inte alltid är värderad utifrån studiens metod och risk för felberäkningar. I denna systematiska litteraturöversikt undersöktes tre studier med hög trovärdighet avseende behandling med tetracykliner och annan vanlig aknebehandling.

Slutsatsen är att många av de läkemedel som kan användas lokalt som krämer eller lösningar på huden som verkar mot bakterier vid akne är lika effektiva som tablettbehandling med antibiotika(tetracykliner). Det potenta läkemedlet Roaccutan är mycket mer effektiv än all annan aknebehandling men ger också fler biverkningar. Motståndskraft av bakterien *Propionibacterium Acnes* har en negativ inverkan på behandlingsresultatet.

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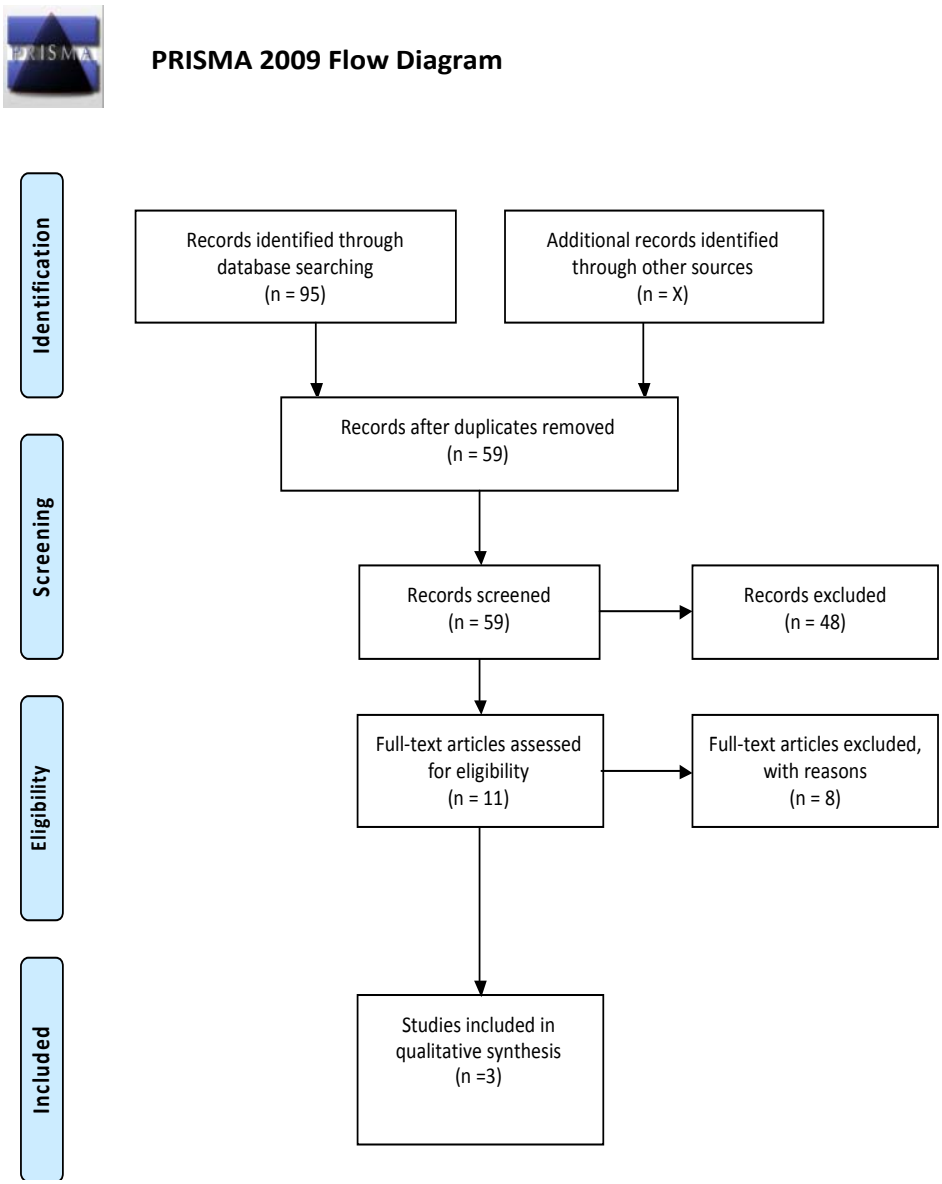
Appendix: Tables and Figures

