Ancient medical texts: a valuable source of knowledge for drug discovery

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Egon Stahl Award Lecture
Geneva 17 August 2009
Pharmacognosy

- Pharmacon + gnosis
- Φάρμακον + γνώσις
- Knowledge of drugs
- Discrimination between therapeutic and toxic plants
- Historically, this knowledge was a result of “trials” on humans
Natural Pharmacopoeia

- The therapeutic use of plants and natural drugs has been systematically exploited for thousands of years all over the world.

- In the western civilization, the most important source of knowledge about the therapeutic use of plants comes from the ancient doctors of the classic Greek and Roman period that created the base of the pharmacopoeia in Europe up to the 16th century.

Dioscorides
Centuries of medical experience on human beings (not animals), described in astonishing details by Hippocrates, Dioscorides, Galenus, Theophrastus, Aetius, Oreibasios, Nikolaos Myrepsos etc have been recorded in thousands of pages including:

- Botanical description
- Mode of extraction
- Dosology
- Disease

More than two thousands of recipes related with more than 1000 taxa have been reported for a great variety of diseases.
Electronic revolution

Thousands pages of knowledge from all the ancient doctors up to the Middle ages are now accessible in electronic form through TLG.
The Thesaurus Linguae Graecae (TLG) Project started in 1972 (University of California, Irvine). Electronic access to the full text of all the ancient Greek literature became available. In 2001, the TLG-team developed a search engine and made the corpus available online. Today, the Online TLG contains more than 100 million words from 9,958 works from the time of Homer up to 1453 AC associated with 2,314 authors and is constantly updated and improved. TLG A was the first compact disk that did not contain music.
Problems

- Language (translation, obscure terms)
- Medical Terminology
- Botanical names
- Complex multidrug prescriptions
- Lost practical experience
It is extremely astonishing that a great number of plants mentioned repetitively from several ancient authors for a period of more than 1500 years have never been studied towards the correct disease and with the proposed mode of preparation.

The systematic, cross checked study of ancient recipes can lead to surprising discoveries.

The success is hidden in the details: plant part, mode of extraction, correct interpretation of disease.

A new concept: ARCHAEOPHARMACOLOGY

Instead of looking at plants from exotic origin or organisms from the deep oceans we should first study the organisms mentioned in medical texts.
Priority criteria for selection of plants

Strategy:
- Collection of plants (>1000)
- Dereplication
- Isolation
- Pharmacological evaluation
- Molecular modelling
- Synthesis of derivatives

*DIOS database for in silico screening
Successful examples

- Paeonia
- Sesame
- Mastic
- Olive - olive oil
- Tyrian purple - Indirubin
Dioscorides in his work De materia medica states that the seeds of Paeonia could be used in wine to calm pains of the womb (uterus) = female genital disorders.

Although there are numerous phytochemical studies on the roots of Asian Paeonia spp, the seeds of European spp. had never been studied or correlated with gynecological problems.
Scientific proof of ancient use

- 50 g of seeds contain
  - trans-resveratrol (100 mg)
  - gnetin-H (550 mg)
  - trans-ε-viniferin (170 mg)
- Amount of resveratrol equal to 100-300 glasses of red wine
- Total resveratrol in Red Wines = 0.30 - 1.07 mg/150mL
- Resveratrol has established estrogen-modulatory effects (Cancer Res. 2001;61(20):7456-63)
- The conclusion is that the ancient text indeed contained important and specific information that nobody had ever studied
Paeonia root-anticonvulsant activity

Phytochemical study of Paeonia spp roots described by Dioscorides and Aetius and in vivo anticonvulsant activity (never tested)

| Table 1: Results of the tested Paeonia extracts at dose of 100 mg/Kg in the 6 Hz test using 32 mA and 22 mA |
|-------------------------------------------------|---------------|---------------|---------------|---------------|---------------|
| Time (h) | 0.25 | 0.5 | 1 | 2 | 4 |
| Tested extract | Intensity | N/F | N/F | N/F | N/F | N/F |
| P. pannassica water extract | 32 mA | 2/4 | 0/4 | 0/4 | 1/4 | 0/4 |
| P. pannassica MeOH extract | 22 mA | 1/4 | 2/4 | 2/4 | 2/4 | - |
| P. pannassica MeOH extract | 32 mA | 1/4 | 1/4 | 1/4 | 1/4 | 0/4 |

Hippocrates describes the action of the whole seed on female genital problems (in combination with flaxseed) and for the secretion of milk during breastfeeding.

The hull (seed coat) of the sesame seed had never been studied and never correlated with the female hormone system.

