TITLE

POTENTIAL ROLE OF THE ADRENOLITIC DRUG MITOTANE IN THE TREATMENT OF HEPATOCELLULAR CARCINOMA (HCC): EFFECT ON CELL PROLIFERATION IN HCC CELL LINES.

Pivonello C.[1], Provvisiero D.P.[1], Negri M.[1], Di Gennaro G.[1], de Angelis C.[2], Simeoli C.[1], Patalano R.[1], De Martino M.C.[1], Colao A.[1], Pivonello R.[1]

[1]Departement of Clinical Medicine and Surgery, University of Naples «Federico II»; Naples, Italy; [2]I.O.S. & COLEMAN S.r.l., Naples, Italy

BACKGROUND AND AIM

HCC is one of the most common malignancies worldwide. Local approaches are generally preferred for patients whose disease is restricted to the liver. In patients with extrahepatic disease systemic therapy can be considered. Chemotherapy did not demonstrate convincing survival advantages in several trails for HCC patients. Presently, the kinase inhibitor sorafenib is the only approved systemic target therapy for the treatment of advanced HCC. Mitotane (dichlorodiphenildichloroethane or o,p'DDD), a chemotherapeutic agent, is the only drug approved for the treatment of advanced adrenocortical cancer (ACC) but no data are available concerning its cytotoxic role in different cancers, including HCC. The aim of this study was to evaluate the effect of mitotane on cell proliferation in HCC cell lines.

METHODS

HepG2, HuH-7, JHH-6, PLC/PRF/5 HCC cell lines, THLE-2 normal hepatocytes cell line and H295R, ACC cell line, were used for the study. DNA assay was conduced to evaluate the rate of inhibition after 6 days of treatment with Mitotane in a concentration ranging between 10^{-6} and 10^{-5} M, beneath the plasma Mitotane levels achieved by ACC patients. To confirm the role of Mitotane in the inhibition of survival, colony forming assay was performed after 21 days of treatment. By assessing the clonogenic assay through crystal violet staining, the percentage of colonies formed was calculated in the different treatment concentrations. Western blot analysis for PARP cleavage was performed to evaluate apoptosis at different concentration used of Mitotane.

FIGURES

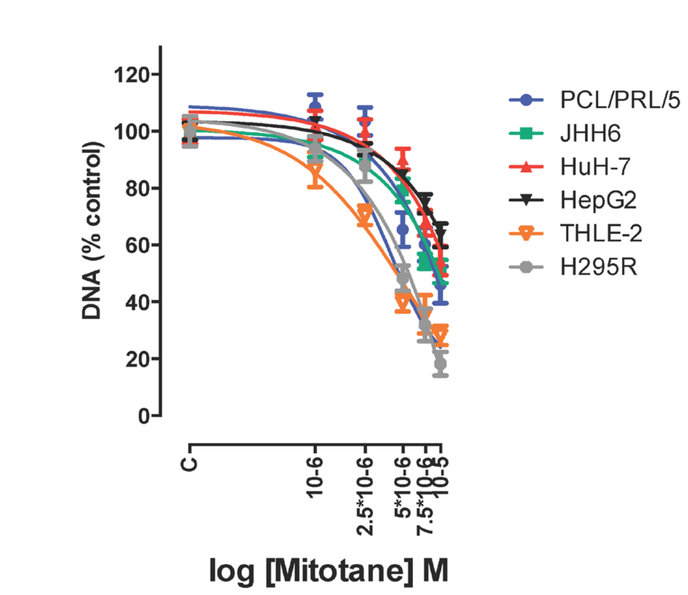
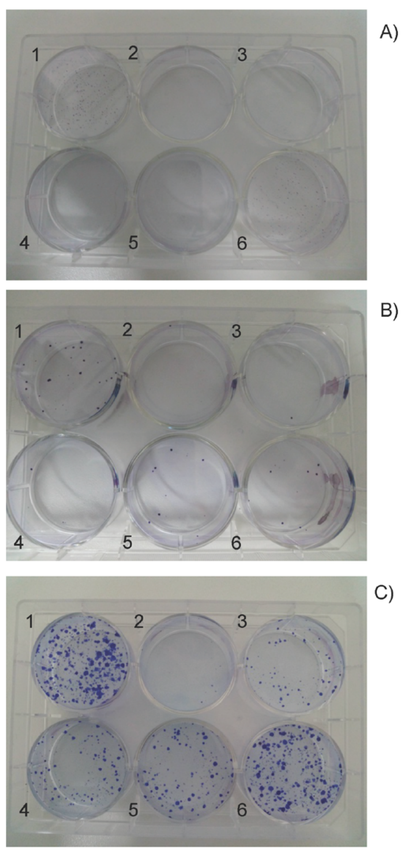


Figure 1. DNA assay in HCC cell lines, normal hepatocytes and H295R cell line.



