Mechanisms of antibacterial herbal action

Dr Christopher Etheridge PhD MCPP
MRSC, CChem, DoIC, ARCS, BSc (Hons) Phyto, BSc (Hons) Chem



Mechanisms of antibacterial herbal action

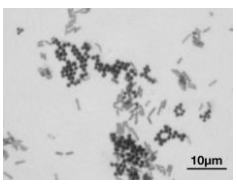
Introduction

- 1. Mechanisms of direct antibacterial activity
- 2. Mechanisms of indirect antibacterial activity
- 3. Mechanisms of potentiation of antibiotic activity

Mechanisms of antibacterial herbal action

Introduction

- Bacteria can broadly be classified into 2 groups, Gram-positive and Gram-negative, depending upon their colour when exposed to Gram's stain.



Gram-positive: Staphylococcus aureus (purple)
Gram-negative: Escherichia coli (red)

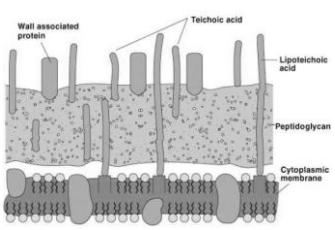
Mechanisms of antibacterial herbal action

Introduction

- Gram-positive bacteria have a single cell membrane (monoderm) made of a single phospholipid bilayer, surrounded by a relatively simple but thick cell wall consisting mainly of peptidoglycans, proteins, teichoic and lipoteichoic acids.

Mechanisms of antibacterial herbal action

Introduction



https://theartofmed.wordpress.com/2015/08/28/bacterial-taxonomy-1-classification-based-on-morphology-and-the-gram-stain/

Mechanisms of antibacterial herbal action

Introduction

- Gram-negative bacteria possess a much more complex cell envelope consisting of three distinct layers: a thin proteoglycan layer sandwiched between inner and outer cell membranes (diderms).
- There is a gap between the inner membrane and proteoglycan layer filled with periplasm which contains proteins and enzymes.

Mechanisms of antibacterial herbal action

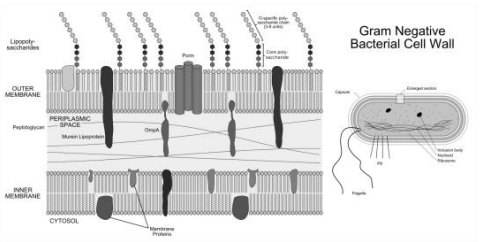
Introduction

- The proteoglycan contains murein lipoprotein rather than teichoic acids.
- Outer cell membrane is typical cell membrane consisting of phospholipid bilayer structure. However it contains lipopolysaccharide (LPS) on outermost side of bilayer.

7

Mechanisms of antibacterial herbal action

Introduction



https://theartofmed.wordpress.com/2015/08/28/bacterial-taxonomy-1-classification-based-on-morphology-and-the-gram-stain/

8

Mechanisms of antibacterial herbal action

Introduction

- The effectiveness of any antibacterial drug (herbal or otherwise) depends both on its ability to penetrate past the protective bacterial membrane(s) and capsules.
- If it can't penetrate these, then it will not work.
- Unsurprisingly, mechanism of antibacterial activity of herbal medicines tend to mirror those already known for antibiotics (most of which of course, are naturally occurring products).

9

Mechanisms of direct antibacterial activity

10

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

1. Inhibition of bacterial wall synthesis:

- Drugs such as β-lactams (penicillins) block peptidoglycan synthesis.
- Bind to penicillin-binding proteins (PBPs) - group of transpeptidase enzymes found in cell membrane involved in cross-linking of bacterial cell wall and cell wall synthesis.
- The β-lactam ring of this group of antibiotics binds to these different PBPs, inhibiting transpeptidation of simple peptidoglycans.

11

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

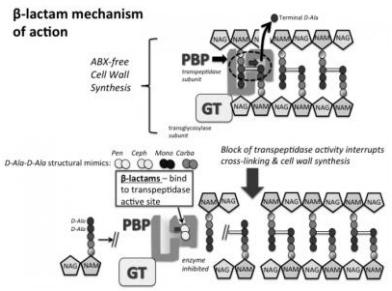
1. Inhibition of bacterial wall synthesis:

- This leads to death of the bacterial cell due to osmotic instability and/or autolysis.

