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

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ABSTRACT

ME-344, a second-generation derivative of a 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 integrity. To gain further insight into this unusual mechanism of action, we compared ME-344 activity in sensitive and naturally resistant lung cancer cell lines, together with primary human lung embryonic fibroblasts (HLEF). Using SeaHorse technology we measured the impact of ME-344 on oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) in these cells under conditions of mitochondrial and glycolytic stress. In a dose dependent manner, the drug caused instantaneous and pronounced inhibition of OCR in drug-sensitive lung cancer cells; significantly less in drug-resistant cells. These results are consistent with targeting of mitochondria by ME-344 with specific effects to the respiratory chain (drug resistance correlated with higher glycolytic indexes in these cells.) Inhibition of OCR did not occur in primary HLEF. ME-344 increased ECAR in drug-resistant lung cancer cells, where this effect was significantly diminished in drug-sensitive cells. Such data suggest that ME-344 specifically targets mitochondrial proton channels (pumps). Only in drug sensitive cells, ME-344 dose-dependently increased the intracellular generation of ROS (detected by H2DCF-DA or Deep Red fluorescent dye staining assays) and caused oxidation of intracellular low molecular weight (mainly GSH) and protein thiols and of NAD(P)H. In vivo studies used the PyMT mouse model, which gives rise to spontaneous breast tumors that are highly glycolytic, useful in assessing the comparative roles of aerobic and glycolytic metabolism. In this model, chronic treatments with the small molecule anti-angiogenic agent BIBF1120 (nintedanib) significantly diminished glycolysis, with the consequence that the growing tumor shifted to reliance on mitochondrial metabolism as the primary energy source. As monotherapy, ME-344 caused minimal inhibition of primary tumors. However, tumors primed by treatment with BIBF1120 showed significantly enhanced sensitivity to ME344, with synergistic antitumor activity. These data indicate complex, but mitochondria-specific effects of ME-344 in cancer cells, differing in extent from normal and linked with drug sensitivity profile with clinical potential through its specific targeting of bioenergetics pathways in cancer cells with the potential for beneficial therapeutic index.

METHODS

Tissue culture.

Primary:

MRC-5 - Human Lung Embryonic Fibroblasts.

Lung cancer:

SHP-77 –Small Cell Lung Carcinoma *(Epithelial Cells, sensitive)*

NCI-H460 - Carcinoma; Large Cell Lung Cancer *(sensitive)*

SW 900 – Grade IV, Squamous Cell Lung Carcinoma ***(resistant)***

NCI-H596 – Adenosquamous Lung Carcinoma ***(resistant)***

Colon cancer:

RKO - Colon Carcinoma *(Epithelial Cells, sensitive)*

HCT-15 - Dukes' Type C, Colorectal Adenocarcinoma *(Epithelial Cells, sensitive)*

SW480 - Dukes' Type B, Colorectal Adenocarcinoma *(Epithelial Cells, **resistant**)*

NCI-H747 - Cecum Colorectal Adenocarcinoma Cells ***(resistant)***

General methodologies:

Seahorse Bioscience™ experiments

For the mitochondria stress test experiments, 96-well plates with attached cells were used in the Seahorse Bioscience™ apparatus. Incubation times and ME-344 dependence of OCR and ECAR was analyzed. The results were normalized for cell number in each well and presented as MEAN ±SE, for n≥3.

Fluorescent detection of ROS and NAD(P)H

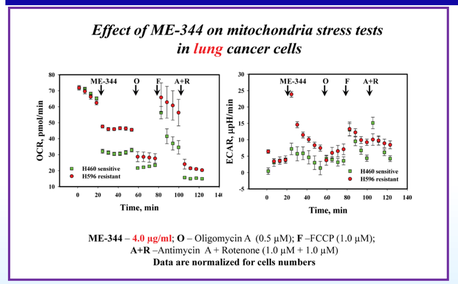
For ROS/ NAD(P)H fluorescent analyses, cells were grown as monolayers on Aclar© plastic slides. Cells were labeled with specific dyes (ROS measurements) and were analyzed in quartz cuvettes (10x10x40 mm) with PBS (pH=7.40, 150μM $CaCl_2$) under constant stirring at 37^{0}C in a QM-4 spectrofluorometer (PTI, NJ).

