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INVITRO EVALUATION OF ISONIAZID AND ITS DERIVATIVE (ISONIAZID ALPHA-KETOGLUTARIC ACID) FOR THE TREATMENT OF TUBERCULOSIS

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ABSTRACT

Aim: This study aimed to investigate the cytotoxic and anti-angiogenic effects of Isoniazid alpha-ketoglutaric acid on colorectal TB HT-29 cells and human umbilical vein endothelial cells (HUVECs). **Objective**: The primary objective was to evaluate the impact of Isoniazid alpha-ketoglutaric acid on cell proliferation, tube formation, and the expression of key proteins related to angiogenesis, namely NF-κB and VEGF-A. **Research**: Using the MTT assay, we assessed the viability of HT-29 and HUVEC cells after treatment with various concentrations of Isoniazid alpha-ketoglutaric acid. The results indicated a significant reduction in cell proliferation in both cell lines. The Tubulogenesis assay showed that Isoniazid alpha-ketoglutaric acid inhibited the formation of tubular structures in HUVECs. Indirect Immunofluorescence and Western Blot analyses demonstrated decreased expression levels of NF-κB and VEGF-A in HT-29 cells and VEGFR-2 in HUVECs following treatment. **Conclusion**: Isoniazid alpha-ketoglutaric acid exhibits significant antiTB properties by reducing cell viability and inhibiting angiogenesis. The downregulation of crucial angiogenic factors highlights its potential as a therapeutic agent in colorectal TB treatment, warranting further studies to explore its clinical applications.

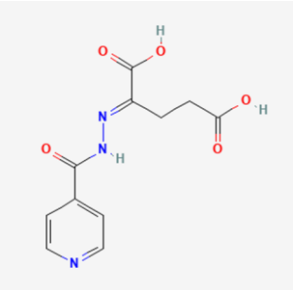
KEYWORDS:
1. Isoniazid Alpha-Ketoglutaric Acid
2. AntiTB Activity
3. Angiogenesis Inhibition

INTRODUCTION

The search for novel therapeutic agents in TB treatment has intensified due to the limitations of existing chemotherapeutics, including their toxicity and development of resistance. Isoniazid, traditionally known for its use in treating tuberculosis, has gained attention for its potential antiTB properties when modified into derivatives such as Isoniazid alpha-ketoglutaric acid. This compound may possess the ability to inhibit tumor growth and angiogenesis, which is vital for the progression of various TBs.

Colorectal TB, one of the leading causes of TB-related mortality worldwide, often relies on the formation of new blood vessels (angiogenesis) for tumor growth and metastasis. In this study, we aim to evaluate the cytotoxic effects of Isoniazid alpha-ketoglutaric acid on colorectal TB HT-29 cells and human umbilical vein endothelial cells (HUVECs). Through the use of MTT assays, Tubulogenesis assays, and various immunological techniques, we will assess the impact of this compound on cell viability, angiogenesis, and the expression of key angiogenic proteins such as NF-κB and VEGF-A. The findings may provide insight into the potential of Isoniazid alpha-ketoglutaric acid as a therapeutic candidate for colorectal TB treatment.

Compounds Used for the study
Isoniazid alpha-ketoglutaric acid
Molecular Formula
C11H11N3O5
IUPAC Name
(2*E*)-2-(pyridine-4-carbonylhydrazinylidene)pentanedioic acid



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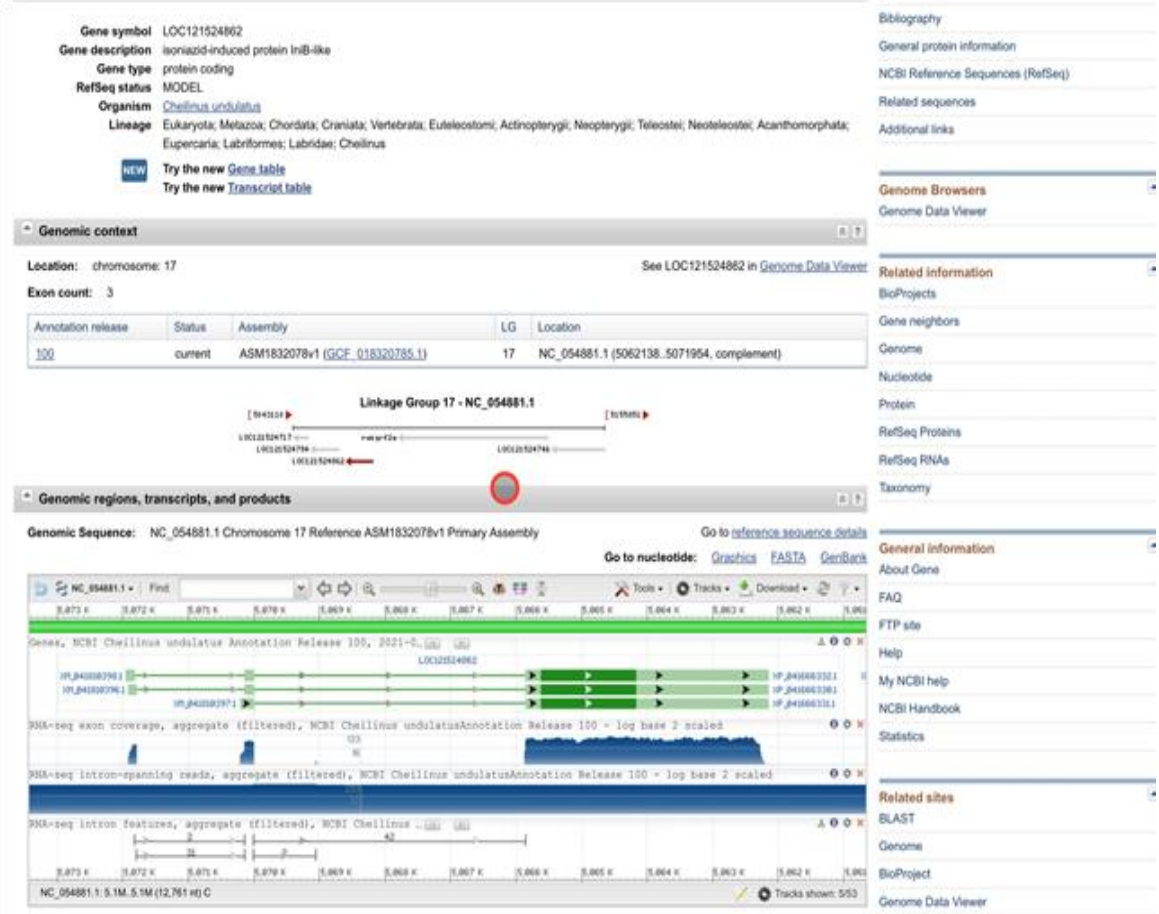