Flow Diagram – Search process



A total of 95 studies were at first provided. After duplicates were removed 59 studies remained. 48 studies were excluded when assessed in title and abstract for meeting inclusion criteria. 11 studies were assessed in full text for eligibility resulting in exclusion of 8 studies. Finally 3 studies were included in the qualitative analysis.

Inclusion list and Outcome tables

1. Oprica C, Emtestam L, Hagstromer L, Nord CE. Clinical and microbiological comparisons of isotretinoin vs. tetracycline in acne vulgaris. Acta dermato-venereologica. 2007;87(3):246-54. PubMed PMID: 17533492.

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Outcome variable 1: Efficacy evaluation of oral tetracycline group vs. control treatment in acne vulgaris

* + No problem
? Some problems
- Major problems

Author, year	Country	Study design	Number of patients n=	With drawals - dropouts	Result Intervention Control 1	Result Intervention Control 2	Comments	Directness*	Study limitations *	Precision *
Oprica et al., 2007	Sweden	RCT, parallell-group	52	13	N=26, Tetracycline plus Adapalene. Improvement was seen (p<0.001), not as high efficacy as in control group 2. A swing up of lesions in the follow up period.	N=26, Oral Isotretinoin More effective than control 1 in the majority of lesions, faster onset of action. Better efficacy than control 1 in the long term (p=0.009).	Tetracycline 500mg 1x2. Topical adapalene 0.1 % once a day. Oral isotretinoin 1mg/kg/day in 2 divided doses. Treatment for 24 weeks with a 2 month follow-up(control 1 received adapalene under the 2 month follow-up period).	-	?	+
Rafiei et al., 2006	Iran	RCT, parallell-group	290	54	N=118, Tetracycline 31.4% showed good/excellent results after 3 months. No statistically significant difference in clinical response compared to control 2 after 3 months. p>0.05	N=118, Azitromycin 44% showed good/excellent results after 3 months. Degree of improvement was greater compared to control 1.	Tetracycline 1g/day for 1 month, thereafter 500mg/day for 2 months. Azitromycin pulse- 500mg/day for 3 consecutive days/week for 4 weeks. Afterwards azitromycin 250mg every other day for 2 months. After 1 month, topical tretinoin 0.05% was added to both groups.	+	?	-
Ozolins et al., 2004	UK	RCT Regimen 1(R1) vs. regimen 4 (R4)	258	102	N=131, Oxytetracycline + topical placebo (Regimen 1) Efficacy between control 1 and 2 were similar. 55% had moderate to great improvement at 18 weeks.	N=127, Oral placebo + topical erythromycin in combo with benzoyl peroxide (Regimen 4) 66% hade moderate tog great improvement at 18 weeks. Odds ratio, (95% CI) control 2 versus control 1 is 1.64 (0.98-2.74).	R1: Oxytetracycline 500mg 1x2 + topical placebo twice a day. R4: Oral placebo once a day + topical 3% erythromycin in combo with 5% benzoyl peroxide twice daily. Treatment for 18 weeks.	+	+	+

Table displaying efficacy of oral tetracycline versus different acne treatments. Oprica et al compared oral tetracycline plus topical adapalene vs oral isotretinoin for 24 weeks. Oral isotretinoin was more effective in the majority of lesions with a better efficacy than the tetracycline plus adapalene group. Rafiei et al compared oral tetracycline versus oral azithromycin for 3 months, thereafter topical tretinoin in both groups for 1 month. There was a greater improvement of lesions in the azithromycin group, however there was no statistically significant difference in the clinical response. Ozolins et al compared oral oxytetracycline plus topical placebo (control 1) versus oral placebo plus topical erythromycin plus benzoyl peroxide (control 2) for 18 weeks. The efficacy between both groups was similar with odds ratio (95% CI) control 2 versus control 1 was 1.64 (0.98- 2.74).

