Staurosporine: A Prototype of a Novel Class of Inhibitors of Tumor Cell Invasion?

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Clinical metastasis is a major cause of cancer mortality, and the development of therapeutic approaches for the prevention of the development and dissemination of metastatic disease is an area of high priority in cancer research today (1). Protein kinase C (PKC) is a Ca2+- and phosphatidylserine-dependent protein kinase that can be activated at physiological Ca2+ concentrations by the second messenger, unsaturated diacylglycerol, and by phorbol-ester tumor promoters (2). Based on recent evidence that PKC activation may contribute to the development of metastatic disease, in their article in this issue of the Journal, Schwartz et al (3) reasoned that PKI inhibitors may be antagonists of tumor cell invasion.

Staurosporine is an indole carbazole produced by a Streptomyces species, which inhibits PKC (4,5) as well as a variety of other protein kinases (6-8) with an exceptionally high potency. Schwartz et al show that non-toxic concentrations of staurosporine can antagonize the invasion of tumor cells through an artificial basement membrane in a dose-dependent manner (3). Their study indicates that inhibition of the invasion of human bladder cancer EJ cells through an artificial membrane by staurosporine involves inhibition of cell motility but not inhibition of cell attachment (3).

Schwartz et al conclude that staurosporine should be a useful tool in studies aimed at elucidating the mechanism of tumor cell invasion, and that the development of staurosporine analogues that are more specific in their molecular action may result in the identification of a derivative that is therapeutically effective in the prevention of metastatic disease (3). In view of the progress already achieved in synthesizing staurosporine analogues that exhibit greater specificity than staurosporine itself at the molecular level (9), this conclusion appears to be well-founded.

The proposed use of staurosporine and related indole carboxazoles in studies concerning the mechanism of tumor cell invasion requires an understanding of their inhibitory activities against PKC and related targets. Staurosporine is best described as a protein kinase inhibitor, because it potently inhibits PKC (IC50 = 6 nM), cAMP-dependent protein kinase (PKA) (IC50 = 15 nM), phosphorylase kinase (IC50 = 3 nM), S6 kinase (IC50 = 5 nM), epidermal growth factor receptor tyrosine protein kinase (IC50 = 25 nM) (9), pp60Src tyrosine protein kinase (IC50 = 6 nM) (6), and insulin receptor tyrosine protein kinase (IC50 = 61 nM) (8). The nonselectivity of staurosporine in its inhibition of protein kinase activity is clearly shown by its ability to potently inhibit widely divergent members of the protein kinase family, i.e., serine/threonine protein kinases and tyrosine protein kinases. This nonselectivity provides evidence that staurosporine functions by binding the protein kinases at a region of considerable homology throughout the protein kinase family. In fact, the catalytic domains of protein kinases bear regions of striking homology, and Gross et al (10) have provided strong evidence that staurosporine inhibits PKC by binding to its catalytic domain.

A fully active catalytic fragment of PKC that contains only the catalytic domain of the enzyme can be produced by limited proteolysis (2), and Gross et al have shown that a radiolabeled analogue of staurosporine binds to the catalytic fragment of PKC with a Kd (2.4 nM) (10), which is similar to its IC50 against the histone kinase activity of the catalytic fragment (IC50 = 1 nM) (11). Taken together with the studies of the catalytic fragment of PKC, the observations that the inhibition of intact PKC by staurosporine is not competitive with allosteric cofactors (Ca2+, phosphatidylserine, diacylglycerol) (4) and that staurosporine does not inhibit the binding of [3H]phorbol 12,13-dibutyrate to PKC (4,11) provide strong evidence that the inhibition of PKC by staurosporine results from direct effects of staurosporine on PKC catalysis and does not involve inhibition of the allosteric activation of PKC. However, the exact mechanism of PKC inhibition by staurosporine remains unclear.

While the structure of staurosporine is closely related to that of the indole carbazole K252a, which inhibits PKC by competition with the substrate ATP (12), kinetic studies of the inhibition of PKC by staurosporine (4) and studies of the equilibrium binding of a radiolabeled analogue of staurosporine to PKC (10) provide evidence that staurosporine does not function by competition with ATP. However, K252a and H-7, an isoquinoline sulfonamide (4), both of which inhibit PKC by competition with the substrate ATP, also compete with the binding of a radiolabeled analogue of staurosporine to PKC (10), suggesting that staurosporine does bind to the ATP-binding region of the active site of PKC. While the results summarized above are inconclusive, they suggest that staurosporine...
sporine may bind to PKC at a region that overlaps with its ATP substrate-binding site. However, the sequences of cDNA's encoding PKC isozymes indicate that the catalytic domain of PKC may contain a second ATP binding site outside of its active site region (2), which therefore presents another potential target for staurosporine inhibition.

Elucidation of the mechanism of protein kinase inhibition by staurosporine should facilitate the design of analogues that are more selective in their molecular action and therefore less toxic in general. Staurosporine itself exhibits a wide range of toxic effects against various cultured cells and is extremely cytotoxic in some cases (IC_{50} against HeLa S3 cells = 4 pM, 72-hour exposure) (4). Thus, although Schwartz et al (3) observed that staurosporine was not cytotoxic in their in vitro system for the study of tumor cell invasion at concentrations as high as 0.1 μM, it is clear that, in studies aimed at designing therapeutically effective antimetastatic drugs based on staurosporine, it will be important to design analogues that are less toxic than the parent compound. Significantly, the staurosporine analogue CGP 41 251, a derivative of the natural product synthesized by CIBA-GEIGY Ltd. (Basel, Switzerland) that is more selective than the parent compound in the inhibition of PKC, is also a more potent antitumor agent than staurosporine in vivo at equally tolerated doses, according to the results of Meyer et al (9) in studies of athymic nude mice bearing T24 human bladder carcinoma xenografts.

Schwartz et al (3) point out that studies aimed at designing more specific and presumably less toxic analogues of staurosporine for use as antimetastatic agents cannot assume that the in vitro anti-invasive effects of staurosporine on human bladder cancer EJ cells result from inhibition of PKC. This point is underscored by reports that staurosporine elicits some effects that parallel responses to PKC activators (such as phorbol-ester tumor promoters) and other effects that inhibit responses to PKC activators. For example, staurosporine and phorbol 12,13-dibutyrate each stimulate the induction of transglutaminase, a marker for differentiation, to the same degree in primary mouse epidermal cells, and both agents also induce cornified envelope production, another marker for differentiation in epidermal cells (13). In addition, staurosporine is a weak tumor promoter for mouse skin (14). On the other hand, staurosporine antagonizes the induction of the respiratory burst by phorbol-ester tumor promoters in human neutrophils (13). Staurosporine also antagonizes the down-regulation of the T-cell receptor and the loss of adhesion that are caused by phorbol-ester tumor promoters in the T lymphoblastoid cell line HPB-ALL (Cheryl Passini and Bradley McIntyre: personal communication).

In conclusion, the effects of staurosporine on PKC-mediated events may vary dramatically, depending on the signal transduction pathways that are involved and the cell culture system employed. Therefore, further study is required to determine the targets of staurosporine action that are responsible for its anti-invasive activity against EJ cancer cells in vitro and to determine whether staurosporine also exhibits anti-invasive activity against other invasive tumor cell lines in vitro and under less artificial conditions.

References