12

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity



Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

1. Inhibition of bacterial wall synthesis:

- A number of important antibacterial herbs are thought to act via this mechanism, including:

1. *Astragalus mongholicus* (huang qi)
2. *Artemisia annua* (qīnghāo)
3. *Bupleurum chinense (*chai hu*)*
4. *Cornus officinale* (shān zhū yú)

(Viswanad et al. IJPSR, 2011; 2(7): 1651-1658)

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

1. Inhibition of bacterial wall synthesis:

5. *Paeonia obovata* (cǎo sháo yào)
6. *Panax pseudoginseng* (san qi)
7. *Prunella vulgaris* (xia ku cao)
8. *Verbena officinalis*

Mechanisms of antibacterial herbal action

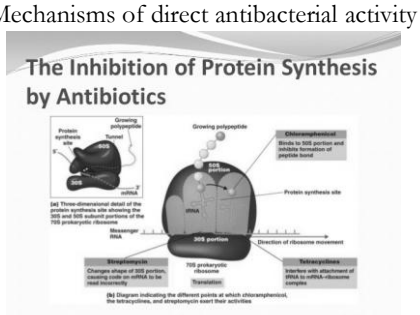
Mechanisms of direct antibacterial activity

2. Inhibition of translation (protein synthesis):

- Some antibacterial drugs act by inhibiting protein synthesis (translation), often by binding with 50s or 30s subunit of bacterial ribosome, preventing either aminoacyl t-RNA binding, peptide bond formation, mRNA reading or translocation.
- Binding causes misreading of m-RNA, disrupting normal protein synthesis.
- Drugs such as tetracycline and macrolides work in this manner.

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity



Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

2. Inhibition of translation (protein synthesis):

- Some antibacterial herbs that are believed to inhibit this include:

1. *Arctium lappa* (niúbàng)
2. *Astragalus mongholicus* (huang qi)
3. *Gentiana triflora* (san hua long dan)
4. *Lonicera japonica* (jinyinhua)
5. *Prunella vulgaris* (xia ku cao)
6. *Saxifraga stolonifera* (huer cao)

(Viswanad et al. IJPSR, 2011; 2(7): 1651-1658)

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

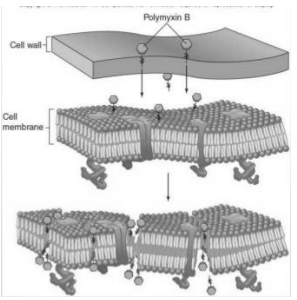
3. Alteration of cell membrane function:

- Cell membranes segregate and regulate the intra- and extracellular flow of substances.
- Disruption or damage to this structure results in leakage of important solutes essential for the cell's survival.
- Examples: polymixin B and colistin.

19

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity



20

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

3. Alteration of cell membrane function:

- Many antibacterial essential oils, due to their lipophilic properties, able to directly disrupt and destabilise bacterial cells membranes. (Xi Yap et al. Open Microbiol J. 2014; 8: 6–14).
- Synergistic effect from different terpenes and phenylpropanoids in essential oils. (Bassole et al. Molecules 2010;5(11):7825–39)
- Activity and antibacterial mechanisms of tea tree (*Melaleuca alternifolia*) essential oil has been studied in detail – act as membrane permeabiliser. (Cox et al. J Appl Microbiol. 2000;88(1):170–5)

21

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

3. Alteration of cell membrane function:

- Saponin-containing antibacterial herbs may also work via this mechanism (Godstime et al. J Pharm Chem Biol Sci , August 2014;2(2): 77-85).
- For example, *Moringa oleifera* (Moyo et al. African Journal of Biotechnology 2012; 12:34-42, Onyekaba et al.. Int JPharm, Chem and Biol Sci 2013; 3(3): 962-973).

