Two Herbal Extracts for Protecting Prostate Cell DNA

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Abstract

During his 50 years of research, biochemist and molecular biologist Mirko Beljanski, PhD, discovered 2 plant extracts that appeared to inhibit the growth of cancer cells without causing any harmful side effects. The research on these products and the preliminary indications from an ongoing clinical trial are the subject of this article.

In summary, Dr Beljanski made great contributions to our understanding of basic life processes and cancer. He determined that, quite apart from the occurrence of genetic mutations of DNA, carcinogens can bind to and damage the DNA double helix, thus creating a destabilized and dysfunctional structure. Dr Beljanski associated this destabilization of the DNA with excess replication, aberrant gene expression, and increased cell multiplication, which are processes that may ultimately lead to cancer. To determine which substances cause DNA destabilization and thus can be considered carcinogenic, Dr Beljanski developed what he called the Oncotest as a way to determine the effect of a compound on the structure of DNA; compounds that enhanced either UV absorption or the level of in vitro DNA synthesis were considered to have carcinogenic properties.

Through use of the Oncotest, Dr Beljanski also discovered 2 natural molecules from the tropical plants *Pao pereira* and *Rauwolfia vomitoria* that specifically recognized and bound to the damaged double helix, thus preventing the process of cell division. In vitro, these 2 natural extracts have been shown to inhibit the proliferation of a wide variety of cancer cells without affecting healthy cells. Experiments with animals confirmed these results, and preliminary work with humans has provided similar indications. Clinical studies using a combination of the pao and rauwolfia extracts have yielded encouraging preliminary results by reducing prostate-specific antigen levels in men and improving symptoms of benign prostatic hyperplasia.

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Other than skin cancers, prostate cancer is the most common cancer in the United States and is the second-leading cause of cancer death in American men.[1] The American Cancer Society estimates that during 2009 some 192 280 new cases of prostate cancer were diagnosed.[1]

Traditionally, chemotherapy and radiotherapy have not been shown to provide significant survival benefits to patients with advanced prostate cancer, and most treatment options available for advanced prostate carcinoma are palliative.[2] Recent studies on derivatives from the drug taxane, alone and in combination with other chemotherapeutic agents, have demonstrated some limited benefit on hormone-independent prostate cancer,[2] but a need for more effective and less toxic means to target and/or prevent this disease clearly exists.

Natural products may prove to be the answer because they have been a bountiful source of bioactive compounds used to treat a variety of ailments and diseases, including cancer.[3] The taxane derivatives (originally of a natural origin) currently being used for treatment are but 1 example among many of the importance of this resource. With an eye toward the future, natural products and herbal remedies with a specific mechanism of action are crucial for developing safe and efficacious therapies for the prevention and treatment of prostate cancer.

The Role of DNA in Carcinogenesis

Molecular biologists have shaped our ideas about the critical role of DNA in the onset of cancer since the mid-1900s. During that time, DNA had been identified as multiples of 4 nucleotides[4] holding genetic information, which are strung together in long, complementary polymers that wrap around each other forming a double helix.[5] Genetic information—the sequences that code for specific proteins contained within DNA—has long been the focus of research on carcinogenesis. Biologists have contended that the coding regions of 2 special sets of genes (oncogenes and tumor suppressors) are corrupted by mutations and thus alter the function of the corresponding proteins that, through unregulated cellular division, lead to cancer. This is considered the mutational theory of cancer.[6]

Another model, which many deem to be much less likely, focuses on DNA's physical properties, suggesting that the initial event leading to carcinogenesis is the disruption of the DNA double helix. French Biochemist and molecular biologist Mirko Beljanski (1923-1998), who received a doctorate in molecular biology from the Sorbonne in Paris in 1951, worked at the Pasteur Institute for about 30 years. He was fascinated by the theory that carcinogens might interrupt DNA and focused his attention and research to identify the physiological differences between normal DNA and cancer DNA at the structural level by testing the stability of the double helix and measuring the conse-

quences of any permanent changes induced by that difference.

