Vincristine archive file

Vincristine is a vinakaloid, which was first discovered 1958 in the tropical plant Catharanthus roseus, native in Madagascar. Its ability to inhibit the metaphase of the mitosis by suppressing the polymerization of the microtubule makes it very important as a chemotherapeutic agent, especially against non-Hodgkin lymphomas and Wilms' tumor.

History:
Vinblastine and Vincristine are bisindole alkaloids and both widely known for their use as antitumor drugs. In former times they were isolated in trace quantities from the leaves of Catharanthus roseus. Because of their importance in the medical field numerous researches were examining the structure, use and synthesis of Vinblastine, Vincristine and their derivates. Their biological effect to inhibit the microtubule formation and mitosis was and still is a very important part of medical cancer therapy. They were both among the first natural products whose structures were identified by X-ray crystallography and among the first for which X-ray analysis of a heavy atom derivative was used to establish their absolute configuration. ($\Rightarrow 2$)

Timeline:
1958 Vinblastine was first discovered as an unexpected myelosuppressive agent by Noble, R. L., C. T. Beer, and J. H. Cuts during the search for an antidiabetic agent in Catharanthus roseus (myelosuppression $\Rightarrow$ decreased activity of the bone marrow)

1959 independently researchers from Eli Lilly Johnson, I. S., J. G. Armstrong, M. Gorman, and J. P. Burnett, Jr., discovered that the extracts of C. roseus Possesses activity against P-1534 leukemia in mice and they isolated Vinblastine as its active entity

1963 July, Vincristine was approved by the United States Food and Drug Administration as Oncovin

1965 the structure of a related compound, Vincristine methiodide, was identified by an X-ray crystallography

$\Rightarrow$ Finally structural studies on Vincristine and Vinblastine revealed that both are bisindole alkaloids, containing Vindoline attached to a tetra cyclic indole, cabemethoxyvelbanamine

Synthesis of Vincristine:

There are a lot of different, but very complex ways to synthesize Vincristine.
The partial synthesis of Vincristine contains the dehydrated coupling of Catharanthine with N-Demethylvindoline. Catharanthine as well as Vindoline are natural alkaloids found in catharanthus roseus. They occur in a larger amount, in comparison to vincristine or vinblastine. In the past, the synthesis of Vincristine contained the coupling of Catharanthine-N-Oxid with Vindoline which leded to Vinblastine. The derivatization of Vinblastine ($\Rightarrow$ by oxidation of the N-methyl group) results in Vincristine:
Later (2004) a stereo controlled total synthesis of vincristine was accomplished “through the coupling of demethylvindoline and the eleven-membered carbomethoxyverbanamine precursor. Demethylvindoline was prepared by oxidation of 17-hydroxy-11-methoxytabersonine, followed by regioselective acetylation with mixed anhydride method.”

Abb. 4 Synthesis of demethylvindoline formamide
Current established synthesis of vincristine (2009):

N-Demethylvindoline and Catharanthine together with Tartaric acid and Iron(3) oxalate react first with FeCl₃, HCL, F₃CCH₂OH, H₂O, second with O₂, H₂O, third with NaBH₄, H₂O and fourth with NH₄OH and H₂O to Vincaleukoblastine, 1 demethyl- 4’α [22%] and Vincaleukoblastine, 1-demethyl-4’deoxy- 4’α [9 %] and vincaleukoblastine, 3’,4’-didehydro-1-demethyl-4’-deoxy- [6%] and Vincaleukoblastine, 1-demethyl- [42 %]. Vincaleukoblastine, 1-demethyl-, and Formic acid react with Ac₂O, HCO₂H to Vincaleukoblastine, 22-oxo (= Vincristine)
Absolute stereochemistry.

Rotation (−).

Absolute stereochemistry.

Reagent (Step 1.2)

1.1 \( R: FeCl_3 \), \( R: HCl \), \( S: F_3CCH_2OH \), \( S: H_2O \), 25°C;
2 h, 25°C

1.2 \( R: O_2 \), \( S: H_2O \), 10 min, 0°C

1.3 \( R: NaBH_4 \), \( S: H_2O \), 30 min, 0°C

1.4 \( R: NH_4OH \), \( S: H_2O \), 0°C

Absolute stereochemistry.

Rotation (+).

Absolute stereochemistry.

Rotation (+).

Absolute stereochemistry.

Rotation (+).

NOTE: regioselective, stereoselective, intermediate from stage 1 added in stage 3.
Reactants: 2, Reagents: 6, Solvents: 2,
Steps: 1, Stages: 4
Since the biosynthetic pathway in C. roseus is very complex and the biosynthesis of Vincristine involves at least 35 intermediates, 30 biosynthetic and 2 regulatory genes and 30 enzymes and because wide parts are still unknown there is no biotechnological synthesis so far.

