Chapter 9

CEPHALOSTATIN 1-INDUCED APOPTOSIS IN TUMOR CELLS
Selective Induction of Smac/DIABLO Release

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Abstract: Cephalostatin 1 is a marine bis-steroidal compound with strong (mean panel GI₅₀: 2.2 (± 1.21) x 10⁻⁹ M) and particular cytotoxic activity as shown in the in vitro 60-cell line screen by the National cancer Institute (USA). Cephalostatin 1 was shown to trigger cell death in human Jurkat T cells with classical morphological (cell shrinkage, membrane blebbing, DNA fragmentation) and biochemical (phosphatidylserine translocation, activation of caspases) signs of apoptosis. Cephalostatin 1-induced apoptosis, however, differs significantly from apoptosis induced by classical chemotherapeutic drugs in that it selectively leads to Smac/DIABLO (second mitochondria-derived activator of caspases/direct inhibitor of apoptosis-binding protein with a low isoelectric point) release from mitochondria. Cytochrome c and apoptosis inducing factor (AIF) is retained in the mitochondrial intermembrane space. Selectively released Smac/DIABLO translocates into the cytosol where it binds to XIAP displacing previously bound caspase-9. Caspase-9 does not associate with Apaf-1 and thus appears to be processed without requirement for apoptosome formation. This unique mitochondrial signaling by cephalostatin 1 is accompanied by mitochondrial matrix condensation instead of mitochondrial swelling. Swelling is assumed to precede outer mitochondrial membrane permeabilization in response to induction of the mitochondrial (intrinsic) apoptosis pathway. These results characterize cephalostatin 1 as a remarkable experimental chemotherapeutic agent with a unique mechanism of action. Further studies will show whether cephalostatin 1 will be a good candidate to be developed against chemoresistant types of cancer.

Key words: cephalostatin, apoptosis, Smac/DIABLO, mitochondria, caspase 9, XIAP

M. Sluyser (ed.), Application of Apoptosis to Cancer Treatment, 209-221.
1. INTRODUCTION

Natural products play a significant role in the discovery and development of drugs for the treatment of human disease. This is particularly evident in the area of cancer, where more than 60% of all drugs are shown to be of natural origin\(^1\). In this context especially compounds of marine origin gained more and more attention over the past decades.

Why are compounds of marine origin promising candidates for anti-cancer drug development? Many marine organisms are soft-bodied and live sedentary. Thus, they developed the ability to produce toxic compounds or to obtain them from marine microorganisms as a chemical defense. The toxicity of these compounds needs to be extremely potent since they are released and diluted into water\(^2\).

Although today the potency of marine compounds especially for the development of new anti-cancer drugs is recognized still difficulties, such as the availability in quantities high enough for preclinical and clinical studies, have to be overcome.

1.1 Cephalostatins

The cephalostatins are a group of structurally strongly related bis-streoidal compounds isolated from the marine organism *Cephalodiscus gilchristi* Ridewood (Cephalodiscidae). The structure of cephalostatin 1 is depicted in Figure 1. Up to now, 19 derivatives could be characterized. The cephalostatins belong to the most cytotoxic marine natural products ever tested by the National Cancer Institute (NCI)/USA. All cephalostatins show the same unique cytotoxicity profile in the NCI-60 panel (see Figure 2), which displays the growth inhibitory potency of substances against 60 cancer cell lines of diverse origin\(^3,4\). Although the cytotoxicity profiles of the cephalostatins do not differ, their potency varies depending on the chemical composition. Cephalostatin 1 proved to be the most potent form in the NCI-60 panel. The two-day NCI-60 screen yielded a mean panel GI\(_{50}\) concentration of 2.2 (±1.21) x 10\(^{-9}\) M for cephalostatin 1\(^5,6\). Interestingly, the obtained cephalostatin cytotoxicity fingerprint did not show comparable correlations to any members of the standard agent database suggesting that the differential cytotoxicity of cephalostatins derives from a unprecedented, but as yet undefined, mechanism of action\(^7\). Beyond *in vitro* testings, cephalostatin 1 was proven to be effective in several xenografts, such as melanoma, sarcoma, in leukemia and even in a human mammary carcinoma model\(^8\).