Beljanski's Discovery and the Oncotest

In the 1980s, Dr Beljanski's first discovery was that cancer DNA is different from normal DNA when compared by UV light absorption or melting point. The UV absorption of DNA from cancer cells was consistently higher than results found for DNA from normal cells. Dr Beljanski concluded that the chemical bonds holding the double helix together are reproducibly disrupted in cancer DNA compared with what is found in healthy DNA. He referred to this pattern of relaxation in the DNA of cancer cells as destabilization of cancer DNA, ie, that the hydrogen bonds that hold together the 2 strands forming the double helix are reproducibly disrupted in cancer DNA.[7]

Dr Beljanski reasoned and later confirmed in experiments that the destabilized cancer DNA served as a more active template for enzymatic reactions—causing excess replication and aberrant gene expression.[7,8] He found that replication occurred more quickly from cancer DNA templates than from the more tightly wound duplexes found in normal cells. In vitro, DNA destabilization positively correlated with enhanced DNA synthesis.[9]

Dr Beljanski used these 2 assays, enhanced UV absorption and enhanced level of DNA replication, to identify molecules that have a carcinogenic potential. And thus was born the Oncotest.[7] For this assay, the following biochemicals are assembled in a test tube:

1. template DNA purified from either healthy or cancerous cells,
2. a combination of all 4 nucleotide building blocks that constitute DNA, and
3. an enzyme called DNA polymerase that links nucleotides together to make a new strand of DNA by copying the sequence of nucleotides in the template strand.

Dr Beljanski already knew that the DNA synthesis reaction in the test tube containing the cancer DNA template goes faster than the reaction with normal DNA. He then added test compounds (various carcinogens) to examine their effects on reactions. He found that known carcinogens significantly enhanced the DNA synthesis reaction from the cancer DNA template while having a lesser effect on the reaction with normal DNA. Dr Beljanski thought that the already open structure of the cancer DNA made it more susceptible to the effect of carcinogens, which apparently acted by promoting even more destabilization of the cancer DNA.

Transcending the application of the Oncotest as an assay for carcinogenic substances, Dr Beljanski reasoned that, whereas carcinogens increase unwinding and duplication in cancer DNA, the opposite might be true for anticarcinogenic agents. Dr Beljanski then used the Oncotest to look for compounds that would interact with the cancer DNA and inhibit DNA synthesis. He focused on screening natural substances. Following an analysis of several hundred compounds, Dr Beljanski discovered 2 specific plant extracts that reduced UV absorption and template activity of cancer DNA in the Oncotest: *Pao pereira* (*Geissospermum laeve*) and *Rauwolfia vomitoria*.

The extracts were subjected to a long series of laboratory tests to examine their effect on cultured cancer cells and animal cells contaminated with various kinds of cancer.[10,11] Ultimately, these studies were repeated as cell studies.[12-15] In the laboratory, the herbal extracts stopped the proliferation of cancer cell lines while sparing healthy cells: They were toxic to cancer cells in mice but did no harm to healthy mice. Their actions selectively targeted cancer DNA and cancer cells with no apparent effect on normal DNA behavior in healthy cells.[16-18] No long-term studies have been performed to determine whether any toxicity or significant side effects exist.

The data in Figure 1 represent experimental evidence demonstrating that in vitro PB-100 (*Pao pereira*) and BG-8 (*Rauwolfia*

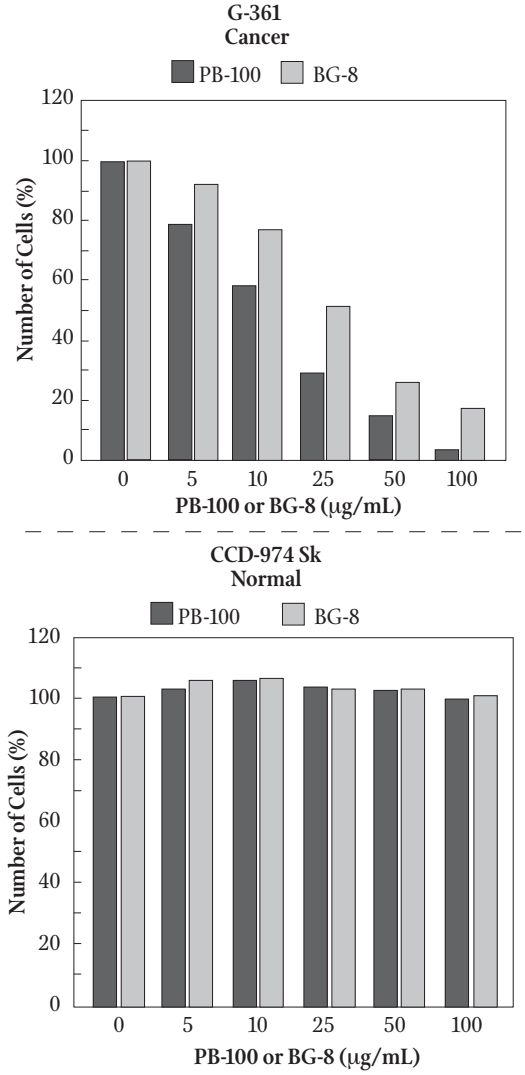

Figure 1. Effect of PB-100 and BG-8 on the multiplication of human melanoma cells and normal human fibroblasts.

