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

By
Neimat Abd Elhakam Elmetwaly*, Mona Younis Youssef**, Amal Abd Elhameed Mohammad***, Karawan Mohammad Abd Elrahman***, Farida Hanem Mohammad Ali Elbanna***

From
*Assistant lecturer of clinical pharmacology, Mansoura faculty of medicine
**Lecturer of pathology, Mansoura faculty of medicine
***Professor of clinical pharmacology, Mansoura faculty of medicine

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 chemembolization 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, strep-tomycin. Cells were maintained at 37°C with5% 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 adriacyine 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 Pd1expression 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 doxorubicin and control groups respectively (P≤ 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≤ 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≤ 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≤ 0.001). There was significant decrease in panobinostat as compared to doxorubicin group (P≤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 Pd1 expression 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-fluouracil (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 form 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.

**REFERENCES**


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COMPARING ANTIPROLIFERATIVE AND PROAPOPTOTIC


المتقبل العربي

المقارنة بين مثبطات الدياستيлиз بانوبينوستات ودوكسوروبيسين في القدرة على تثبيط نمو خلايا سرطان الكبد

مقدمه من

تغنت عبد الحكيم التوني - مدرس مساعد الفارماكولوجيا الأكلينيكية كلية الطب - جامعة المنصورة
منى يونس يوسف - مدرس بالبيولوجي كلية الطب - جامعة المنصورة
أمل عبد الحميد محمد - أستاذ الفارماكولوجيا الأكلينيكية كلية الطب - جامعة المنصورة
كروان محمد عبد الرحمن - أستاذ الفارماكولوجيا الأكلينيكية كلية الطب - جامعة المنصورة
فوزي هانم محمد على البنا - أستاذ الفارماكولوجيا الأكلينيكية كلية الطب - جامعة المنصورة

مرض سرطان الكبد من المشاكل الصحية الخطيرة وهو السبب الثالث للوفاة المتعلقة بالسمنة. ويتطلب علاجه عن طريق الاستئصال الجراحي والحقن الكيمياوي وزرع الكبد.

يعتبر عقار الدوكسوروبيسين مريض سرطان الكبد عن طريق الحقن في السرطان الكبيدي وقد أثبت بعض الفاعليات دون اللجوء إلى أعطاه عن طريق الحقن الوردي ولا يتم توزيعه على باقي أجزاء الجسم.

من الأدوية الجديدة في علاج السرطان مجموعة مثبطات انزيم الدياستيليز والتي تتحكم في الجينات المسببة للسرطان ومنها سرطان الكبد وقد أثبتت فعاليتها في علاج حالات سرطان الدم وبعض السرطانات الأخرى.

في هذه الدراسة تم زراعة خلايا سرطان الكبد الإنساني (Hep G2) في وسط ملام أنواع الدوكسوروبيسين والبانوبينوستات ومقارنة تأثير هذه الأدوية على نمو الخلايا السرطانية. وقد أثبتت التجربة فعاق عقار بانوبينوستات على الدوكسوروبيسين في تثبيط نمو خلايا سرطان الكبد عن طريق قياس محضزات الاستفادة الخلوية (pdl) وقياس (P53).

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نسبة الخلايا الخاضعة لعملية الانقسام الخلوي (G2/M) والتي زادت نسبتها في الخلايا المعالجة بالدوكسوروبيربيسين عن الخلايا المعالجة بالباوبيونيستات مم يؤكد قدرة باوبيونيستات على إيقاف نمو وتكاثر خلايا سرطان الكبد وتتفوق في ذلك على الدوكسوروبيربيسين.