**Mechanism of action:**

The first thing to understand the mechanism of action is to consider the property of cancer cells. The cancer cell is a fast growing and dividing cell type which spreads into healthy cell tissue and destroys it. To stop the division of those cells cause an improvement of controlling the cancer decease. Vincristine (VCR) turns the growing advantage against the cancer cell itself. During the mitosis vincristine binds to specific regions in the cells inside, which are necessary to make a
cell division. When it has bond the cell is not able to make a division and the cell will die by apoptosis. The problem of vincristine is that it is not specific to cancer cells and interferes with healthy non-cancer cells too. When it is used in chemotherapy a lot of side effects can occur.

To understand the mechanism of action it is important to be aware of the molecule structures which are involved during the cell-division, the mitosis. In all eukaryotic cells there's one structural macromolecule which is important for a lot of essential function. These so called microtubules give stability to the cytoskeleton and move the chromosomes through the cell during the mitosis they are also involved in protein moving mechanisms. Microtubules are polymers of α-tubulin and β-tubulin. Tubulin is one member of a small family of globular proteins which got their name by their “globe-like” shape. This type of proteins are well known for the so called globin fold. The three-dimensional globin fold is formed by eight β-helices and is classified as an all-alpha protein fold. This structural domain usually folds in angles of fifty degrees. The angle can vary depending on the amino acids in the primary structure, their sterics and hydrophobic interactions with their side chains.

An other important characteristic of the globular proteins (spheroproteins) is that they are normally well soluble in aqueous solutions. To gain this solubility and be able to form dipol-dipol interactions the primary protein structure has got a special amino acid setup. Apolar, hydrophobic amino acids are bounded towards the molecules inside and the polar, hydrophobic ones are bound outwards.

To form microtubules in the cell the α-tubulin and β-tubulin which have an approximate molecular weight of 55kiloDalton (kD) are connected to a helical structure with alternating tubulin types of α-tubulin and β-tubulin dimers. The microtubules have a diameter of 25nm an a length from 25nm up to 200nm. To create a microtubule the tubulin dimers polymerize to this imperfect helical structure with 13 tubulin dimers packed in one turn of the helix.

Microtubules are nucleated in microtubule organization centers (MTOCs) in the cell. During the mitosis the mitotic
spindle is also involved to segregate the chromosomes. The important structural part here is the Centrosome which controls the length, number distribution and polarity. It is the major microtubule organization center in most eukaryote cells. The polarity of the microtubule is one important feature. The α subunit of one tubulin dimer is connected to the β subunit of an other dimer. The β ends are termed as (+) and the α ends as (−). The exposed (−) end is capped so the elongation process is performed only on the exposed (+) end. To get a closer view on the mechanism of action of vincristine it is important to look closely at the elongation process of microtubule polymerization. To perform their function microtubules do a process called dynamic instability. Which means that the tubulin dimers can be attached or replaced at the end of the microtubule structure. Tubulin structures are able to bind and hydrolize nucleoside triphosphates. In this case they use GTP instead of ATP. A new polymerized microtubule consist only of GTP tubulin bond dimers. After the microtubule has reached its final length at the (+) end of the microtubule the GTP can be hydrolized to GDP. The GDP tubulin dimer bond is less stable. In some cases the (+)-end of the microtubule is capped with GTP-tubulin-dimers to stabilize the microtubule. This capping mechanism is also used to stop a depolarizing microtubule from shrinking. This is called rescue. When the length of the microtubule begins to shrink and in case of the mitotic spindle it is a reason why the chromosomes during mitosis gets separated, the microtubule splits of GDP-tubulin dimers. To perform this function other proteins like kinesin (moves towards (+)-end) and dynein (moves towards (−)-end) help to provide this process. They are able to generate forces and have got their role during chromosome-movement and other movement processes in the cell. Other co-factors interfere with the microtubules too. For example a protein called MAD2 helps to bind the microtubules to the chromosomes. During movement processes in the cell these proteins have got other special function. When a microtubule switches from elongation to shrinking it is called catastrophe. 

Vinca alkaloids like vincristine as mitosis inhibitors can bind to the tubulin- GTP-complex and inhibits the hydrolization of GTP-dimers into GDP-dimers. The microtubules does not shrink any more an the cell will rest in the M-phase (metaphase) of the cell-cycle. When this happens the cell will die of apoptosis. The inhibition of microtubule depolarization is one of the most efficient function provided by anti-cancer pharmaceuticals. At the moment the vinca alkaloids are the most effective drugs in the fight against cancer. As mentioned earlier the problem of this type of chemicals is that they are highly effective on fast dividing cells as the cancer cell, but the drug is not specific. So side effects are still a problem. In the future an other groups of cellular proteins could be interesting too.
Kinetochores polymers from the mitotic spindle which also helps to attach the chromosome to the microtubule, could be another mechanism to look at closer. To generate the most efficient drug a high specificity to the cancer cell itself is necessary.