RCT: Randomized Controlled Trial, R1: Regimen 1, R4: Regimen 4

Outcome variable 2: Side effects evaluation of oral tetracycline group vs. control treatment in acne vulgaris

* + No problem
? Some problems
- Major problems

Author, year	Country	Study design	Number of patients n=	With drawals - dropouts	Result Intervention		Comments	Directness*	Study limitations *	Precision *
					Control 1	Control 2				
Oprica et al., 2007	Sweden	RCT, Parallell-group	52	13	N=26, Tetracycline plus adapalene. 10% complained of transitory nausea or abdominal pain. 15% experienced dry skin, itching and redness of the skin at one point during the 24 weeks.	N=26, Oral Isotretinoin Side effects: dry skin 91.4 %, chelitis 95.8%, dry eyes 75%, epitaxis 54% after 2 months of therapy. After discontinuation of therapy side effects disappeared in 83%. Most had transitory increase in triglycerides and cholesterol, although within normal range.	Tetracycline 500mg 1x2. Topical adapalene 0.1 % once a day. Oral isotretinoin 1mg/kg/day in 2 divided doses. Treatment for 24 weeks with a 2-month follow-up (control 1 received adapalene under the 2 month follow-up period).	-	?	+
Rafiei et al., 2006	Iran	RCT, Parallell-group	290	54	N=118, Tetracycline 6.8% complained of of epigastric pain, 4.2 % complained of diarrhea, 2.5% complained of vulvovaginal pruritus. In summary: 11% gastro intestinal side effects.	N=118, Azitromycin 5% complained of heartburn and epigastric pain, 5.9 % complained of diarrhea. In summary: 10.9% gastrointestinal side effects.	Tetracycline 1g/day for 1 month, thereafter 500mg/day for 2 months. Azitromycin pulse- 500mg/day for 3 consecutive days/week for 4 weeks. Afterwards Azitromycin 250mg every other day for 2 months. After 1 month, topical tretinoin 0.05% was added to both groups.	+	?	-

Table displaying side effects evaluation of oral tetracycline group versus different acne treatments. Oprica et al compared oral tetracycline plus topical adapalene (control 1) to oral isotretinoin (control 2) for 24 weeks of treatment. Control 1 in Oprica et al experienced typical side effects produced by oral antibiotics such as gastrointestinal upset. Out of the 26 participants in this group 15 % experienced dry skin, itching and redness. Control 2 in Oprica et al experienced more side effects than control 1. The most common complaint was dry skin produced in 91.4 %, chelitis and dry eyes. These side effects disappeared in most cases after cessation of therapy. Most of the patients in control 2 had a transitory increase in lipid levels, however within the normal range. Rafiei et al compared oral tetracycline (control 1) to oral azithromycin (control 2) for 3 months with topical tretinoin for 2 months after 1 month of oral therapy in both groups. Both groups experienced similar gastrointestinal side effects at an equal amount.

RCT: Randomized Controlled Trial

Outcome variable 2: Side effects evaluation of oral tetracycline group vs. control treatment in acne vulgaris

* + No problem
? Some problems
- Major problems

Author, year	Country	Study design	Number of patients n=	With drawals - dropouts	Result Intervention Control 1	Result Intervention Control 2	Comments	Directness*	Study limitations *	Precision *
Ozolins et al., 2004	UK	RCT Regimen 1 vs. regimen 4	258	102	N=131, Oxytetracycline + topical placebo (Regimen 1) Adverse events: After 6weeks 22 patients experienced GI upset, 11 CNS symptoms, 5 skin irritation. More systemic side effects compared to control 2. Control 1 and 2 had similar mean- patient- assessed – irritation score.	N=127, Oral placebo + topical erythromycin in combo with benzoyl peroxide (Regimen4) Adverse events: After 6 weeks 8 patients experienced GI upset, 4 CNS symptoms, 11 skin irritation. More skin irritation compared to control 1.	R1: Oxytetracycline 500mg 1x2 + topical placebo twice a day. R4: Oral placebo once a day + topical 3% erythromycin in combo with 5% benzoyl peroxide twice daily. Treatment for 18 weeks. Gastrointestinal symptoms: nausea, upset stomach. CNS symptoms: Headache	+	+	+

Table displaying side effects evaluation of oral tetracycline group versus different acne treatments continuation. Ozolins et al compared oral oxytetracycline plus topical placebo (control 1) to oral placebo plus topical erythromycin plus benzoyl peroxide (control 2) for 18 weeks. There were more systemic side effects in control 1 compared to control 2. Control 2 produced more skin irritation compared to control 1. Both groups had similar mean-patient-assessed- irritation score.

