Protozoa

Protozoa (in Greek proto = first and zoa = animals) are single-celled eukaryotes, many of them showing characteristics usually associated with animals (mobility, heterotrophy). They are not animals, though.

- Flagellates (Giardia, Trichomonas, Trypanosoma, Leishmania)
- Amoeboids (Entamoeba)
- Sporozoans
  - Apicomplexa (Babesia, Plasmodium, Cryptosporidium, Toxoplasma)
    - Myxozoa (mainly in fishes)
    - Microsporidia (mainly fishes, crabs, not important human pathogens)
- Ciliates, not important human pathogens (Balantidium)

Life forms:

- Trophozoite: active, feeding stage of protozoan parasites.
- Cyst: protozoa in a protective capsule under unsuitable conditions.

Reproduction:

- Asexual: schizont (mother cell) → merozoite (daughter cells)
- Sexually: ♀ and ♂ gametocytes → zygotes

Distribution and risk of malaria

- About 36% of the mankind is affected, 40-45 millions of new infections/year, 1-1.1 millions deaths, of those 90% children.
- Plasmodium vivax, P. malariae, P. ovale, P. falciparum
- Geographical distribution of resistance is diverse.
- No effective vaccination so far.
Life cycle of malaria Plasmodium

- Mosquito (female anopheline):
  - oocysts
  - sporozoites
  - hepatocytes
  - gametocytes
  - zygotes
- Human:
  - liver
  - liver cell
  - parasites in red blood cells
  - asexual cycle
  - sexual cycle
  - stomach epithelium
  - male and female gametocytes
  - merozoites
  - oocysts
  - Proguanil, Pyrimethamine, Sulfonamides
  - Primaquine
  - Quinine, Chloroquine, Mefloquine, Artemisinin

Malaria problems

- Elimination of malaria:
  - different resistance of the four Plasmodium strains against drugs → WHO recommendations of drug combinations for prevention (travellers) and cures.
  - elimination of Anopheles mosquito (vector): use of insecticides, use of physical prevention methods.
- AIDS and malaria

Cinchona alkaloids, quinine

- Cinchona succirubra (Peruvian bark) from South America → quinine and other cinchona alkaloids. Used by the Indians. 17th century → Europe. Isolation 1820, structure elucidation 1908, synthesis 1944.
- Quinine is the most active against schizonts and gametocytes. Not very good for prevention. Used again in case of otherwise resistant Plasmodiums.
- Other physiological effects: tonic, adstringent, may cause spasms of smooth muscles (abortion), febrifuge, treatment of cardiac arrhythmias (quinidine is better).

4- and 8-amino-quinolines

- Chloroquine discovered in 1934, based on the structure of quinine.
- Very widely used for treatment and prophylaxis.
- Nowadays widespread resistance against chloroquine (used in combinations).
- Acts only against parasites in erythrocytes:
  - During digestion of hemoglobin: hem → hematin → hemazoin (malaria pigment). Chloroquine binds to the hem in the parasite and prevents it from digesting.

Chloroquine
Mefloquine i

2-Trifluoromethyl aniline

Ethyl 4,4,4-trifluoroacetoacetate

Mefloquine ii

Mefloquine ii

Folic acid antagonists + DFH reductase inhibitors

- Combination of sulfonamides or sulfones with dihydrofolate reductase inhibitors are effective against both sexual and asexual forms.
- Sulfonamides are similar to those used against bacteria.
- Dapsone: used against leprosy since 1943.

DFH reductase inhibitors

- Pyrimethamine: dihydrofolate reductase inhibitor used specifically for parasites.
DFH reductase inhibitors

- Guanidine
- Biguanidine
- 1,3,5-Triazine

Proguanil (Paludrin)

Chlorproguanil

New antiplasmodium agents

- Artemisinin (Quinghaosu)
- Artemether
- Artesunate

- From Artemisia annua (sweet wormwood)
- The peroxide bond interacts with the hem Fe^{2+} iron in the parasite and produces reactive O species
- Artemether and Artesunate are more stable semisynthetic derivatives

New antiplasmodium agents

- Atovaquone inhibits the mitochondrial electron transport through the cytochrome C complex. Used in combination with proguanil (Malarone®)
- Lumefantrine (benflumethol) is always combined with artemether (Co-Artemether®, Riamet®)
- Both are used mainly in SE-Asia with multiresistant P. falciparum

Resistance and prophylaxis

Malaria Endemic Areas

Prophylaxis

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Trypanosomiasis

- African sleeping sickness (affects the CNS). Vector: tsetse fly (Glossina Genus)
  - Trypanosoma brucei gambiense in west and central Africa, 90% of the cases, slow progress chronic infection,
  - Trypanosoma brucei rhodesiense (T.b.r.) in eastern and southern Africa, 10%, rapid acute infection.