Fluorescent detection of LMW(GSH) and protein Thiols

Intracellular thiol redox status in cell lines was performed using ThioGlo-1 fluorescent probe (Calbiochem, CA) as an established protocol in our laboratory..

SeaHorse experiments were performed with the help of the Metabolomic Core of COBRE in Oxidants, Redox Balance and Stress Signaling. The authors wish to thank Dr. G. Beeson, G. Beeson and B. Hoover for their help and advice.

RESULTS



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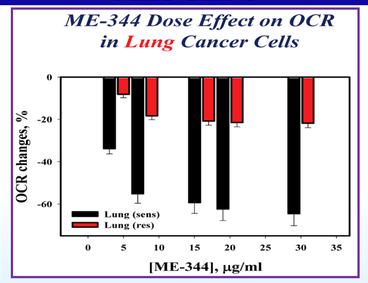

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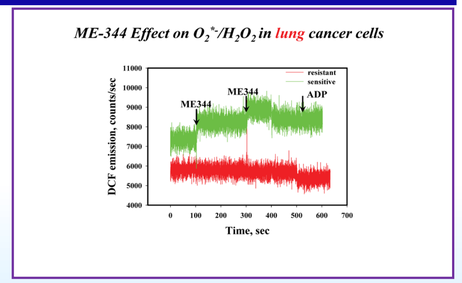

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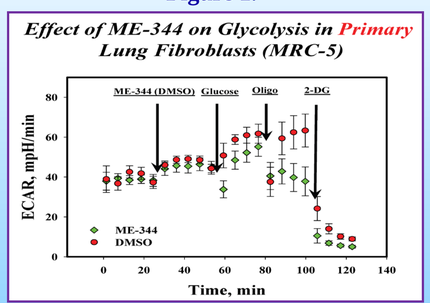

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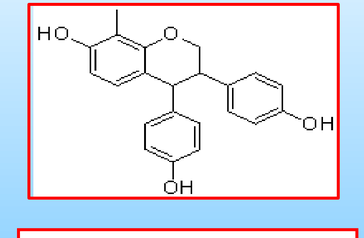

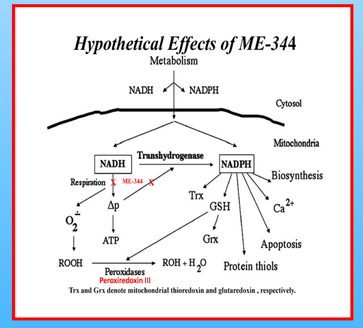

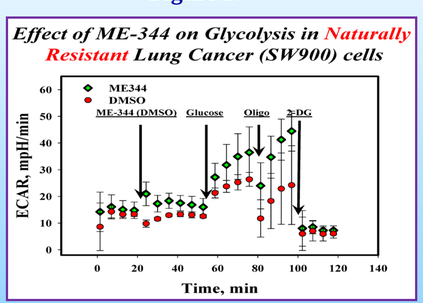

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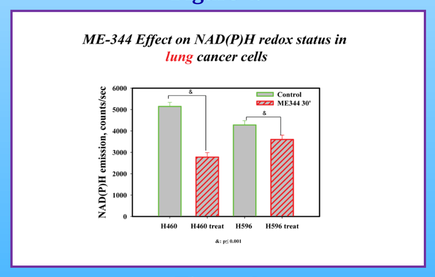