22

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

4. Inhibition of nucleic acid synthesis:

- DNA and RNA essential for the replication of all living forms, including bacteria.
- Some antibacterials work by binding to components of DNA or RNA synthesis, causing interference with the normal cellular processes leading to compromised bacterial multiplication and survival. Antibiotic examples: quinolones, metronidazole, and rifampicin.

23

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

4. Inhibition of nucleic acid synthesis:

- Berberine found in herbs such as *Berberis vulgaris*, *Berberis aquifolium*, *Coptis chinensis* (duǎn è huánglián) and *Hydrastis canadensis* has been demonstrated to directly bind with DNA and RNA and prevent transcription and translation in bacteria. (Karaosmanoglu OMICS. 2014 Jan;18(1):42-53)

24

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

5. Inhibition of type II topoisomerase

- Type II Topoisomerases are a class of enzymes that regulate the overwinding or underwinding of DNA, important in translation of the DNA strand.
- Antibiotics such as ciprofloxacin work as type II topoisomerase inhibitors.

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

5. Inhibition of type II topoisomerase

- Antibacterial herbs that are believed to operate via the inhibition of this enzyme include:

1. *Camellia sinensis* (Godstime et al. J Pharm Chem Biol Sci , August 2014; 2(2): 77-85)
2. *Citrus x limon*
3. *Lavandula latifolia*
4. *Mentha arvensis*
5. *Pulsatilla koreana*

(Viswanad et al. IJPSR, 2011; 2(7): 1651-1658)

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

6. Antimetabolite activity:

- Antimetabolites - block key metabolic pathways by inhibiting use of metabolites by enzymes.
- Folic acid essential vitamin for synthesis of DNA and RNA precursors in bacteria. Without folic acid the bacterium cannot grow.

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

6. Antimetabolite activity:

- Bacteria must synthesise own folic acid.
- Bacteriostatic drugs such as sulphonamides compete with p-aminobenzoic acid (PABA) for the enzyme dihydropteroate synthase (DHPS) (necessary for synthesis of folic acid), blocking folic acid production, or trimethoprim, which inhibits the enzyme dihydrofolate reductase (DHFR) - also needed for folic acid manufacture.

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

6. Antimetabolite activity:

- Antibacterial herbs that are believed to work by inhibiting folic acid synthesis include:

1. *Camellia sinensis* – EGCG inhibitor of DHFR (Sánchez-del-Campo et al. Int J Mol Sci. 2009 Dec; 10(12): 5398–5410).
2. *Cornus oblonga* (substitute for shān zhū yú)
3. *Crataegus pinnatifida* (shānzhā) (Wu et al. Molecules 2014, 19(2), 1685-1712)
4. *Lycium barbarum* (gǒuqǐ) (Mocan et al. Molecules 2014;19(7):10056-10073)

(Viswanad et al. IJPSR, 2011; 2(7): 1651-1658)

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

6. Antimetabolite activity:

5. *Morus alba* (sang ye) (Devi et al. Int J Pharm and Pharmaceut Sci 2013 5(2):14-18)
6. *Solanum indicum*
7. *Sinapis alba* (Bai Jie Zi) (Kim et al. J Korean Soc Appl Bio Chem; 52 (5), 555-559)
8. *Thlaspi arvense* (Bai Jiang Cao)
9. *Thuja occidentalis*

- The activity is probably mediated by polyphenols in the herbs, with either DHPS- or DHFR-inhibiting activity.

(Viswanad et al. IJPSR, 2011; 2(7): 1651-1658)

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

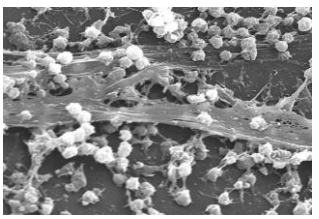
7. Biofilm disruption/formation inhibition:

- Biofilm is group of microorganisms in which cells stick to each other and often these cells adhere to a surface. Cells are usually embedded within self-produced matrix of extracellular polymeric substance (EPS).
- Biofilms involved in wide variety of microbial infections in the body and linked to quorum sensing (bacterial cell-cell communication).