- Chagas disease (American trypanosomiasis) caused by Trypanosoma cruzi in central and south America. Affects the internal organs and muscles.
Sleeping sickness, Central Africa 1926-2004

1998: ~39,000 new cases (10% of the population under surveillance)
2005: ~18,000 new cases

Sleeping sickness, 1st stage treatment

- Pentamidin (1941) T.b. gambiense sleeping sickness
- Suramin (1921) T.b. rhodesiense

Sleeping sickness, 2nd stage treatment

- Melarsopol (1949) arsenic derivative with many undesired toxic side effects (encephalopathy, 3-10% lethal)
- Eflornithine (1990), less toxic. Effective only against T.b. gambiense
- Eflornithine + Nifortimix (1964) combination is recommended nowadays

Chagas disease (American trypanosomiasis)

- Parasites are invading most organs of the body, often causing fatal heart, intestinal and oesophageal damage
- Cases: ~16 to 18 million
- Deaths: 21,000 annually
- New cases/year: 300,000

Chagas disease (American trypanosomiasis)

- Nifurtimox and benznidazole can be used for the early chronic phase.
- Side effects because of long therapy of the chronic phase

Leishmaniasis

- Leishmania donovani, tropica, infantum etc. flagellata
- Vector: infected female sandflies (Plebotomus sp.)
- Leishmaniasis has several manifestations:
  - Visceral leishmaniasis (VL) or kala azar, destroying visera, intestinal organs – 100% lethal. Bangladesh, Brazil, India, Nepal and Sudan.
  - Mucocutaneous leishmaniasis (MCL), or espundia, destroys mucous membranes of the nose, mouth and throat cavities - Bolivia, Brazil and Peru.
  - Cutaneous leishmaniasis (CL) produces large numbers of skin ulcers - Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria
- Cases: ~12 million (all continents, incl. South Europe)
- Deaths: 21,000 annually
- New cases/year: 1.5 to 2 million
Leishmaniases

- There is as yet no effective vaccine for prevention of any form of leishmaniasis.
- Leishmaniasis/HIV co-infection is emerging as an extremely serious, new disease – AIDS and VL are locked in a vicious circle of mutual reinforcement.
- Classical drugs have serious drawbacks, side effects
  - Sodium antimony gluconate inj.
  - Paramomycin
  - Liposomal amphotericin B inf.
  - Miltefosine tabl. (2002) India: highly effective, achieving cure rates of up to 98% (VL)

Five-valent antimony derivatives

- Glucuronic acid Na salt

Amoebiases

- Amoebiasis is an enteric infection caused by a protozoan parasitic organism Entamoeba histolytica (trophozoites or cysts in stool).
- Transmitted fecal-orally by ingestion of cysts (contaminated food or water or through person-to-person spread).
- Amoebiasis is of minor importance in Europe, North America etc (tourism!), but highly prevalent in the Third World.

Emetine

- Old drug: the alkaloid emetine from the extract of Ceph. ipecacuanha. Inhibits protein synthesis.
- Not used today because of toxicity.
- In small doses emetine is a powerful expectorant.

8-Hydroxychinolin derivatives

- Especially iodoquinol (Diquinol, Yodoxin) is recommended for amoebiases.
- Possible side effects: neuropathy, blurred vision, iodine allergy – not registered in every country.

Nitroimidazole derivatives
Nitroimidazole derivatives

- Very good antiprotozoal compounds. Especially useful against *Trichomonas vaginalis* vaginitis, as well as amoebiasis.
- Active against several Gram + and – anaerobes (*Bacteroides*, *Fusobacterium*, *Clostridium* spp.)
- Mode of action: not clear in details. The nitro group is probably reduced to a hydroxylamine derivative by ferredoxine and the DNA is damaged in the ribosomes.

Other protozoal infections

- *Trichomoniasis* (*Trichomonas vaginalis*) – Metronidazol (STD → all sexual partners should be treated simultaneously).
  - Immunodeficient adults.
  - Congenital infections of neonates (blindness)
  - Pyrimethamine + sulfa drug, or spiramycin for pregnant (found to be safe in the pregnant woman, fetus, and newborn)

Other protozoal infections

- *Cryptosporiasis* (*Cryptosporodium parum*), (1976)
  - Explosive diarrhea, upset stomach
  - Severe in case of weakened immune systems, children
  - Healthy body can defeat the infection by itself.
  - Contaminated water, pools
  - Pyrimethamine, azithromycin, nitazoxanide
- *Giardiasis* (*Giardia lamblia*), beaver fever
  - Tinidazole (2 g single dose), quinacrine, nitazoxanide

Anthelmintics (taeniciads)

- Drugs that expel parasitic worms (helminths) from the body by killing or stunning them.
  - 1. Flatworms – *Plathelmintes*
    - a) Flukes – *Trematoda*
      - Liver fluke (*Fasciola hepatica*)
      - Blood flukes (*Schistosoma mansoni* etc.)
      - Schistosomiasis, bilharzia
    - b) Tapeworms – *Cestoda*
      - Pork and beef tapeworms (*Taenia* sp.)
      - Fish tapeworm (*Diphyllobothrium latum*)
      - Dog worm or Hydatid disease (*Echinococcus granulosus*)
  - 2. Roundworms - *Nemathelmintes*
    - Large roundworm - *Ascaris*
    - Pinworm or threadworm (*Oxyuris vermicularis*)
    - Lymphatic filariasis or elephantiasis (*filariae, Wuchereria sp.*)
    - Onchocerciasis or river blindness (*Ochocerca volvulus*)
    - Trichinosis or trichinellosis (*Trichinella spiralis*)
    - Whipworm (*Trichuris trichiura*)
  - 3. Arthropods – *Artropoda*