Phytochemical study of sesame seed coat
In vivo studies

- 10 times more rich in lignans than the dehulled seed
- The lignan extract of sesame hull in mice showed estrogenic activity
Mastic

- Galen (22 volumes and over 20,000 pages in length):
- Among the resins he states that mastic is the most powerful for the treatment of stomach inflammation
- Only from the island of Chios (Pistacia lentiscus var.chia)
Phytochemical study of mastic resin

Isomasticadienonal NEW
**Phytochemical study of mastic resin, mastic oil, mastic water (poster)**

**Isolation of triterpenic acids**

**In vitro- in vivo studies**

- Although mastic does not eradicate H. pylori as initially believed (Huwez et al., N Engl J Med 339(26):1946, 1998) it reduces in vivo (in mice) the colonization of H. pylori and the grade of inflammation as originally stated in the ancient texts!!

- The activity is attributed to the acid fraction and to specific triterpenic acids.
Olive- olive oil

- Dioscorides and after him all the ancient doctors insist that the best health effects come from the fresh olive oil from unripe olives and especially from the wild variety (Olea europaea, var. sylvestris)
- Numerous applications are reported including headache, toothache
- Obvious indications of antinflammatory activity

Olive harvest. Pot of the 6th century BC.
Oleocanthal

- Oleocanthal possesses antiinflammatory activity similar to Ibuprofen
- Following the ancient guidelines we have recently identified olive oil varieties with high concentration of oleocanthal

Oleuropein

- The bitter principle of olive leaves and fruits
- The ancient texts mention the use of the olive leaf decoction and the brine of the table olives
- Phytochemical study
- Protects from myocardial infarction and lowers cholesterol

Ten years ago, indirubin was identified as the main active ingredient of a traditional Chinese medicinal recipe, Danggui Longhui Wan, used successfully to treat chronic myelocytic leukemia (CML). The activity of indirubin against CML was confirmed in clinical trials performed in China.

The action of indirubin was first identified to be mediated through the inhibition of cyclin-dependent kinases (CDKs) but it was also quite potent on glycogen synthase kinase (GSK-3) inhibition.
Based on the importance of CDKs or GSK-3 inhibition in the treatment of cancer or several other serious diseases, in our laboratory in the Univ. of Athens we decided to start a research for new natural indirubin derivatives as kinase inhibitors.
Natural sources of indirubins

- Indirubins have been found in over 200 species of indigo-producing plants.
- Indirubins, along with indigo, are also produced by various bacterial strains.
- But the most famous source of indirubins is the Gastropod mollusks, of the Muricidae family that have been used as the source of the vivid purplish red dye, known as “Tyrian purple” or “Royal Blue” around the Mediterranean Sea.

Byzantine Emperor Justinian clad in Tyrian purple
The main chemical constituent of the Tyrian dye was discovered by Paul Friedländer in 1909 to be 6,6′-dibromoindigo.

Dioscorides states 2000 years ago that the tyrian dye is similar to indigo!!

Numerous pharmacological activities are reported from all ancient medical texts including Malignant ulcers, sarcomas, hair loss, wound healing, burns, spleen oedema etc.....
Gastropods used for the production of Tyrian purple

1. *Murex brandaris* (synonym *Bolinus brandaris*)
2. *Hexaplex trunculus* (synonym *Phyllonotus* or *Trunculariopsis*)

But Aristotle (*Historia animalium*) clearly states two different types of dye coming from two different species:

1. Κήρυξ = *Ceryx* = herald’s trumpet (Friedländer)
2. Πορφύρα = Purple shellfish (not studied)
Extraction procedure

The snails were removed by hand from the shell

Exposed to sunlight for 1 h

Then the material was lyophilized

and extracted repeatedly with dichloromethane.

The hypobranchial glands and their excretions changed from colorless to green and rapidly to purple.

Crucial information by Aristotle
After several chromatographic separations we isolated four pure compounds with vivid red color (2-3 mg each).

NMR and MS study led to the structure elucidation of each compound.
Final purification

6-bromo-indirubin
First time described as natural product
HPLC analysis of the extracts, using the above isolated authentic samples revealed surprisingly that 6-bromo-indirubin was found only in *H. trunculus* and not in the other Tyrian purple producing gastropod.

“Identification of the Coloring Constituents of Four Indigoid Dyestuffs” Karapanagiotis, I.; V Violaine de Villemereuil; Magiatis, P.; Polychronopoulos, P.; Vougogiannopoulou, K.; Skaltsounis, AL. *J. Liquid Chromatography*, 2006, 29, 1491-1502
A long history

The oldest sample of “Tyrian” purple (17th century BC) was recently identified in frescos from Santorini island before the volcano eruption. The research was performed by a Greek art diagnosis center (Ormylia) and was based on the authentic samples of colorants from *Hexaplex trunculus*.

The complex chemical technology for the production of the purple dye was first discovered by the Aegean civilization and then transferred to Pheonicia.
**Pharmacological evaluation of pure natural compounds**

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<th>Pure compounds</th>
<th>CDK1</th>
<th>CDK5</th>
<th>GSK3β</th>
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<td>indirubin</td>
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*Magiatis, P. et al, Chemistry & Biology, 2003, 10, 1255-1266*
The small quantity of the natural indirubins led us to investigate their total synthesis as well as the synthesis of several derivatives.