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

7. Biofilm disruption/formation inhibition:



S. aureus biofilm on catheter

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

7. Biofilm disruption/formation inhibition:

- Lemon grass, xiāng máo cǎo (*Cymbopogon citratus*) has been shown to inhibit and destroy bacterial biofilms, as well as disrupting quorum sensing. (Moore-Neibel et al. 2012.J Appl Microbiol 112: 485-492)
- Other herbs which may also have this effect include:

Mechanisms of antibacterial herbal action

Mechanisms of direct antibacterial activity

7. Biofilm disruption/formation inhibition:

1. *Allium sativum*
2. *Camellia sinensis*
3. *Chelidonium majus*
4. *Hypericum perforatum*
5. *Panax ginseng* (rénshēn) (Lee at al, Carbohydr. Res. 2006. 341: 1154-1163,)
6. *Zingiber officinale* (gan jiang, sheng jiang)

(Rabin et al. Future Med Chem. 2015;7(5):647-71)

Mechanisms of indirect antibacterial activity

Mechanisms of antibacterial herbal action

Mechanisms of indirect antibacterial activity

1. Immunomodulation:

- Some herbs have indirect antibacterial action via interaction with human innate and adaptive immune system:

1. *Aloe vera* (lu hui): selectively stimulates cytokines and activates lymphocytes.
2. *Angelica polymorpha* (dong quai): modulates cytokines.
3. *Astragalus mongholicus*: cytokine modulation and increases macrophage level.

(Tan et al. Cur Med Chem 2004,11(11)1423-1430)

Mechanisms of antibacterial herbal action

Mechanisms of indirect antibacterial activity

1. Immunomodulation:

4. *Ganoderma lucidum* (língzhī): modulates cytokines, increases lymphocytes and natural killer cells.
5. *Panax ginseng*: increase production of IL-1, IL-6 IL-12, TNF-α and IFN-γ, increases lymphocyte and macrophage activity
6. Scutellaria baicalensis (huang qin): Stimulates TNF-α, activates iNOS.
7. *Zingiber officinale*: increases IL-1 and IL-6 production.

Tan et al. Cur Med Chem 2004,11(11)1423-1430)

Mechanisms of antibacterial herbal action

Mechanisms of indirect antibacterial activity

1. Immunomodulation:

- Antibacterial herbs with adaptogenic and healing activities will also work (ultimately) by their effect on the immune system.

Mechanisms of antibacterial herbal action

Mechanisms of indirect antibacterial activity

2. Adherence inhibitors:

- These act by preventing the binding of bacteria to tissue such as the bladder, urethra, stomach and teeth, reducing the risk of UTIs, gastric ulcers and dental caries.
- Such herbs are often rich in polyphenolic compounds e.g. polyphenols in *Camellia sinensis* can prevent binding of *Streptococcus mutans* to teeth (Limsong et al. J Ethnopharmacol. 2004 Jun;92(2-3):281-9).

Mechanisms of antibacterial herbal action

Mechanisms of indirect antibacterial activity

2. Adherence inhibitors:

- *Glycyrrhiza glabra* has been demonstrated to prevent the adherence of *Helicobacter pylori* to human gastric mucosa (Wittschier et al. "*J ethnopharmacol* 125.2 (2009): 218-223).
- *Vaccinium macrocarpon* has been demonstrated to reduce the binding of P-fimbriated *E. coli* to urothelial tissue, reducing risk of UTIs (e.g. Kaspar et al. Food Funct., 2015,6, 1212-1217) and to reduce the binding of *Campylobacter jejuni* to human epithelial cells (Ramirez-Hernandez et al. J Food Protection 2015; 8,1428-1617).

Mechanisms of potentiation of antibiotic activity

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity

- A great deal of research has revealed that combining herbal medicines with antibiotics can cause a synergistic effect, enhancing the activity of antibiotics and overcoming antibiotic resistance (Hemaiswarya et al. Phytomed 2008, 15(8):639-662).
- There are believed to be four main mechanisms for the synergistic effects:

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity

1) Attack on bacterial cell wall

- Polyphenols such as EGCG from *Camellia sinensis* can damage the bacterial cell, as discussed previously, making it more porous and allowing antibiotics such as β-lactams to enter the bacteria more easily, thereby re-enabling susceptibility to antibiotic.
- Key in antibiotic resistant bacteria such as MRSA (Zhao et al. Antimicrob. Agents Chemother 2001. 45(6):1737-1742).

