Camptothecin

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What is Camptothecin?

- **Camptothecin (CPT)**
- Used to treat CANCER
- Inhibits DNA enzyme topoisomerase I.
Chinese doctors have used *Camptotheca acuminata* for hundreds to thousands of years in treating...

- colds
- psoriasis
- cancers
- leukemia of liver, gallbladder, spleen, and stomach
Camptotheca acuminata

“Happy Tree”/“Tree of Life”
In Mandarin - xi shu
Camptothecin was discovered in 1966 by M.E. Wall & M.C. Wani in a systematic screening of natural products for anti-cancer drugs.

Comes from the Chinese tree, *Camptotheca acuminata* (a.k.a. Happy Tree or Tree of Life).
described camptothecin as a “novel alkaloidal leukemia & tumor inhibitor”
Camptothecin can be found in

- Barks
- Fruits
- Roots
- Leaves of *Camptotheca acuminata*

(Has also been found in fungi that live under the bark)
Other Sources?

After the initial discovery in China, a family of trees in Western India have also been found to produce Camptothecin.

Nothapodytes nimmoniana & Nothapodytes foetida
The harvesting of this plant for the pharmaceutical industry has decimated the population of this once numerous tree.

This tree is now considered as “endangered” by the Chinese government.

Estimated that less than 4,000 of the trees remain in the wild in China.
Extraction/Isolation

- **Hairy root cultures of *C. acuminata***. The hairy roots produce and secrete CPT as well as the more potent and less toxic natural derivative, 10-hydroxycamptothecin (HCPT). Up to 1.0 and 0.15 mg/g dry weight for CPT and the HCPT, respectively.

- **A micro-assay**. This assay utilizes thin-layer chromatography in conjunction with fluorescence imaging to obtain reproducible measurements in the nanogram range.

- **With *N. foetida***, it comprises of drying, grinding and hot defatting of the twigs and stems with a sequential hot extraction with two solvents. Removal of solvents under vacuum followed by a precipitation and filtration of crude extracts that give camptothecin with up to a 0.15% yield.
CANCER

All cancer cells never stop dividing.

Topoisomerase are enzymes that wind & unwind DNA in order to facilitate DNA replication.
DNA Structure

• The double-stranded DNA is extremely intertwined.

• It requires the 2 strands to be untwisted in order to access the genetic information (transcription or replication)
How it Kills Cancer Cells

- Previous cancer drugs target topoisomerase II, which is present at the same levels in both healthy and cancer cells.

- Camptothecin targets topoisomerase I, which is over-expressed in cancer cells (high levels).
  - Higher levels because of the growth rate difference.
  - Making camptothecin more selective for cancer cells and a more effective drug.
Topoisomerase I

- Binds to DNA & cuts the phosphate backbone.
- This intermediate break allows the DNA to be untangled or unwound.
- DNA is then reconnected at the end of this process.
Camptothecin binds neither to topoisomerase I alone or nor DNA alone.

Binds only to the complex formed by topoisomerase I when it cleaves DNA.

Instead of blocking the binding of these two macromolecules, CPT prevents the dissociation.
Mechanism of Action

- CPT binds to DNA topoisomerase I through H-bonding to inhibit its activity and transform it into a “poison”
- Chiral Carbon: (S)
- (R) is inactive
Cell Death

- CPT binds to the Top1-DNA covalent complex, leaving one of the DNA strands cleaved.
- The replication fork collides with the cleavage complex, resulting in double strand break.
Camptothecin includes:

- Planar pentacyclic ring structure (most important factor in topoisomerase inhibition)
  - Includes:
    - Pyrrolo[3,4-β]-quinoline moiety (rings A, B and C)
    - Conjugated pyridone moiety (ring D)
    - One chiral center at position 20 within the alpha-hydroxy lactone ring with (S) configuration (the E-ring)
Mass Spectrometry
Carbon NMR
Side Effects

- The severity depends on the number of doses taken.

- Common effects
  - constipation
  - diarrhea
  - fatigue
  - hair loss
  - loss of appetite
  - nausea
  - stomach pain
  - tiredness
  - vomiting

- Other effects include fever, weight loss, short of breath, insomnia, cough, headache, skin rash, mouth sores, heartburn, & swelling of feet.
Additional Side Effects

- **Loss of red & white blood cells** causes half of the side effects, which occurs after treatment.

- **Extreme diarrhea** is common in those treated with camptothecin – because camptothecin doesn’t dissolve in water.

- After 24 hours, patients become dehydrated, and electrolytes, or solutions of acids, base, and salts in the body become imbalanced.
Analogos & New Uses

Sam Richards
While CPT is excessively potent at inhibiting TopI, it still has a high degree of cytotoxicity. Kills so many cells because...

- Lipophilicity - The drug lacks enough areas where hydrolysis can occur and thus remains in the human body killing healthy cells in the process.
- A high degree of lipophilicity means the drug cannot be excreted through the urine.
Camptothecin & Derivative Structures

Camptothecin

Topotecan

Irinotecan
Methyl, Ethyl, Futil...

- **Topotecan**
  - p 7 increases water solubility & decreases toxicity
  - p 10 increases activity dramatically

- **Irinotecan**
  - p 7 greatly increases activity adds some lipophilicity
  - p 10 increases water solubility
Issues Associated with CPT Derivatives

pH mediates between active & inactive forms of drug

ACTIVE FORM
lactone

INACTIVE FORM
carboxylate
New Uses

- From a study in *Journal of Food and Agriculture*...

- **ANTIFUNGAL ACTIVITY**

- CPT exhibits strong activity against fungi.

- While more common (flavinoids, trifolin and hyperoside) require a much higher concentration to be effective.
Antifungal Activity? How??

- Fungi, Plants, & Human DNA barely differ in sequencing.

- Each utilize TopI in the synthesis of new DNA strands, & thus the utility of CPT becomes apparent for anti-fungal activity.

- Some plants are immune to CPT’s inhibiting effects.

- More research is necessary for suppressing fungi growth in humans & plants.
any questions?
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