Personal Recollections on the Early Development of Taxol[⊥]

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All of us who have worked with taxol in the laboratory and the clinic, and the many patients all over the world who have benefited from the drug, owe a great debt of gratitude to Monroe Wall, Mansukh Wani, and their colleagues for the initial isolation and characterization of this compound.

In the spring of 1977, when I was an assistant professor at The Albert Einstein College of Medicine in New York, I received a letter from the National Cancer Institute requesting that I study the mechanism of action of taxol, a molecule that was totally unknown to me. One piece of information came with this letter, a reprint of the article[1] published in 1971 in the *Journal of the American Chemical Society* by Monroe Wall and Mansukh Wani and their colleagues. In this landmark paper, the isolation of taxol from the bark of *Taxus brevifolia* was described as well as the structure of the compound and its cytotoxic activity against KB cells growing in tissue culture. The paper was published nearly a decade after the first samples of the yew tree were collected as part of a search for new plant products with antitumor activity. In 1967, during the period that the drug was being isolated, Dr. Wall had given the compound the name taxol (now known by the generic name paclitaxel and the trade name Taxol).

One could certainly ask why the National Cancer Institute wrote to me, a relatively unknown scientist who was at that time working hard to establish an independent career. There were two reasons that come to mind. First, I had already made it clear that I was interested in small molecules, particularly natural products that had the potential to become useful antitumor agents. I had published some of the earliest papers on camptothecin,[2] the epipodophyllotoxins,[3] and bleomycin.[4] Second was that I had received a special grant from the National Cancer Institute that was referred to as a CREG, a Cancer Research Emphasis Grant. As I understood this grant, it was part research grant and part contract. I had been asked to look at other compounds, but they were always analogues of known drugs that were of no special interest to me.

My decision to study the action of taxol was based solely on the unique chemical structure of the drug. My reading of the literature at that time indicated that there were a number of molecules with the taxane ring system, all isolated from the plant family Taxaceae. However, the biological properties of none of these compounds had been studied. I had hoped that since the drug had a novel structure, it might have a unique cytotoxic mechanism of action. With 10 mg of taxol that I had requested from the National Cancer Institute, and a new graduate student, Peter Schiff, who was searching for a Ph.D. thesis project, a study of the mechanism of action of taxol was initiated in my laboratory in 1977. I made a deal with Peter, and that was that we would work on the drug for one month, and if at the end of that time it did not seem interesting, we would drop the project and go on to something else. As it turned out, after one month we were convinced that taxol had a novel mechanism of action for a small molecule and were hoping that this would translate into an active agent in the treatment of human cancer.

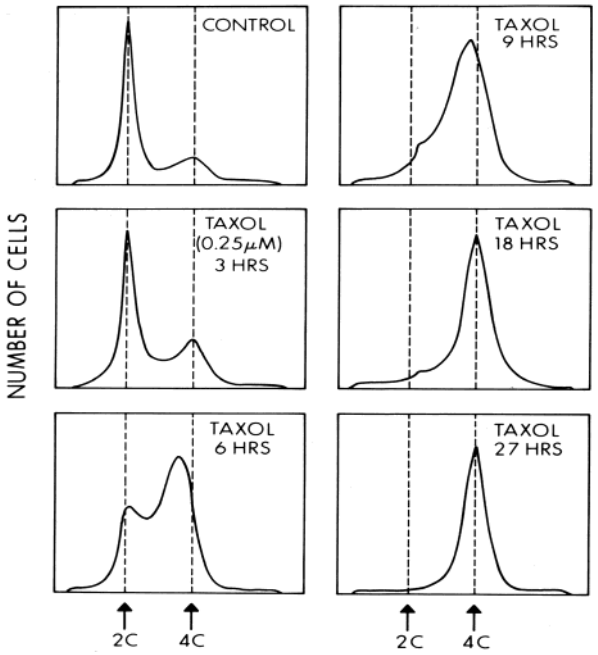

Figure 1. Taxol blocks cells in mitosis. Flow cytometry of the DNA content of HeLa cells after incubation with taxol. The arrows indicate the modal positions of cells having diploid (2C) and tetraploid (4C) DNA contents. In control cultures, the proportions of cells with various DNA contents did not vary significantly during the time course of the experiment.