Both cell lines were grown in triplicate for 48 h at 37° C in the absence and presence of indicated concentrations of PB-100 and BG-8. Data are an average of 3 separate experiments.

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vomitoria) destroy human g-361 melanoma cells in a dose-dependent manner by inhibiting the multiplication of unhealthy cells, but they do not affect human nonmalignant CCD-974Sk fibroblasts used as controls.

The image shown in Figure 2a reveals that pao remains outside of the healthy cell, unable to penetrate it. And Figure 2b shows pao entering the cancerous cell, localizing in the nuclei of cancer cells (glioblastoma) and the nucleoli. This demonstrates selectivity of action.

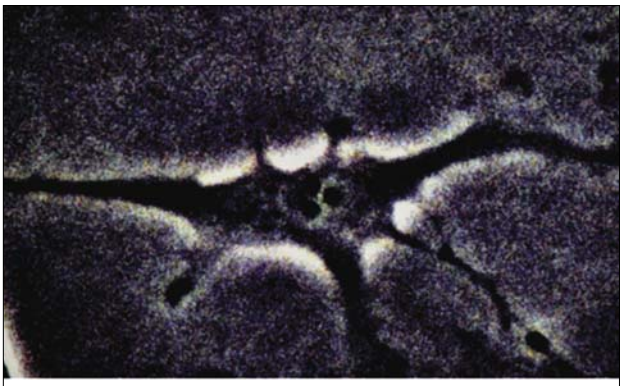

Figure 2a. Naturally fluorescent *Pao pereira* outside a healthy cell (astrocyte), unable to penetrate its nonporous membrane.

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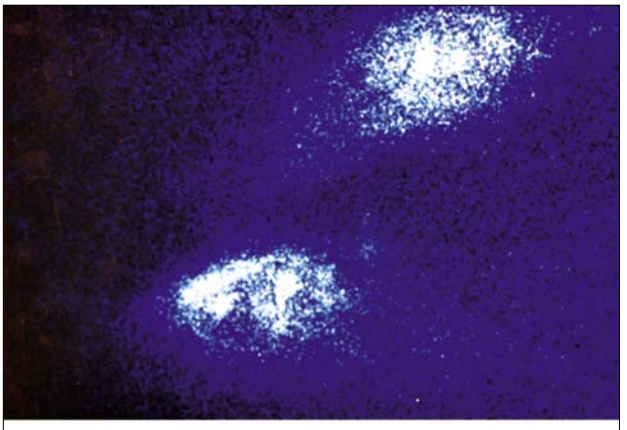

Figure 2b. *Pao pereira* extract seen penetrating the cancerous cell (glioblastoma).

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DNA behavior is a microcosmic reflection of the whole cell's behavior. Inhibiting cancer DNA duplication prevents cancer cell multiplication. This was extensively tested in both normal and cancer cells cultured in vitro in either the presence or absence of the 2 purified plant extracts.[15] Healthy cells were not affected in every scenario. Dr Beljanski showed that carcinogenic compounds (or hormonal compounds) had to compete with the cancer-fighting extracts at both the DNA level and at the cellular level (during multiplication).

These selective cancer-fighting substances have been used concurrently with chemotherapy or radiation therapy by many doctors in Europe to treat numerous cancers. The process of confirming and extending Dr Beljanski's research in the United States is now under way at well-known institutions. This work, involving both preclinical and clinical studies, has focused initially on protection of prostate health.

