COMPARING ANTIPROLIFERATIVE AND PROAPOPTOTIC EFFECTS OF THE PAN-DEACETYLASE INHIBITOR PANOBINOSTAT WITH DOXORUBICIN ON HEPG2 HEPATOCELLULAR CARCINOMA CELL LINE

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ABSTRACT

Panobinostat is a new histone deacetylase inhibitor approved for treatment of advanced multiple myeloma. In this study we compare its effects with doxorubicin which is already used for trans-arterial chemoembolization of hepatocellular carcinoma. *Methods:* anti-proliferative and pro-apoptotic effects of panobinostat and doxorubicin are tested by measuring cell viability, P53, pd1 expression and cell cycle analysis in HepG2 cells cultured in suitable media and incubated with both drugs for 24 hours. *Results:* Panobinostat was found to highly significantly induce

apoptosis via increasing expression of both P53 and pd1 and decrease cell viability and mitosis more than doxorubicin. *Conclusion:* These results suggest that panobinostat may be a potent alternative to doxorubicin for treatment of hepatocellular carcinoma.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a major health problem worldwide as the third cause of cancer-related mortality (1). In HCC chemotherapy, doxorubicin is given by the hepatic artery route associated with some form of hepatic artery occluding

agent, usually tumour shrinkage and partial responses are seen (2). Histone deacetylase (HDAC) inhibitors are new promising anticancer agents (3). HDAC was found to be greatly concerned with regulation of many physiological processes of cell proliferation, differentiation and apoptosis by controlling gene transcription (4). Hep G2 cells are derived from an embryonal malignancy of hepatocellular origin known as hepatoblastoma (5). HepG2 cell line is used in a variety of fields including drug development, and hepatotoxicity (6).

In this study we compare effects of panobinostat with doxorubicin on HepG2 cell line via study of the expression of the pro-apoptotic gene P53 on the cell surface and also the programmed cell death 1 (pd1) pro-apoptotic gene. And we analyze the cell cycle to detect the effect of both drugs on mitosis via checking the percent of cells in the G2/M phase of cell cycle.

MATERIALS AND METHODS

Cell lines and culture conditions

The human hepatocellular carcinoma cell line HepG2 (p53 wt), obtained from Mansoura experimental

research center, were maintained in Dulbecco's modified Eagle medium (DEMEM F12) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin, streptomycin. Cells were maintained at 37°C with 5% CO₂ (7). Cells were treated with 100 nM of either panobinostat (Purchased from Selleck Chemicals in the form of brownish powder dissolved in DMSO (Dimethyl sulfoxide 2%) according to manufacturer instructions) or doxorubicin (in the form of adriacycline 10mg/5ml vial) and all groups are processed for 24 hours before cell collection for further investigations (8).

Analysis of cell viability and apoptosis

Proliferation rate was determined by counting the number of viable cells after Trypan blue staining (9). Flowcytometry technique has been used to estimate cellular DNA content and measure percentage of cells in G2/M phase of the cell cycle (10). The marker P53 and Pd1, both were purchased from abcam chemicals, were mixed well with the cells obtained from cell suspension and measured by flowcytometry (10).

For statistical analysis one way ANOVA followed by post hoc tukey test was used and P value ≤ 0.05 was considered statistically significant.

RESULTS

Panobinostat significantly decreased cell viability compared to both control and doxorubicin at 100 nM concentration in HepG2 cells 24 hours after treatment. Panobinostat also, induced apoptosis significantly more than doxorubicin by induction of both P53 and Pd1 expression on the surface of HepG2 cells. Panobinostat was found to inhibit mitosis and significantly decreased the percentage of cells in G2/M phase of the cell cycle.

Cell count

There was a highly significant decrease in the Number of viable cells in HepG2 cell culture at panobinostat group as compared to that of doxorubi-

cin and control groups respectively ($P \leq 0.001$) (Figure 1).

P53 expression

There was a highly significant decrease in P53 at doxorubicin group as compared to that of control and Panobinostat groups respectively ($P \leq 0.001$) (Figure 2).

Pd1 expression

There was a highly significant increase in Pd1 at panobinostat group as compared to that of control and doxorubicin groups respectively ($P \leq 0.001$) (Figure 3).

G2/M %

There was a highly significant decrease in G2/M phase % at doxorubicin and panobinostat groups as compared to that control group ($P \leq 0.001$). There was significant decrease in panobinostat as compared to doxorubicin group ($P \leq 0.001$) (Figure 4).

Figure (1) : Number of viable cells in HepG2 cell culture and after incubation with either doxorubicin or panobinostat at 100 nM for 24hrs.

Figure (2) : Flowcytometric analysis of P53 expression in HepG2 cells either control or after incubation with doxorubicin or panobinostat at 100nM concentration for 24hrs.

Figure (3) : Flowcytometric analysis of Pd1expression in HepG2 cells either control or after incubation with doxorubicin or panobinostat at 100nM concentration for 24hrs.

Figure (4) : Cell cycle analysis showing G2/M phase percentage in HepG2 cells either control or after incubation with doxorubicin or panobinostat at 100nM concentration for 24hrs.

