Serine Proteases in Histone Deacetylase Inhibitor-Induced Apoptosis Still an Unresolved Question – Response

In response to Sonnemann and colleagues’ letter, first, we agree with the evidence that 4-(2-aminoethyl) benzene-sulfonylfluoride (AEBSF) has a direct effect on trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA), as the authors mention. However, we believe that, on the basis of the data shown by these authors, this effect has been overestimated. When they compare the effects of TSA and SAHA on histone deacetylase (HDAC) activity in vivo and in vitro, it is quite clear that AEBSF is able to practically abolish the inhibitory effect of TSA and SAHA in intact cells in vitro. However, AEBSF shows a significant lesser effect in cell-free assays in vitro at the same concentration. To clarify this discrepancy, we extrapolated the data from their Fig. 1A and B and combined it as our Fig. 1, to emphasize the differential effect of AEBSF on the hydroxamic acids’ (TSA and SAHA) inhibition of HDAC activity in vivo and in vitro. We propose two possible explanations for these discrepancies: On the one hand, the interaction of hydroxamic acids with AEBSF could be favored in vivo through an unknown mechanism. On the other hand, and most likely, a direct effect of AEBSF on TSA or SAHA takes place only in vitro, whereas AEBSF has additional effects in intact cells in vivo, related to its serine protease inhibitory activity. On the basis of this second explanation, we believe that the authors’ assumption affirming the completely unrelated effects of TSA and SAHA to serine protease activity in the cells is, at least, somewhat risky. The authors do not take into account our findings indicating that HDAC inhibitor (iHDAC)-induced apoptosis in pancreatic cancer cells can be related to serine protease activity (1). We build this argument not only on the AEBSF effect, but mostly on the effect of another serine protease inhibitor, Ucf-101, which specifically inhibited the mitochondrial serine protease Omi-Htr2 (2). Moreover, this compound was able to inhibit the iHDAC-induced apoptosis not only in pancreatic cancer cells, but also in myeloma cell lines, as shown by Fandy and colleagues (3).

In addition, in Fig. 2 we show the effect of tosyl-l-lysine chloromethyl ketone (TLCK) and tosyl-l-phenylalanine chloromethyl ketone (TPCK), other serine protease inhibitors, on TSA-induced apoptosis in leukemic murine L1210R cells. As shown in this figure, both inhibitors are able to abrogate the TSA-induced apoptosis when cells are treated for 1 or 3 hours with one of the inhibitors and TSA, followed by incubation up to 24 hours after the drugs are removed. This protective effect decreases after 6 hours of treatment, and disappears after longer treatments, either because the half-life of TLCK and TPCK in the culture media is much shorter than the TSA half-life, or because these two inhibitors are able to produce apoptosis in HL60 and other cell lines by themselves after longer periods of incubation (24-72 hours), as it has been suggested (4). Taken together, these data suggest that serine protease activity plays a role in iHDAC-induced apoptosis, although in agreement with Sonnemann and colleagues, the results obtained after AEBSF treatment should be analyzed very carefully, because of its direct effect on TSA and SAHA.

This direct effect of AEBSF on TSA and SAHA seems to be quite moderate, because AEBSF treatment at 100 μmol/L, which is the concentration that we used in our previous article (1), is able to block only 20% of the TSA effect on HDAC activity in vitro. If the TSA concentration in these experiments is 1 μmol/L, we can assume that a molecular ratio of 100:1 of AEBSF versus TSA, is able to only partially block the TSA effect in vitro.

Furthermore, we did DNA microarrays analysis in L1210R cells in the presence and absence of TSA and AEBSF, and we found at least 600 genes induced by TSA. This phenomenon is not abolished by AEBSF, which suggests that at least some of the TSA effects are not inhibited by its interaction with AEBSF.

We find Sonnemann and colleagues’ Letter to the Editor very interesting, because it represents a novel finding about some undesirable effects of AEBSF derived from its interaction with iHDACs.

Maria Piedad Menéndez-Gutierrez
José Antonio Ferragut
Instituto de Biología Molecular y Celular de la Universidad Miguel Hernández de Elche, Alicante, Spain

Figure 1. Data from Fig. 1A and B of Sonnemann and colleagues letter were extrapolated and combined to highlight the differential effect of AEBSF on TSA-inhibited HDAC activity in vivo and in vitro.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Maria del Pilar García-Morales
Fundación para la Investigación Biomédica del Hospital general Universitario de Elche, Elche, Alicante, Spain

Miguel Saceda
Instituto de Biología Molecular y Celular de la Universidad Miguel Hernández de Elche and Fundación para la Investigación Biomédica del Hospital general Universitario de Elche, Elche, Alicante, Spain

Isabel Martinez-Lacaci
Instituto de Biología Molecular y Celular de la Universidad Miguel Hernández de Elche, Alicante, Spain and Unidad AECC de Investigación Traslacional en Cáncer, Hospital Universitario Virgen de la Arrixaca, Murcia, Spain

Figure 2. L1210R cells were incubated with or without 1 μmol/L TSA, in the presence or absence of the serine protease inhibitors TLCK (100 μmol/L) or TPCK (1 μmol/L) for 1, 3, 6, or 24 hours. Then, the media was replaced with fresh media up to 24 hours. Cells were collected and stained with propidium iodide, and cell cycle distribution of DNA content was analyzed by flow cytometry. The percentage of cells in sub-G1 phase of the cell cycle is presented in the figure as a measure of the percentage of apoptotic cells. Data are presented as mean ± SD of three independent experiments.
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