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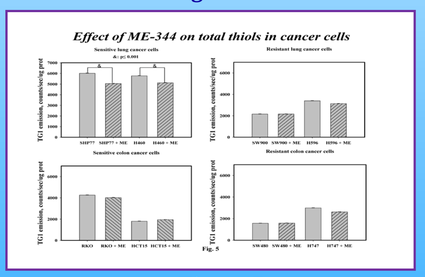

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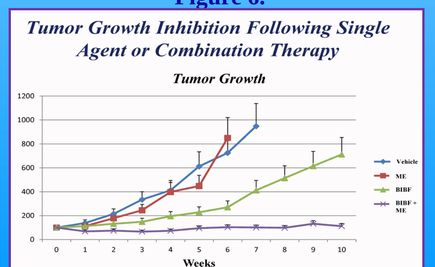

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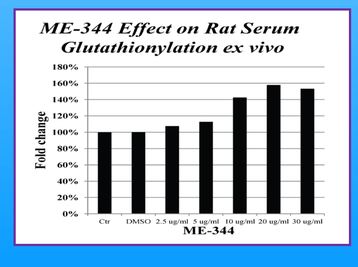

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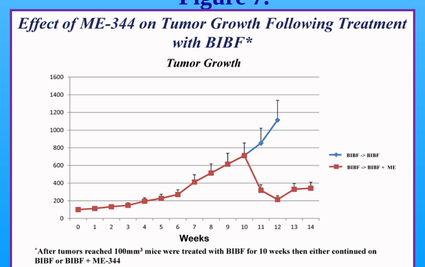

Figure 10.

CONCLUSIONS

ME-344 caused instantaneous inhibition of oxygen consumption rates (OCR) in cancer cell lines. The effects were more pronounced in lung cancer cells and correlated with sensitivity of the cells to the drug (Fig. 1, left panel). Conversely, ME-344 instantaneously stimulated extracellular acidification rates (ECAR) only in drug resistant cancer cells. This effect was more pronounced in lung cancer cells (Fig.1, right panels). The rapidity of these ME-344 effects is quite unusual and might imply effects occurring at the cell surface/plasma membrane. The inhibitory effects of ME-344 on OCR are concentration-dependent (Fig. 2) for those lung cancer cells sensitive to drug but were substantially smaller in resistant cells. These observations are interesting and perhaps reflect some component of aerobic vs. anaerobic metabolism. ME-344-mediated intracellular generation of $O^{\bullet-}/H_2O_2$ and NAD(P)H oxidation in lung cancer cells sensitive to the drug (Fig. 3 and 6). 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 S-glutathionylation *ex vivo* (Fig. 9), which provides a focus for continued studies. The correlation between drug-induced ROS generation (Fig. 3) and either the redox status of intracellular or cell surface thiols can be directly linked to cellular antioxidant capacity (glutathione peroxidases, peroxiredoxins) through supply of NADPH to mitochondria as well as redox signaling potential (e.g. S-glutathionylation/ deglutathionylation). The influence of ME-344 on intracellular thiol status does seem to correspond with its impact on cellular redox homeostasis.

In general we hypothesize that ME-344, through its regulation of proton gradients, is regulating the supply of NADPH to mitochondria. This is critical for antioxidant protection and regulation of metabolic switches between oxidative phosphorylation and glycolysis in cancer cells (see general scheme).

To test this hypothesis, the PyMT mouse model which gives rise to spontaneous, highly glycolytic breast tumors was employed. (Fig.8). 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 this is followed by an acceleration, perhaps consistent with a switch to mitochondrial reliance (M. Quntela-Fandio). Application of BIBF with 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 those characteristics of ME-344 that regulate redox homeostasis, providing a possible explanation for the beneficial therapeutic index of this compound now undergoing Phase II testing in ovarian cancer at MUSC.

SUMMARY

- ❖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 natural 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 influences metabolic switching between oxidative phosphorylation and glycolysis in cancer cells as well as in the PyMT mouse model *in vivo*.
- ❖We will focus on studying specific mechanism(s) involved in these metabolic effects.

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