The first few experiments that were performed indicated to us that taxol was worth further exploration. Low concentrations of taxol, in the nanomolar range, inhibited that replication of HeLa cells. On examination of the effect of the drug on the progression of HeLa cells through the cell cycle, it became obvious that taxol was an antimitotic agent blocking cells in metaphase[5] (Figure 1). After 18 h in the presence of 250 nM taxol, essentially all of the cells had replicated their DNA, had a tetraploid DNA content, and were blocked in metaphase. Although other drugs such as colchicine and the Vinca alkaloids blocked cells in the mitotic phase of the cell cycle, only cells treated with taxol reorganized their microtubules so that distinct bundles of microtubules could be seen in cells (Figure 2). The formation of microtubule bundles, which are highly stable, are diagnostic of taxol treatment and a hallmark of taxol binding to microtubules in cells.

[⊥] Dedicated to the late Dr. Monroe E. Wall and to Dr. Mansukh C. Wani of Research Triangle Institute for their pioneering work on bioactive natural products.

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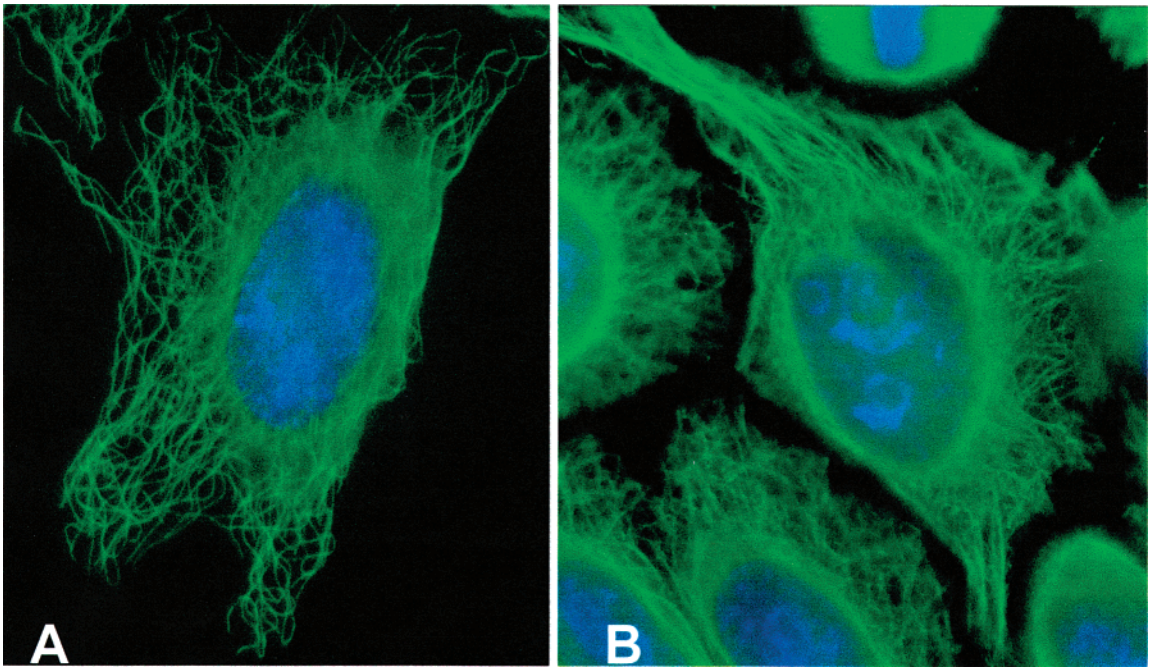

Figure 2. Taxol causes the formation of microtubule bundles in HeLa cells. Indirect immunofluorescence of HeLa cells, using a monoclonal antibody against α-tubulin (green) and DAPI staining (blue) for DNA in the nucleus. (A) Control. (B) 10 μM taxol for 30 min. Courtesy of Dr. Laura Klein, Albert Einstein College of Medicine.

