EFFECT OF THE TRIAZOLE ANALOG TAN ON GDF-15 EXPRESSION IN HUMAN COLORECTAL CANCER HCT116 CELL LINE

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Abstract

A tumor marker is a term usually refers to proteins that is indicating the presence of a tumor biochemically. They are mainly found in blood or urine and other body fluids, those markers may be used to detect expected primary or secondary tumor. GDF_15 is one of the tumor markers that engaged in controlling apoptosis & inflammatory response during disease & tissue injury). It is assumed to be a biomarker for p53 pathway enhancement in human CA. In the current study we try to assess the effect of our investigational drug TAN on the expression of GDF-15 in in human colorectal cancer HCT116 cell line by comparing its effect with the standard anticancer agents doxorubicin(DOX) and the traditional triazole member; itraconazole (ITC).

Objectives: To assess the effect of the triazole analog TAN on GDF-15 expression in human colorectal cancer cell line HCT116.

Results: TAN shows a significant decrease in GDF15 level at concentration of 20 and 40 μg/ml, and a significant increase at 80 μg/ml concentration.

Conclusions: TAN significantly decreased the GDF15 level in HCT 116 cells in low and moderate concentrations.

Keyword: TAN, GDF15 , HCT116, CRC
Introduction
markers are almost helpful in assessing the progress of the tumor status after primitive chemotherapy and radiotherapy to plan for next management strategies(1).
The typical tumor marker should be both specific and sensitive to diagnose small tumors to permit early detection or aid in tumor screening, little number of markers are specific for a specific tumor. Tumor (1). It is assumed to be a biomarker for p53 pathway enhancement in human CA (2; 4; 5). Its level increase in many tapes of cancers including colorectal cancer(6) and this is associated with poor clinical outcomes, its serum levels in CRC indicates reduced disease overall survival rates & lymph node penetration(7). In this study we try to assess the effect of our investigational drug TAN on GDF-15 expression in comparison with the traditional triazole member ITC and the standard anticancer agent DOX.

GDF-15 is belonged to the β super family of the transforming growth factor & it is correlated with P53 pathway activation in human CA. GDF-15 is engaged in controlling apoptosis & inflammatory response during disease & tissue injury. Normally, GDF-15 expressed in significant amounts by the placenta only; although it is found in little amounts in some tissues like pancreatic, renal & colonic tissues.

Materials and methods
Cell line
HCT-116 colorectal carcinoma cell line(ATCC® CCL-247™) USA

TAN and ITC solutions:
These solutions were prepared in a final concentration 1000 µg/ml for application of biomarker assay, micro titration of these compounds was done as the experiment required.

Doxorubicin (DOX):
The molecular weight for this drug is 580.0 and chemical formula: C27 H29NO11,HCl. This solution was prepared in a final concentration of 1000 µg/ml for application of biomarker assay, and for use micro titration of this compound was done as the experiment required.

GDF-15
By using Human GDF-15 ELISA kit (Enzyme linked immune assay for GDF-15 Cloud clone, USA):
A. Principle: In these kits The micro-plate supplied has been pre-covered with a specific antibody to GDF-15. Standards or specimens are then added to the proper micro-plate wells with a biotin-conjugated antibody specific to GDF-15. After that , a conjugation of Avidin conjugated and Horseradish Peroxidase (HRP) is introduced to each micro-plate well and incubated.
When TMB substrate solution is applied, only the wells which have GDF-15, biotin-conjugated antibody and enzyme-conjugated Avidin will show a color difference. The enzyme-Substrate reaction is ended by the applying of sulphuric acid solution and the color difference is detected by spectrophotometer at a wavelength of 450 nm. GDF-15 concentration in the specimens is then detected by comparing the O.D. of the specimens to the standard curve.
B. GDF-15 concentration is measured for untreated cells and that treated with 20,40, and 80 µg/ml of different compounds including TAN, ITC, and DOX by final absorbance reading at 450 nm wavelength ELISA reader

Statistical analysis
Statistical descriptive measures (mean and median ) and scattering measures(SD and SE ) in addition to R² and ANOVA were done using IBM SPSS software for windows of version 21.0. The P value considered < 0.05 for all tests.
**Results**

After 24 hours incubation of drug treated and untreated HCT 116 cells the cells are submitted to the standard procedure of tumor marker GDF-15 assay. IC50 concentration of standard and test drugs (that obtained from our research Evaluation of the apoptotic effect of the triazole analog TAN on human colorectal Cancer cell line HCT 116 by flow cytometry) (8), IC50 which was for TAN: 63.94 µg/ml, for ITC: 21.85 µg/ml and DOX: 345.176 µg/ml).

TAN shows a significant decrease in GDF15 level at concentration of 20 and 40 µg/ml, and a significant increase at 80 µg/ml concentration with negative R² (table 1 and figure 1).

Itraconazole shows a significant decrease in GDF15 level with positive R, (table 2 and figure 2). DOX reveals a significant decrease in GDF15 level but with negative (table 3, and figure 3).

**Discussion**

TAN shows a significant decrease in GDF-15 level at 20 and 40 µg/ml concentrations and a significant increase at concentration of 80 µg/ml with negative R. This reveals that TAN has concentration independent effect on GDF-15 level. Lakhal et al., mentioned that hypoxia and iron depletion in the cell are responsible for elevating GDF-15 levels (9), this may explain the unexpected increase in the GDF-15 levels in cells treated with high concentrations of TAN as it could be due to hypoxia that results from apoptotic ITC shows a significant decrease in GDF-15 level with positive R, so it demonstrates a dose dependent effect on GDF-15 level.

DOX reveals a significant decrease in GDF-15 level but with negative R, this means that DOX has a dose independent ability to decrease GDF-15 level, however, in an invitro study on isolated cardiomyocytes GDF-15 shows a very interesting profile and responds to doxorubicin with a significant dose dependent up-regulation at day 1, 2, 7, and 14. Based on the gene expression profile analysis of GDF-15 (10).

**Conclusions**

1. TAN showed a significant dose independent decreasing ability on the GDF-15 levels in low and moderate concentrations in HCT116 cells.
2. ITC appeared a significant dose dependent reducing effect on the GDF-15 in HCT116 cells.
3. DOX revealed a significant dose independent reducing effect on the GDF-15 in HCT116 cells.

**Acknowledgement**

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**References**


Table (1): The Effects of Different Concentrations of TAN on GDF-15 Levels

<table>
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<th>TAN Dose (μg/ml)</th>
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<th>Level of significance</th>
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Table (2): The Effects of Different Concentrations of ITC on GDF-15 Levels

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Table (3): The Effects of Different Concentrations of DOX on GF15 Levels

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Figure (1): The Effects of Different Concentrations of TAN on GDF-15 Levels in Pg. / ml obtained from HCT116 homogenate 24 hr. after addition of TAN.

Figure (2): The Effects of Different Concentrations of ITC on GDF-15 Levels. Levels in Pg. / ml obtained from HCT116 homogenate 24 hr. after addition of ITC.
Figure (3) The Effects of Different Concentrations of DOX on GF15 Levels in Pg./ml obtained from HCT116 homogenate 24 hr. after addition of DOX.