RCT: Randomized Controlled Trial, R1: Regimen 1, R4: Regimen 4

Outcome variable 3: Evaluation of Propionibacterium acnes resistance to oral tetracycline group vs. control treatment in acne vulgaris

+ No problem
? Some problems
- Major problems

Author, year	Country	Study design	Number of patients n=	With drawals - dropouts	Result Intervention		Comments	Directness*	Study limitations *	Precision *
					Control 1	Control 2				
Oprica et al., 2007	Sweden	RCT, Parallel-group	52	13	N=26, Tetracycline plus adapalene. Higher probability of gaining clindamycin/tetracycline resistant PA's after the follow-up	N=26, Oral isotretinoin Patients with resistant PA are treated with ISO kept the already existing resistant strains or lost them.	Tetracycline 500mg 1x2. Topical adapalene 0.1 % once a day. Oral isotretinoin 1mg/kg/day in 2 divided doses. 24 weeks therapy with a 2-month follow-up (control 1 received adapalene under the 2 month follow-up period).	-	?	+
Ozolins et al., 2004	UK	RCT Regimen 1 vs. regimen 4	258	102	N=131, Oxytetracycline + topical placebo (Regimen 1) 47% of patients colonized with resistant strains reported moderately improvement at 18 weeks compared to 56% that had no resistant strains to tetracycline. Reduction in mean skin lesion count at 18 weeks was significantly less effective for control 1 versus control 2 for those with resistant strains. 23.1(11.8-34.5)	N=127, Oral placebo + topical erythromycin in combo with benzoyl peroxide (Regimen 4) 48% was colonized at baseline with erythromycin-resistant PA's. A reduction of 9 % in number of colonized participants was seen after 18 weeks. Topical erythromycin gave the largest reduction (16%) in population of all PA strains after 18 weeks.	R1: Oxytetracycline 500mg 1x2 + topical placebo twice a day. R4: Oral placebo once a day + topical 3% erythromycin in combo with 5% benzoyl peroxide twice daily. Treatment for 18 weeks. (Measured as effect of colonization with tetracycline-resistant PA's on treatment outcome). Simplification: A significantly decreased effect of therapy with oxytetracycline was seen in participants colonized with tetracycline resistant PA's strains.	+	+	+

Table displaying evaluation of Propionibacterium acnes resistance to oral tetracycline group versus other treatment in acne. In Oprica et al they compared oral tetracycline plus topical adapalene (control 1) to oral isotretinoin (control 2) for 24 weeks of treatment. In control 1 in Oprica et al there was a higher probability of gaining clindamycin and tetracycline resistant PA's after the follow-up. However in control 2 in Oprica et al those who already had resistant PA kept the resistant strains or lost them.
Ozolins et al compared oral oxytetracycline plus topical placebo (control 1) to oral placebo plus topical erythromycin plus benzoyl peroxide (control 2) for 18 weeks. In control 1 in Ozolins et al, those that had no resistant strains to tetracycline reported moderately improvement to a higher extent compared to participants colonized with resistant strains. Reduction in mean skin lesion count was significantly less effective in control 1 compared to control 2, 23.1 (11.8- 34.5). There was a significantly decreased effect of therapy with oral oxytetracycline in participants colonized with tetracycline resistant PA's.

