Taxol production in plant cell culture comes of age

John Pezzuto

Cancer is the world's most prevalent killer disease. Furthermore, as deaths resulting from cardiovascular disorders continue to decline, cancer is expected to become the leading cause of death in the United States by the turn of the century. Curative treatment of malignant metastatic conditions is still uncommon, but natural-product drugs provide the greatest therapeutic hope. One of the most promising natural-product drugs currently in the clinic is paclitaxel (Taxol®); however, obtaining adequate amounts of this drug from scarce environmental sources has always been problematic. In this issue, Yukimune et al.[1] describe one approach for alleviating this problem, generating significant levels of paclitaxel and baccatin III in plant cell culture by the addition of the cell signal transducer methyl jasmonate.

Work on paclitaxel formally began in August 1962, when specimens of Pacific yew [*Taxus brevifolia* Nuttall (Taxaceae)] bark were collected in the northwestern United States. Through the efforts of the research team of Wall and Wani, pure drug was obtained in 1966 and the chemical structure was reported in 1971 (ref. 2). Additional antitumor evaluations of paclitaxel were then performed, but the next great step forward was definition of the mechanism of its action as a promoter of microtubule assembly by Horwitz and coworkers in the late seventies[3]. In 1984, an investigational new drug application was approved by the US Food and Drug Administration (FDA, Rockville, MD), and this culminated in approval of a new drug application for the treatment of refractory ovarian cancer in 1992 (ref. 4). In 1994, FDA approval for use of the drug in the treatment of metastatic breast cancer was obtained. Most recently (May 1996), a paclitaxel derivative, Taxotere®, has been approved for the treatment of metastatic breast cancer. Paclitaxel and Taxotere®, either singly or in combination, are in various ongoing clinical trials[5], with activity indicated against cancers of the ovary, breast, lung, esophagus, bladder, endometrium, and cervix, as well as Kaposi's sarcoma and lymphoma. Accordingly, approval for additional uses is likely.

John Pezzuto is professor and director of the Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The University of Illinois at Chicago, 833 South Wood Street, Chicago, IL 60612-7231 (john.m.pezzuto@uic.edu).

As with other natural-product cancer chemotherapeutic agents, the structure of paclitaxel is rather complicated (see Figure 1). Its source, for the conduct of initial preclinical and clinical studies, was the natural one: the bark of the Pacific yew tree. In

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Figure 1. *Taxus brevifolia*, the source of paclitaxel (Taxol).

1991, for example, about 2 kg of paclitaxel were procured from about 60,000 pounds of dry bark (0.007% yield)[6]. Following establishment of a cooperative research and development agreement between the National Cancer Institute (Bethesda, MD) and Bristol-Myers Squibb (Princeton, NJ) in 1989, several million pounds of bark were collected and processed, and this quickly led to one of the most intense and highly publicized outcries of recent times[7]. The dilemma was apparent: A clinically useful antitumor therapy was known, but provision of sufficient quantities of drug, estimated at 200–300 kg per year[8], would cause considerable depletion of Pacific yew reserves. In essence, the question became should one sacrifice human life or the environment?

Reports of the total synthesis of paclitaxel, independently published by the groups of Holton[9] and Nicolaou[10], were celebrated achievements that required incredible insight and talent. As anticipated, however, total synthesis does not provide an economically feasible solution to the paclitaxel supply problem. Another well-publicized development was the discovery of *Taxomyces andreanae*, a fungal endophyte isolated from *Taxus brevifolia* capable of biosynthesizing paclitaxel[11]. The amount of paclitaxel produced was extraordinarily low, but the implication was that an organism had been identified that could be used in a large-scale fermentation process, such as is common for the production of antibiotics. The practical and functional aspects of such a process remain to be demonstrated.

A mutually acceptable solution to the paclitaxel dilemma was found through the process of semisynthesis, the original concept of which is generally attributed to Potier[12]. Yews, such as *Taxus baccata* Linnaeus (Common or European yew) or *Taxus wallichiana* Zuccarini (Himalayan yew), are renewable resources that can be cultivated on a commercial scale without difficulty or environmental complications. The needles and stems, for example, are harvested and used for the isolation of baccatin III or 10-deacetylbaccatin III, which can be converted by semisynthetic procedures to paclitaxel. Production of paclitaxel through this process for clinical use was approved by the FDA in 1994, and this continues to serve as the predominant method by which the drug is produced.

Until now, the production of paclitaxel in plant cell culture has been too low to be economically viable. But the levels of synthesis described by Yukimune et al.—about 0.6% dry weight—give a paclitaxel yield that clearly overshadows that of the Pacific yew and exceeds production levels of previous cell culture reports by about a factor of six. The issue of possible loss of efficiency on obligatory scale-up has also been partially addressed, at least on a 200 liter scale, which is promising; of course, commercialization would require scale-up production in the range of 100,000 liters.

It is clear, however, that this is a promising discovery, worthy of thorough pharmacoeconomic analysis. The paclitaxel story, as fascinating as it has been, is certainly not complete. Perhaps the next chapter will describe large-scale production through biotechnological advances involving plant tissue culture.

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