Extracts Being Studied in the United States

Aaron Katz, MD, is a nationally recognized urology surgeon, researcher, author, and director of a center for holistic urology in the United States (for an interview with Dr Katz, please see "The Center for Holistic Urology, an Inside Look Where Research Meets Practice" *IMCJ.* 2006;5.4:46-49). His group has developed scientific protocols to take Dr Beljanski's body of work and study it for efficacy. He has worked closely with Debra Bemis, PhD, who also specializes in urology. Katz's group is focusing on complementary treatment modalities for men experiencing both benign and malignant prostate problems. Their goal is to identify extracts and natural products amalgams that would benefit prostate and bladder cancers. Dr Katz and Dr Bemis believe that *Pao pereira* and *Rauwolfia vomitoria* are extremely promising.[16]

Initially, Katz and Bemis tested the 2 extracts in cell culture models of prostate cancer using an androgen-sensitive prostate cancer cell line. They observed that both extracts inhibited prostate cancer cell growth in tissue culture. In an interview, Dr Bemis explained significant findings from the preclinical trial: "Interestingly, the pao extract more potently induced cell death (apoptosis) than the rauwolfia extract. However, the rauwolfia extract more specifically inhibited cell cycle progression of the prostate cancer cells, hence suppressing their ability to grow and divide."[17]

The team then went on to test both of the extracts in a mouse model of prostate cancer, which involved implanting human prostate tumor cells into mice (tumor xenografts model). The test mice were fed the individual extracts for 6 weeks, and their tumors were compared with control mice that did not receive the extracts. Both the pao and rauwolfia extracts reduced the overall tumor volume in the test mice; when compared with controls, the results were statistically significant.[18]

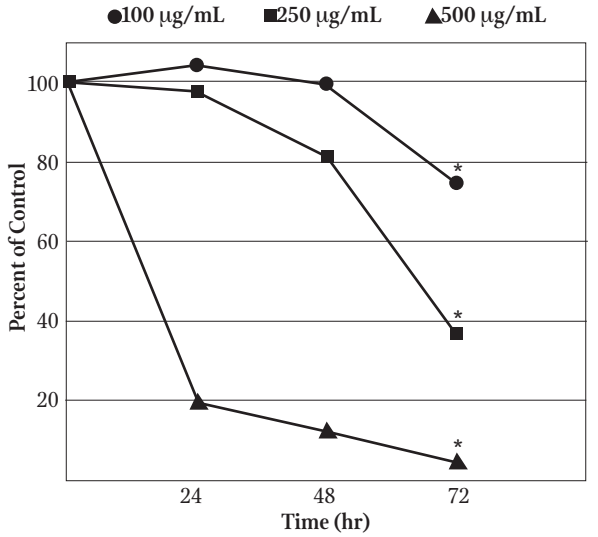
As shown in Figure 3, rauwolfia extract inhibited cell growth in LNCaP cells (a line of human cells commonly used in the field of oncology). The cells treated with rauwolfia extract demonstrated a dose-dependent growth inhibition over a 72-hour period. The highest concentration tested, 500 µg/mL, elicited a marked reduction in cell growth from 24 hours onward.

To further investigate the effects of the extract on cell growth, Figure 4 shows a cell cycle analysis revealing that the rauwolfia extract significantly impeded G1 to GS phase progression ($P<.0005$, Student's t test).

To assess whether the rauwolfia extract induces apoptosis, the amount of subgenomic (Sub-Go) DNA present was quantified by flow cytometric analysis following propidium iodide staining. As shown in Figure 5, this study confirmed the increase in cell death following treatment with 500 µg/mL rauwolfia; when compared with the control cells, the results were statistically significant ($P<.0005$; Student's t test).

The Phase 1 Clinical Trial

These preclinical results prompted Dr Katz and his group to organize a clinical trial using a combination of pao and rauwolfia



*$P \leq .0001$ compared with control cells using the Student's *t* test.

Figure 3. Rauwolfia extract reduces LNCaP cell growth over 72 hours.

Following 24-, 48-, or 72-h incubation with the rauwolfia extract (100, 250, and 500 μg/mL), cells grown in a 96-well format were pulsed for 3 h with WST-1 reagent and absorbances were measured at 450 nM. Values are expressed as means ± SEM (n=8).

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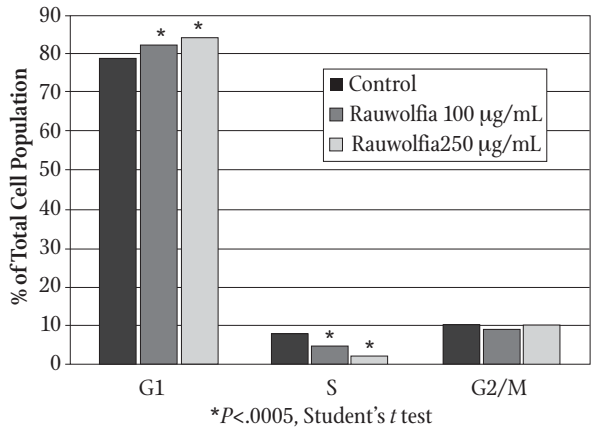

*$P < .0005$, Student's *t* test

Figure 4. Effects of rauwolfia extract on a cell cycle progression.