RCT: Randomized Controlled Trial, R1: Regimen 1, R4: Regimen 4, PA: Propionibacterium acnes

GRADE analyse

Side effects of tetracycline vs. other regimen										
3	RCT	No serious limitations (0)	No important inconsistency	No uncertainty	No Imprecision	Unlikely	Not relevant	Not analysed	Not analysed	Moderate ⊕⊕⊕◯
Efficacy of tetracycline vs. other regimen										
3	RCT	Serious limitations (-1)	Some inconsistency (0?)	Some uncertainty (0?)	Uncertain precision (0?)	Unlikely	Not relevant	Not Statistically significant	Not analysed	Low ⊕⊕◯◯
Evaluation of resistant PA's in therapy with oral tetracycline vs. other treatment										
2	RCT	No serious limitations (0)	Very serious inconsistency (-2)	Serious indirectness (-1)	Uncertain precision (0?)	Unlikely	Not relevant	Not Statistically significant	Not analysed	Low ⊕⊕◯◯

Evidence through out studies assessed for each outcome measure. Side effects of tetracyclines versus other regimen had a moderate evidence grade. Efficacy of tetracyclines versus other regimens provided low evidence. There was a low evidence concerning evaluation of the PA's resistance.

Exclusion Table

Providing studies that were excluded and the reason for exclusion.

Study (Author, publication year)	Reason for exclusion
Khorvash et al., 2012	Case-Control study.
Leyden et al., 2011	Intervention not concurrent with PICO.
Purdy et al., 2011	Review.
Lipozenic et al., 2011	Not concurrent with PICO (perioral dermatitis)
Yoon et al., 2010	Not concurrent with PICO (Stevens-Johnson Syndrome)
Jang et al., 2010	Case study.
Ochsendorf et al., 2010	Review.
Geddes et al., 2010	Intervention not concurrent with PICO.
Sugita et al., 2010	Not concurrent with PICO. (Antifungals)
Del Rosso JQ et al., 2009	Review.
Purdy et al., 2008	Review.
Simonart et al., 2008	Review.

Guay et al., 2007	Review.
Tehrani et al., 2007	Case study.
Benjamin et al., 2007	Case-control study.
Somani et al., 2006	Case study.
Ochsendorf et al., 2006	Review.
Friedman et al., 2005	Review.
Tan et al., 2004	Review.
Mouton et al., 2004	Not concurrent with PICO.
Bikowski et al., 2003	Review.
Tan et al., 2003	Review.
Garner et al., 2003	Review.
Moon et al., 2012	Intervention not concurrent with PICO.
Song et al., 2011	Intervention not concurrent with PICO.
Gonzalez et al., 2011	Intervention not concurrent with PICO.
Hassanzadeh et al., 2008	Intervention not concurrent with PICO.
Margolis et al., 2007	Participants and outcome not concurrent with PICO.

Tan et al., 2005	Review.
Shalita et al., 2012	Intervention not concurrent with PICO.
Wainwright et al., 2012	Intervention not concurrent with PICO.
Holst et al., 2011	Case study.
Leyden et al., 2011	Review.
Ingram et al., 2010	Review.
Geria et al., 2009	Intervention not concurrent with PICO.
Branley et al., 2009	Case study.
Tabibian et al., 2009	Case study.
Amin et al., 2007	Review.
Riddle et al., 2007	Review.
Webster et al., 2007	Review.
Rao et al., 2006	Review.
No author name available, 2006	Intervention not concurrent with PICO.

Del Rosso et al., 2006	Review.
Van Zuuren EJ et al., 2011	Subject not of interest. (Rosacea)
Arowojolu et al., 2012	Subject not of interest. (Oral contraceptives)
Thevarajah et al., 2005	Observational study.
Adawiyah et al., 2010	Observational study.

Kinney et al., 2010	Observational study.
Owczarek W Fau et al., 2011	Review.
Kircik LH., 2010	Review.
Ochsendorf F., 2010	Review.
Adisen E Fau-Kaymak et al., 2008	Intervention not concurrent with PICO.
Song SJ., 2007	Subject not of interest. (Ear point blood-letting)
Leyden Jj Fau et al., 2007	Review.
Ma Xh Fau-Zhu et al., 2004	Subject not of interest. (Intervention with Qingre Cuochuang tablet)
Del Rosso etal., 2007	Review.

Exclusion References

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