Anthelmintics

- The extract of the rhizomes and stipes of *Dryopteris filix-mas* (European aspidium or male fern), or of *Dryopteris marginalis* (American aspidium or marginal fern). Active agent: filixic acid. Toxic, not used today.
- Stibium derivatives – toxic.
- A few plant proteinase enzymes, e.g. papaine (from papaya), ficine (from fig).
- CCl₄, chlorinated hydrocarbons – very toxic (liver): gasoline.
- Piperazine. Derivatives are recently used agents.

History

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Agents against trematodes and cestodes

- Tapeworms (cestodes) are humanity’s largest intestinal inhabitants, however, usually unnoticed and harmless.
- Flatworms (trematodes) Schistosomiasis (bilharzia)
  - Cases: 200 millions
  - Deaths: 14-15,000 annually

http://www.parasitecleanse.com/gallery.htm
http://www.who.int/topics/en/

Niclosamide
- Niclosamidum

5-Chloro-N-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide

- Used since the ‘60th.
- Does not absorb from the GI tract.
- Very effective against all kind of enteral cestoda infections.
- Inhibits the oxidative phosphorylation of the cestodes

Niclosamide

Schistosomiasis

http://www.parasitecleanse.com/gallery.htm
http://www.who.int/topics/en/

Agents against nematodes (roundworms)

- Piperazine and its salts – obsolete, but still used against pinworm (Oxyuris vermicularis) and roundworm (Ascaris lumbricoides).
- Diethylcarbamazepine and its salts – Far more effective than its parent compound. Used against ascariasis and especially filariasis and onchocerciasis.

Diethylcarbamazepine
Broad spectrum anthelmintic drug widely used to control internal parasites in livestock. In human medicine as an anthelmintic in a single dose form at 2.5 mg/kg.

Benzimidazol derivatives

Synthesis of mebendazole:

Mode of action of benzimidazoles:

Inhibition of formation of microtubules through binding to β-tubulin monomers and preventing polymerization.

Lymphatic filariasis or elephantiasis

Caused by thread-like worms of genus *Wuchereria bancrofti* and *Brugia malayi*, known as filariae, that lodge in the nodes and vessels of the lymphatic system → lymphoedema of the limbs, swelling of the scrotum and penis, vulva and breasts, etc. → bacterial and fungal "superinfection" of tissues with compromised lymphatic function.

- Cases: ~120 millions (1/3 India, 1/3 Africa).
- Seriously ill: ~40 millions.
- Global Programme to Eliminate Lymphatic Filariasis (GPELF) of the WHO has made rapid progress, reaching almost half of the estimated global population at risk (~1200 millions) by the end of 2005 with mass drug administration (MDA).
Onchocerciasis (river blindness)

- Onchocerciasis - caused by the parasite *Onchocerca volvulus* and transmitted by blackflies *Simulium damnosum* (close to the fast-running streams and rivers in the inter-tropical zones).
- Adult worms live in nodules, microfilariae migrate to the subepidermal layer of the skin (flies' bites) and the eye leading to eye lesions, inflammations and blindness.
- About half a million blind people mainly in Africa.

To eliminate microfilariae from the blood of infected individuals so that transmission of the infection by the vector insect can be interrupted:
- Filarial: Albendazole + diethylcarbamazepin or ivermectin + carbamazepin (once yearly for the whole population!)
- Onchocerciasis: earlier suramin or diethylcarbamazepin, recently ivermectine.

Ivermectin

- *Streptomyces avermitilis* ssp.: milbemycin and avermectin family of antibiotics (Merck)
- Agonist of glutamate-controlled periferal, neuromuscular post synaptic chloride channels → long-lasting depolarization, paralysis.

Ectoparasites

- Scabies – skin infestation with the arthropod mite, *Sarcoptes scabiei* causing severe pruritis (“itchy mite”) → skin damage → portal of entry for pathogenic bacteria.
- Old remedies: sulfur compounds, soaps, oils etc.
- Lindane: contact poison, insecticide. Used in locally in cremes etc.
- Benzyl benzoate: natural constituent of Peru balsam.
- Dimethyl phthalate: repellent.
Three types of sucking lice are important for human health: Pediculus humanus capitis (head louse), P. humanus humanus (body louse) and Pthirus pubis (crab louse).

Body lice survive in the clothing and is also the vector (transmitters) of epidemic typhus.

Synthetic pyrethroids disrupt sodium channels of nerve cell membranes.

Pyrethrum

Chrysanthemum cinerariaefolium (Tanacetum cinerariaefolium), pyrethrum, Dalmatian chrysanthemum