**Usage and side effect:**

Vincristine is commonly used for the treatment of two types of cancer, the Hodgkin's Lymphoma and Wilm's Tumor. Hodgkin's Lymphoma is a type of cancer attacking the lymph system, which is spread out all over the body. The disease can be recognised by the swelling of lymph knots all over the body, in addition there may be so called B-symptoms (e.g. Pyrexia). Characteristically so called Sternberg reed cells are found in the microscopical tissue image. The chances for curing the disease is good till very good, especially if children are concerned.

Abb. 1: Lymphocyte. These leukocytes could be struck by an Non-Hodkin lymphoma.

Wilm's Tumor is placed in the kidney and mostly affecting children around the age of three.
If the nephroblastome forms metastasis, usually the lung is affected. It contains of several different types of tissue and is in the most cases aggressive.

[Image: http://medsavailable.com/files/kidney_cancer_program_2_0.jpg]

In chemotherapy a single agent is rarely used alone. This includes the treatment of both types with Vincristine. For the treatment of Hodkin's Lymphom a special combination of agents called CHOP is used. The name is a shortform containing the starting letters of each agent, or its brand name, in the case of Vincristine this is Onconvin.

Chemotherapy is often combined because it increases the efficiency, by not changing the grade of the side effects. This, obviously, has a great impact on the quality of life of the patient. Vincristine is used in this polychemotherapy because of its lack of bone-marrow suppression and because of its unique toxicity, which then can not add up with other agents side effects. Vincristine is not usable in the spinal cord because it can not penetrate the membrane around it.

The agent is given intravenously and its terminal half-life in the body adds up to about 85 hours. The main part of the dose is processed in the liver. The substance is metabolized by Cytochrome P450 isoenzymes in the CYP3A subfamily. About 80% of an injected dose appears in the dejection, 10% – 20% can be found in the urine. About 15–30 minutes after the injection, 90% of the drug distributed from the blood into tissue, where it stays tightly but not irreversibly bound. A Cytochrome P450 isoenzyme

The main side effects of Vincristine are peripheral neuropathy,
Hyponatremia and hair loss.

Peripheral neuropathy is describing an illness that affects the peripheral nerves of the body. Peripheral nerves are those who connect organs and limbs to the central nerve system (brain and spinal cord). The myelin sheath can be damaged which affects the senses and the motorical abilities also the connection between Axon and the neuronal cell nucleus. This can lead to numbness, tremor, and gait abnormality. It can also be very painful.

Hyponatremia is the common term for the disturbance of the sodium concentration in the extra cellular serum. Sodium is the dominant extracellular cation and if its level sinks this can lead to nausea, headache, vomiting and cramps or even spasms.

Sources:

11. http://www.staff.ncl.ac.uk/i.r.hardcastle/antibiotics.html, 20.06.2010

The chemotherapeutic agent Vincristine is most
Abb. 1 + Abb. 3 + 2α Total Synthesis of Vinblastine, Vincristine, Related Natural Products and Key Structural Analogues
Hayato Ishikawa, David A. Colby, Shigeki Seto, Porino Va, Annie Tam, Hiroyuki Kakei, Thomas J. Rayl, Inkyu Hwang, and Dale L. Boger*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037
4904 J. AM. CHEM. SOC. 2009, 131, 4904–4916,
10.1021/ja809842b CCC: $40.75 α 2009 American Chemical Society)

Abb. 2 + Abb. 4 +1 (line 1–6) α Stereo controlled total synthesis of ( )-Vincristine
Takeshi Kuboyama*, Satoshi Yokoshima*, Hidetoshi Tokuyama*†, and Tohru Fukuyama*‡

*Graduate School of Pharmaceutical Sciences, University of Tokyo, 7–3–1 Hongo, Bunkyo–ku, Tokyo 113-033, Japan; and †Precursory Research for Embryonic Science and Technology, Japan Science and Technology Agency, 7–3–1 Hongo, Bunkyo–ku, Tokyo 113-0033, Japan

Abb. 6, 7, 8: (Copyright © 2010 ACS. In addition to reactions indexed by CAS, CASREACT contains reactions derived from the following sources: ZIC/VINITI database (1974-1999) provided by InfoChem, INPI data prior to 1986, and Biotransformation database compiled under the direction of Professor Dr. Klaus Kieslich.)

Abb. 9: www.germanlipa.de
Abb. 10: α p. Schönfelder

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