Additionally to the natural indirubins we synthesized:

A total of >250 derivatives bearing: Br, Cl, F, I, NO₂, CH₃, CH=CH₂, COOH, CH₂OH, CHO, at positions 4,5,6,7, 5’,6’ in combination with =O, =NOH, =NOAc, =NOCH₃ at position 3’ and with N1-H or N1-CH₃.

The most interesting among all the synthesized derivatives was 6-bromo-indirubin-3'-oxime (6BIO) which combined a high potency (5 nM) 10-100 fold selectivity for GSK-3.
6BIO into the ATP pocket of GSK-3.
Crystallographic studies
Explanation of the selectivity of 6BIO against GSK3 in comparison to CDKs

6-Bromoindirubin 3'-oxime
The solubility problem

- The solubility of BIO in water is about 12 mg/l. This solubility is sufficient for in vitro tests or tests in cellular level but not for in vivo administration.

- **Target:** amelioration of water solubility (minimum 40 mg/l) with retention of low nanomolar activity (100 nM) and high selectivity (10 fold).
Molecular modeling design

There was enough space to attach a hydrophilic side chain on the 3’-oxime group of BIO without affecting the binding sites in the ATP pocket.
SYNTHESIS AND IN VITRO KINASE INHIBITION OF 3'-DERIVATIVES WITH AMINO SIDE CHAINS

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<tr>
<th>ΠΑΡΑΓΟΝΤΟ ΙΝΤΕΡΟΥΜΕΝΗΣ</th>
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Increase of selectivity for GSK-3 by 180%!

Water Solubility = 4.253 g/l!!

- Selectivity index = -log IC50 (GSK-3) / IC50 (CDK1)

APPLICATIONS OF INDIRUBINS FROM OUR LAB AND COLLABORATORS

- Proliferation of Stem Cells: *Nature Medicine*, 2004, 10, 55-6
- Indirubin is one of the most powerful ligands of AhR (dioxin receptor) causing G1 arrest. "Independent actions on cyclin-dependent kinases and aryl hydrocarbon receptor mediate the anti-proliferative effects of indirubins" *Oncogene* 2004, 23, 4400-4412.
- Against Leishmania: *Antimicrobial Agents & Chemotherapy*, 2004, 3033-3042
- 6-Br-5-methyl indirubin -3'-oxime against Leishmania. *Int. J. Parasitology* 2009
- Against human Papilloma virus: *Oncogene*, 2004, 23, 8206-8215
APPLICATIONS FROM LABS ALL OVER THE WORLD

- Replication and survival of pancreatic beta cells: J.Biol. Chem. 2007, 282, 12030-12037
- Proliferation of Mammalian Cardiomyocytes: Chem. Biol. 2006, 13, 957-963
- regulation of melanogenesis, 2008 Cellular Signalling 20 (10), pp. 1750-1761
- Adipocyte differentiation, 2008 BMC Cell Biology
- Downregulation of total tau proteins in cultured neurons 2009, Brain Research, 1252, 66-75
Applications

More than 50 publications exploring the pharmacological applications of 6BIO have appeared during 2004-2009.

More than 300 citations of the two main publications concerning the isolation of natural 6-bromoidirubin, the synthesis of 6BIO and the first bioactivity tests on GSK3.
About five years ago, in the website of Nature appeared a hot article entitled:
“stem-cell secret of youth found”

That article described the action of a small molecule named BIO coming from a “humble” Mediterranean snail on the differentiation of stem cells. This molecule was reported as a highly potent and selective inhibitor of an enzyme named GSK-3 which was found to possess a key role in the procedure of cell differentiation in the Wnt pathway. The addition of this compound in the culture media of the stem cells permitted their proliferation, without losing their pluripotency, something that had never been achieved before.
Pilot scale production of BIO

50 gr 6BIO
6BIO, N-Me-6BIO, 6BIO-acetoxime and 7BIO are now commercially available by SIGMA-ALDRICH, MERCK, ALEXIS etc.

Before many centuries Tyrian purple was as valuable as gold.

Today BIO (20€/mg) and its derivatives are 1000 times more valuable than gold (20€/g).
Indirubin and Malassezia

Surprisingly we found that indirubin is a metabolite of Malassezia spp, a yeast that causes seborrheic dermatitis, pityriasis versicolor and dandruff and lives on the skin of everybody.

Role unknown

Potential implication in development of basal cell skin cancer
University of Athens, Faculty of Pharmacy
ACKNOWLEDGEMENTS

- Prof. Leandros Skaltsounis
- Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, University of Athens, GREECE: Panos Polychronopoulos, Marina Kritsanida, Nantia Vougogiannopoulou, Raphael Grougnet, Georgia Stathopoulou, Sotiris Paraschos
- Laboratory of Pharmaceutical chemistry of the University of Athens: Prof. Emmanuel Mikros and Vassilis Myrianthopoulos
- My wife Dr. Eleni MELLIOU and MY FAMILY
ACKNOWLEDGEMENTS

- Society for Medicinal Plant and Natural Product Research
- Egon-Stahl committee