LNCaP cells were exposed to 100 and 250 μg/mL rauwolfia extract for 24 h. Floating and adherent cells were then collected and fixed in a 2:1 ratio (v/v) chilled ethanol for 2 h before staining with propidium iodide in the presence of RNAse. Cells were then analyzed by flow cytometry. Data analysis was performed using CellQuest PRO software. Percent of cells from treated populations that partitioned out into each cell cycle phase were compared with control populations using the Student *t* test. Each condition was repeated in triplicate.

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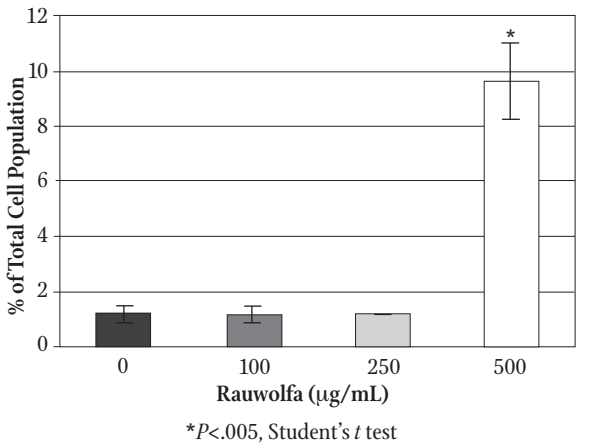

*$P < .005$, Student's *t* test

Figure 5. Rauwolfia extract induced apoptosis in LNCaP cells at the highest concentration tested (500 μg/mL).

Following treatment of LNCaP with the extract, cells were fixed and stained with propodium iodide. Cells were then analyzed by flow cytometry to determine the percentage of the cell population containing sub-genomic DNA. Data analysis was performed using CellQuest PRO software. Each condition was repeated in triplicate and compared to control populations using the Student *t* test.

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for men at high risk for developing prostate cancer. These are men with elevated levels of prostate specific antigen (>2.5 ng/dL) but no clinical signs of prostate cancer (based on a negative biopsy report). Nationally, a very large number of men fit this description.[19] Since both of the extracts reduced overall tumor volume in mice, the data suggest that a combination of *Pao pereira* and *Rauwolfia vomitoria* might be especially effective at inhibiting the ability of precancerous prostate cells to grow and divide.

The researchers enrolled some 30 patients with elevated PSA readings and a negative biopsy. The total number of patients was divided into 7 cohorts of 3. The first group was given 3 capsules/d for 2 months; at this point, if no adverse reactions were registered, a second group was given 3 capsules/d for 2 months. The last group was given 8 capsules/d.

This dose escalation helps determine the optimal dosage in terms of safety and maximum efficiency. Each cohort took the pao/rauwolfia combination for 1 year following a dose escalation protocol. As the trial was not completed until the summer of 2009, only preliminary results have been tabulated. In a recent interview, Dr Katz described his optimism from the early results: "From what the results show so far, [a combination of pao and rauwolfia] can produce favorable health benefits and give men the opportunity to do something positive to reduce their risk for more serious outcomes. Yet, [the plant extracts have] no side effects, [are] not a drug, and [are] well tolerated."[19] It is worth noting that the rauwolfia extract has been purified and does not contain any reserpine, a toxic alkaloid known for its negative effect on blood pressure.

Katz continues to explain some of the preliminary results, "[W]e now know that [the two extracts, combined], significantly lowered PSAs in a 12-month period. Also, we have had very few patients convert to prostate cancer and have found a number of patients who have had a dramatic improvement in their urinary symptoms. Men are clearly having less frequency, better streams, and better flow rates."[19] It is noted that data are preliminary.

Katz is enthusiastic about the extracts' potential for helping many men. In the-above cited interview, he further states, "[Pao and rauwolfia extracts have] all the genetic studies showing why [they] work, and how [they] actually recognize the 3-dimensional structure through the laddering and bonding of cancer DNA. [Dr

Beljanski] really did get it right. [These have] great potential to help patients."[19]

Patients should bear in mind that Dr Beljanski conceived of these plant extracts as adjuncts to conventional cancer treatment. By considering complementary and alternative medicine therapies, such as the plant extracts discovered by Dr Beljanski, clinicians are able to offer more options to their patients and improve their overall care.

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