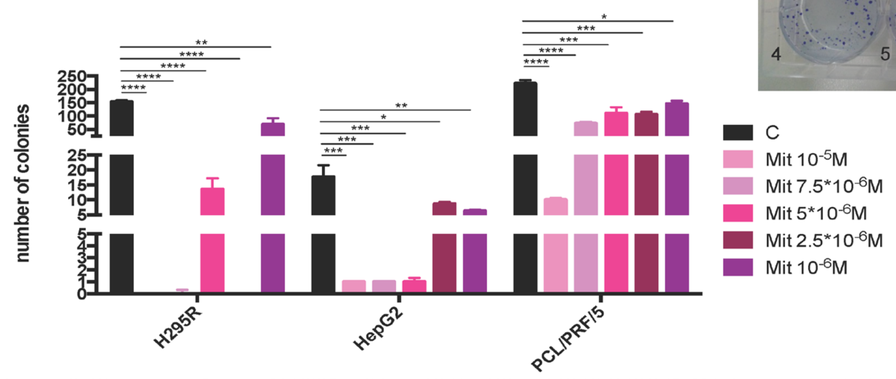


Figure 2. Clonogenic Assay: A) H295 cell line; B) HepG2 and C) PLC/PRF/5. Mitotane concentrations in the wells: 1) Control + vehicle; 2) 10^{-5}M; 3) $7.5*10^{-6}$M; 4) $5*10^{-6}$M; 5) $2.5*10^{-6}$M and 6) 10^{-6}M

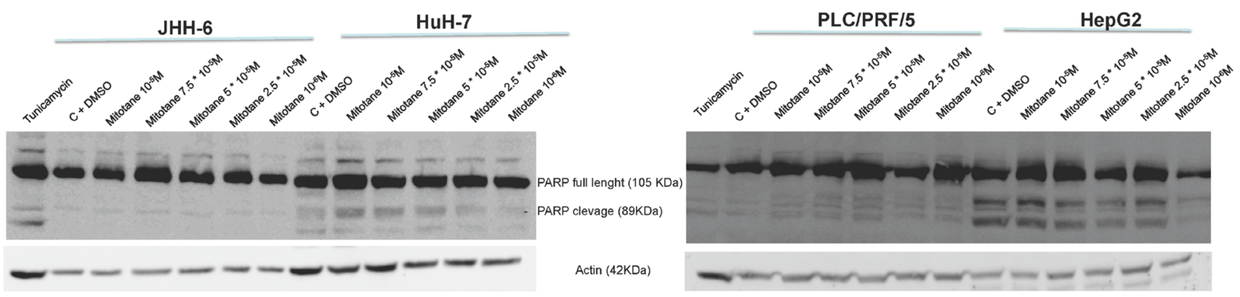


Figure 3: PARP cleavage in HCC cell lines.

RESULTS

Mitotane was able to inhibit cell proliferation in a dose-dependent manner: PLC/PRF/5 with a maximum effect of 54% ($p<0.001$) at 10^{-5}M and minimum effect of 35% ($p<0.01$) at $5*10^{-6}$M; JHH-6 with a maximum effect of 49% ($p<0.001$) at 10^{-5}M and minimum effect of 20% ($p<0.001$) at $5*10^{-6}$M; HuH-7with a maximum effect of 45,5 % ($p<0.001$) at 10^{-5}M and minimum effect of 32% ($p<0.001$) at $7.5*10^{-6}$M; HepG2with a maximum effect of 36% ($p<0.001$) at 10^{-5}M and minimum effect of 15,4% ($p<0.01$) at at $5*10^{-6}$M; THLE-2 with a maximum effect of 72%($p<0.001$) at 10^{-5}M and minimum effect of 29,5% ($p<0.001$) at $2.5*10^{-6}$M and H295 with a maximum effect of 82% ($p<0.001$) at 10^{-5}M and minimum effect of 52% ($p<0.001$) at $5*10^{-6}$M (Figure 1).

Mitotane significantly inhibited the formation and the survival of colonies of HCC cell lines able to form colonies: HepG2 (B) and PLC/PRF/5 C) after three weeks in a dose dependent manner as shown in Figure 2, in which H295 (A) was used as control.

Moreover, Mitotane triggered cell apoptosis as demonstrated by PARP cleavage after 2h of treatment. In particular this clivage is clearly visible in HuH7 and HEPG2 cell at the highest drug concentration 10^{-5}M, as shown in Figure 3.

CONCLUSIONS

In conclusion, these preliminary data demonstrated the *in vitro* antiproliferative and anti survival role of mitotane in HCC cell lines suggesting its potential use in the treatment of HCC. Further studies are mandatory to investigate the molecular mechanisms of action of Mitotane in the regulation of HCC progression.

ECE 2016
620-EP
Endocrine tumours and neoplasia
Claudia Pivonello
Poster presented at: ECE2016 28 – 31 May 2016 MUNICH, GERMANY 18th EUROPEAN CONGRESS OF ENDOCRINOLOGY