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity

2) Membrane disruption

- *Thymus vulgaris* contains various phytochemicals such as thymol and carvacrol that act as membrane permeabilisers, enabling antibiotics to penetrate into gram-negative bacteria more effectively, increasing their bactericidal activity (Wagner et al. PhytoMed 2009. 16(2-3):97-110).

Mechanisms of antibacterial herbal action

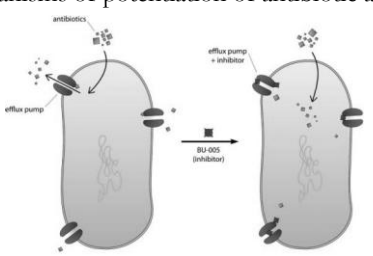
Mechanisms of potentiation of antibiotic activity

3) Inhibition of efflux pump

- Efflux pumps are proteinaceous active transporters found in the cytoplasmic membrane of bacteria (and all kinds of cells).
- Involved in important mechanism of bacterial drug resistance – in drug resistant bacteria genes for one or more efflux pumps are often overexpressed – allowing bacteria to pump antibiotics out of cell before they can have an antibacterial effect.
- Many antibiotics act as inducers and regulators of bacterial efflux pumps.

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity



https://news.brown.edu/articles/2011/11/bacteria

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity

3) Inhibition of efflux pump

- *Thymus vulgaris* and *Scutellaria baicalensis* contains the flavonoid baicalein that directly inhibits bacterial efflux pumps. Demonstrated to reverse resistance to ciprofloxacin in MRSA (Chan et al. J Ethnopharmacol 2011. 137(1):767-773) and tetracycline (Fujita et al. Microbiol Immunol. 2005. 49(4):391-396).
- *Sophora alopecuroides* (ku dou zi) has been shown to make resistant *E. coli* susceptible to ciprofloxacin by inhibiting efflux pumps (Zuo et al. Molecules 2012. 17:2955-2967).

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity

3) Inhibition of efflux pump

- *Rosmarinus officinalis* similarly has been shown to inhibit efflux pumps in resistant *Campylobacter jejuni*, restoring susceptibility to various antibiotics (Klančnik et al. PLoS One 2012. 7(12): e51800).

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity

4) β-lactamase inhibition

- Ability of drug resistant bacteria to penicillins is often due to the presence of the *β-lactamase* gene that easily spreads between bacterial populations.
- Gene encodes for β-lactamase enzyme that enables bacteria to cleave β-lactam ring in penicillins, rendering them harmless.
- Conventionally β-lactamase inhibitors such as clavulanic acid used in combination with penicillins (e.g. co-amoxiclav) to block this resistance.

49

Mechanisms of antibacterial herbal action

Mechanisms of potentiation of antibiotic activity

4) β-lactamase inhibition

- *Alpinia officinarum* (gao liang jiang) contains flavanol galangin which has been shown to reverse β-lactam antibiotic resistance of *S. aureus* to penicillins by acting as β-lactamase inhibitor (Eumkeb et al. Phytomedicine 2010. 18:40-5).
- *Allium sativum* and *Calotropis procera* have also been shown to have this effect (Akkiraju et al. Intl J Sci & Eng Res 2015. 6(2):68-70).

50

Mechanisms of antibacterial herbal action

Conclusion

- Herbal medicines have demonstrated antibacterial action; mechanism for activity elucidated in many cases.
- With current concerns about multidrug resistant bacteria, herbal medicines may offer:

1. Safe and effective option to improve activity of current antibiotics by reversing resistance and potentiating activity.
2. Safe and effective alternative to antibiotics, as antibacterial agents in their own right.

51

Mechanisms of antibacterial herbal action

Dr Christopher Etheridge PhD MCPP
MRSC, CChem, DoIC, ARCS, BSc (Hons) Phyto, BSc (Hons) Chem