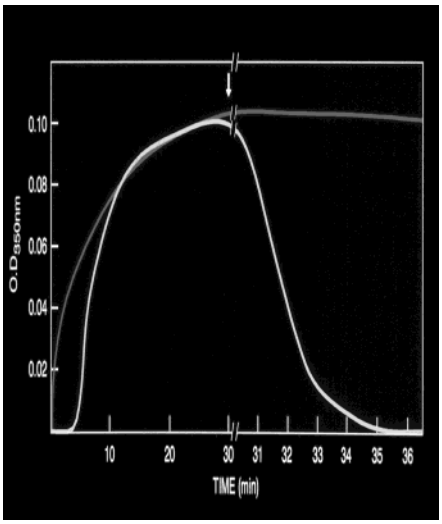

Figure 3. Taxol enhances in vitro tubulin polymerization and microtubule stabilization. No additions (white line); 10 μM taxol (gray line). $CaCl_2$ is added at a concentration of 4 mM at 30 min (↓).

Microtubules are composed of α- and β-tubulin dimers that can be easily studied in a cell-free system. Tubulin was purified from calf brain, a rich source of the protein, and its assembly into microtubules, which occurred at 37 °C in the presence of GTP, was monitored by an increase in absorption at 350 nm. When this experiment was done in the presence of taxol, the 3−4 min lag period that was present in the absence of drug was eliminated[6] (Figure 3). Taxol clearly enhanced the initiation phase of microtubule polymerization. The decrease in lag time was dependent on the taxol concentration. The drug was able to polymerize tubulin in the absence of GTP and at cold temperatures. Most important was the observation that the microtubules formed in the presence of drug were stable to depolymerization at 4 °C and by Ca^{2+}, conditions that normally depolymerize microtubules. The maximum effect of taxol on tubulin stabilization was always seen when the taxol concentration was stoichiometric to that of the tubulin dimer concentration.[7] Today we know that taxol binds to β-tubulin in the microtubule and its mechanism of action in cells is dependent on the concentration of drug. There is no evidence that taxol can bind to the tubulin dimer. At low taxol concentrations (<10 nM), where only a fraction of the total taxol-binding sites are occupied, there is no obvious effect on polymer mass and the principal effect of the drug is suppression of microtubule dynamics.[8,9] Under these conditions, aneuploid populations of cells are produced in the absence of mitotic block.[10,11] At higher taxol concentrations, the drug alters the equilibrium between soluble tubulin dimers and microtubules, resulting in an increase in polymer mass. It became obvious to us that taxol was an amazing small molecule in its ability to promote tubulin polymerization in the absence of GTP, stabilize microtubules against depolymerization, and induce the formation of stable microtubule bundles in cells. We suggested that taxol was a protoype for a new class of antitumor drugs.[12]

Our one month of work had convinced us that taxol was here to stay, even though at that time we had no indication that the drug would have clinical activity. If nothing else, it would be a superb tool for cell biologists interested in the functions of microtubules in cells and for the biochemists intent on purifying tubulin. In the ensuing years, my laboratory and those of many other scientists have carried out extensive studies on various aspects of taxol including its binding site on the microtubule, its effects on cell signaling pathways, and the mechanisms by which cells become resistant to the drug.[13]

The clinical success of taxol has followed a tortuous route.[14] Originally the compound was scarce, due to its low abundance in the bark of the tree. The aqueous insolubility of the drug led to continual formulation problems, and the unexpected allergic reactions in patients resulted in a five-year hiatus in clinical trials. Twenty-one years after the original publication on taxol, the drug was approved by the FDA for treatment of ovarian cancer. The latter remains

a tribute to the many scientists and clinicians who pursued taxol, a task that required unusual persistence and dedication.

Acknowledgment. The author thanks her many colleagues who have worked with her over the years for their interest and contributions to studies on taxol.

References and Notes

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