DISCUSSION

The treatment of HepG2 cells with both panobinostat and doxorubicin showed decreased proliferation and survival, and induced apoptosis and G2/M cell cycle arrest. The P53 protein is a transcription factor related to DNA damage repair, growth arrest and apoptosis (11). To which extent the tumor's p53 status influences the anticancer effects of HDAC inhibitors has not been clearly resolved. The majority of studies point to a largely p53-independent pro-apoptotic action of HDAC inhibitors (12), however, other studies suggest an essential role of p53 in the anticancer effect of HDAC inhibitors treatment. These conflicting observations may be due to the use of different HDAC inhibitors or due to detection methodological differences (13). The cytotoxicity of doxorubicin on the HepG2 cells was determined previously and showed better results on induction of necrosis and apoptosis in different HCC cell lines than other anticancer agents like cisplatin and 5-fluorouracil (14). In the present study panobinostat was found to significantly increase expression of P53 more than doxorubicin by the same concentration and duration. This may explain the lower cellular viability

of HepG2 cells after 24 hours incubation with panobinostat than doxorubicin.

In addition to P53 another apoptotic marker, which is the programmed cell death 1 (Pd1), was tested on the surface of HepG2 cells and found to be highly significantly expressed on Panobinostat treated cells. This marker was formerly discovered and highly expressed on the surface of T lymphocytes and has a high role in development of cancers and increased susceptibility to infections like HBV infection. Its expression on T lymphocytes negatively regulates immune response (15). Pd-1 activation by ligand binding produces a number of intracellular effects that result in T-cell inactivation and reduced proliferation (16). There are very limited studies about the possibility of Pd-1 expression on the surface of cells other than immune cells as its expression anywhere must be associated with higher rates of apoptosis and necrosis. In our study we tested the possibility of its expression on HepG2 cell line in response to drug effects and it was found to be highly and very significantly increased under panobinostat treatment than with doxorubicin.

On study of the effects of panobinostat and doxorubicin on cell cycle progression and affection of mitosis, it was found that both drugs decrease the percentage of cells reached the G2 phase and entered into mitosis in comparison with control. But, this arrest in mitosis was significantly more with panobinostat than doxorubicin.

The cell cycle is an ordered process of events occurring in four stages. During the G1 and G2 (gap) phases, the cell is not dividing but actively metabolizing. In synthesis (S) phase, the DNA replicates resulting in chromosomal duplication. During the mitosis (M) phase, there is separation of chromosomes in the nucleus and division of the cytoplasm (17). There are two main DNA damage checkpoints in the cell cycle at the end of both G1 and G2 phases that can prevent the cell from entering the next S or M phase respectively. There is a family of protein kinases controlling this checkpoint system called the cycline dependent kinases (Cdks) that are further controlled by another complex protein array including the cyclins (18). Doxorubicin was proved to decrease expression of cell division cycle

cdc25C, cdc2/p34, and cyclin B1 in various cell lines. This association suggests that doxorubicin effect on cell growth and apoptosis induction may be due to down-regulation of the G2/M cell cycle regulators and the following mitosis arrest (19). Panobinostat was also found to induce G2/M cell cycle arrest at 24 h of incubation with prostate cancer cell lines. This arrest was induced by phosphorylation of certain cyclins/ Cdks involved in the G2/M phase check point including cdc2 and cyclin B1 (20).

Conclusion

Panobinostat is a new promising anticancer agent and by further studies may be proved to be a more potent alternative to doxorubicin in treatment of HCC.

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الملخص العربى

المقارنة بين مثبط انزيمات الدياتيستيليز بانوبيينوستات

ودوكسوروبيسين فى القدره على تثبيط نمو خلايا سرطان الكبد

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مرض سرطان الكبد من المشاكل الصحية الخطيره وهو السبب الثالث للوفاة المتعلقة بالسرطان. ويتم علاجه عن طريق الاستئصال الجراحي والحقن الكيماوى وزرع الكبد.

يعطى عقار الدوكسوروبيسين لمريض سرطان الكبد عن طريق الحقن فى الشريان الكبدي وقد اثبت بعض الفاعليه دون اللجوء الى اعطائه عن طريق الحقن الوريدي ولا يتم توزيعه على باقى اجزاء الجسم.

من الأدوية الجديده فى علاج السرطان مجموعة مثبطات انزيم الدياتيستيليز والتي تتحكم فى الجينات المسببة للسرطان ومنها سرطان الكبد وقد اثبتت فاعليتها فى علاج حالات سرطان الدم وبعض السرطانات الأخرى.

فى هذه الدراسه تم زراعة خلايا سرطان الكبد الانسانيه (Hep G2) فى وسط ملائم ومعالجتها بكل من عقار الدوكسوروبيسين والبانوبيينوستات ومقارنة تاثير هذه الأدوية على نمو الخلايا السرطانية. وقد اثبتت التجريه تفوق عقار بانوبيينوستات على الدوكسوروبيسين فى تثبيط نمو خلايا سرطان الكبد عن طريق قياس محفزات الاستماته الخلويه (P53) و (pdl) وقياس

نسبة الخلايا الخاضعة لعملية الانقسام الخلوى (G2/M) والتي زادت نسبتها في الخلايا المعالجه بالدوكسوروبيسين عن الخلايا المعالجه بالبانونبينوستات مم يؤكد قدرة البانونبينوستات على ايقاف نمو وتكاثر خلايا سرطان الكبد وتفوقه في ذلك على الدوكسوروبيسين.