ME-344, a Novel Isoflavone with Activity as a Mitochondrial Oxygenase Inhibitor

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ABSTRACT

ME-344, a second-generation natural product isoflavone is being developed as a clinical candidate in small cell lung and ovarian cancer by MEI Pharma (San Diego, CA). Treatment of tumor cells in culture with low micromolar ME-344 decreased mitochondrial ATP production and increased ROS, with subsequent disruption of mitochondrial inner membrane homeostasis, providing a possible explanation for the beneficial therapeutic index of this drug.

RESULTS

ME-344 caused mitochondrial oxygen consumption rates (OCR) to cancer cell lines, which were more pronounced in lung cancer cells and correlated with sensitivity of the cells to the drug (Fig. 1, left panel). Conversely, ME-344 simultaneously stimulated extracellular acidification rates (ECAR) only in drug-resistant cancer cells. This effect was more pronounced in those lung cancer cells (Fig. 5, right panel). The rapidity of these responses and the subsequent therapeutic effects is quite unique and might imply effects occurring at the cellular surface instead of the mitochondrial membrane. The effects of ME-344 on OCR and ECAR were concentration-dependent, with those lung cancer cells sensitive to drug but were substantially smaller in resistant cells. These observations are interesting and indicate potential for multiple mechanisms of action. ME-344-induced mitochondrial oxygen consumption rates (O2-OCR) and NAD(P)H oxidation in lung cancer cells sensitive to drug but were substantially smaller in resistant cells. These observations are interesting and indicate potential for multiple mechanisms of action. ME-344-mediated intracellular ROS generation and modulation of NAD(P)H levels.

Hypothetical Effects of ME-344

We have established relevant cell lines, animal models, and experimental protocols needed for ongoing and future experimental plans for ME-344 studies. Our hypothesis is that ME-344 through modulation of proton gradients in mitochondria mimics the effects of glutathionylation. The influence of ME-344 on mitochondrial stress test results in rapid tumor cell death (Fig. 10). To test this hypothesis, the PyMT mouse model which gives rise to spontaneous, highly metastatic tumors was employed. (Fig. 6). This model shows the minimal effects of ME-344 on tumor growth. Nintedanib (BIBF), an anti-angiogenic tyrosine kinase inhibitor, initially inhibits tumor growth, but the tumor burden remains consistent with a switch to mitochondrial reliance (M. Quintela-Fandino). Application of BIBF for ME-344 completely prevents tumor cell growth. Addition of ME-344 at the time of tumor cell switch to mitochondrial reliance results in rapid tumor cell death (Fig. 10).

We are planning future studies to define these characteristics of ME-344 that regulate tumor homeostasis, providing a possible explanation for the beneficial therapeutic index of this compound now undergoing Phase II testing in ovarian cancer at MUC.

CONCLUSIONS

We have established relevant cell lines, animal models, and experimental protocols needed for ongoing and future experimental plans for ME-344 studies.

Our experiments show strong immediate effects of ME-344 on OCR in cancer cells, which correspond with normal cellular drug sensitivity.

Our data show ME-344-mediated intracellular ROS generation and modulation of NAD(P)H levels.

Our hypothesis is that ME-344 through modulation of proton gradients in mitochondria mimics the effects of glutathionylation. In vivo studies used the PyMT mouse model which gives rise to spontaneous, highly metastatic tumors. In this model, chronic treatments with the small molecule anti-angiogenic agent BIBF1120 (nintedanib) significantly diminished glycolysis, with the consequence that the growth of tumors was reduced. This study is the first to show that ME-344, a novel isoflavone, is being developed as a clinical candidate in small cell lung and ovarian cancer by MEI Pharma (San Diego, CA). Treatment of tumor cells in culture with low micromolar ME-344 decreases mitochondrial ATP production and increases ROS, with subsequent disruption of mitochondrial inner membrane homeostasis.

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In general we hypothesize that ME-344, through its regulation of proton gradients, is appropriate for application of NAD(P)H as a mitochondrial source. As monotherapy, ME-344 caused minimal inhibition of primary tumors. However, tumors primed by treatment with BIBF1120 showed significantly enhanced sensitivity to ME-344. In general we hypothesize that ME-344, through its regulation of proton gradients, is appropriate for application of NAD(P)H as a mitochondrial source. As monotherapy, ME-344 caused minimal inhibition of primary tumors. However, tumors primed by treatment with BIBF1120 showed significantly enhanced sensitivity to ME-344. The correlation between drug-induced ROS generation and either OCR or ECAR was analyzed. The results were normalized for cell number in each sample and presented as MEAN±SE, for n≥3.

The inhibitory effects of ME-344 on OCR are concentration-dependent (Fig. 2). The effects of ME-344 on glycolysis are specific for cancer cells compared to primary cells (Fig. 4 and 5). ME-344 exposure (30 min) induces oxidation of total thiols in drug-sensitive lung cancer cells (Fig. 7) and thiol-dependent cytochrome c release (Fig. 9), which provides a focus for additional studies. The correlation between drug-induced ROS generation and either OCR or ECAR was analyzed. The results were normalized for cell number in each sample and presented as MEAN±SE, for n≥3.

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