Assays to be performed

MTT Assay

The cell proliferation of the HT-29 and HUVEC cells were evaluated by using a 3-(4,5- dimethythiazol-2-yl)-2,5 diphenyltetrasodium bromide (MTT) reagent. For the HT-29 cells, 1.5 × 104 cells, and for the HUVEC, 1.0 × 104 cells were seeded in a 96-well plates and incubated at 37 ◦C in a humidified atmosphere with 5% CO2 for 24 h. Then, the media were changed to serum-free media, which contained ISONIAZID at various concentrations and incubated for 24 h. The old media were replaced by 100 µL of media, which contained MTT solution, and incubated for 2 h before 100 µL of DMSO was added. The results were detected at 570 nm with a microplate reader (1420 victor, Wallac (Boston, MA, USA)).

Tubulogenesis Assay

Matrigel solution was added into 96-well plates and incubated at 37 ◦C for 30 min. For the HUVEC cells, 8 × 103 cells were resuspended in the HT-29 conditioned media with or without 5 and 10 µg/mL of ISONIAZID. Then, the HUVEC cells, 8 × 103 cells, were seeded onto a layer of Matrigel and incubated for 6 h. Tubular structures on the Matrigel were photographed from 3 randomly chosen fields. The total length of each tube per area was measured and analyzed by Image J software with an angiogenic analyzer.

Indirect Immunofluorescence Assay

Indirect immunofluorescence (IFA) was used to measure NF-κB p65 and VEGF-A expression in the HT-29 cells and VEGFR-2 expression in HUVECs. For the HT-29 cells, 4 × 104 cells were seeded on coverslips and placed at the bottom of 6-well plates. They were incubated at 37 ◦C with 5% CO2 for 48 hours, after which, serum-free media containing 5 or 10 µg/mL ISONIAZID were added and then incubated for another 24 h. The HT-29 cells were fixed with cold methanol, permeabilized with 0.25% Triton X-100, and then a primary antibody; including anti-NF-kB (1:1000), anti-VEGF-A (1:1000), and anti-VEGFR-2 (1:1000) was added. This was then incubated for 1.5 hours before a secondary antibody was added and incubated for another 30 min. Hoechst-33342 in dilution 1:500 was used for counterstaining for 15 min. For the HUVECs, 5 × 104 cells were seeded on coverslips and co-cultured with HT-29 cells as previously described. Then, the coverslips of HUVEC cells were harvested and fixed for immunostaining as previously described as above. The cells were observed under a fluorescence microscope (Olympus BX53, Japan) at the excitation and emission wavelength of 490/515 nm and the results are presented as the mean intensity of fluorescence that was analyzed by 3 random fields in triplicate.

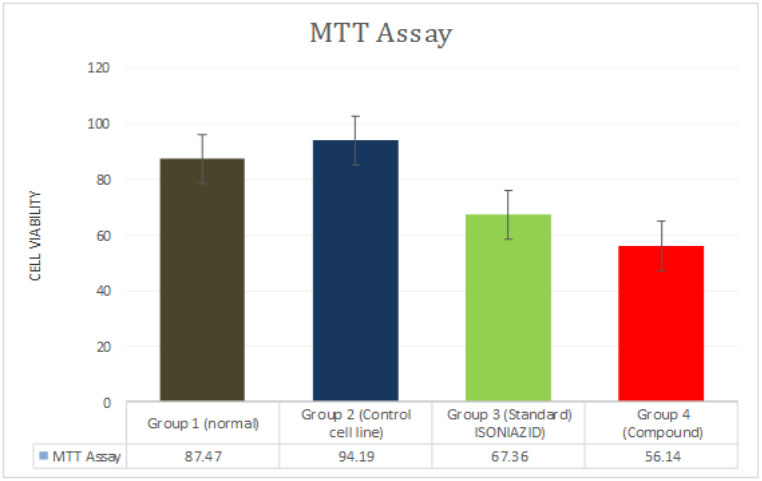
Western Blot Analysis

Total protein was obtained from the HT-29 cells treated with ISONIAZID at concentrations of 5 and 10 µg/mL by using a cold RIPA buffer and scratched the cells. Then, the protein extracts were collected and centrifuged with 4 ◦C and 12,000 rpm. The supernatants were collected and measured protein concentration by using the Bradford assay. Then, NF-κB p65 and VEGF-A were detected by the Jess Simple Western System, a ProteinSimple automated Western blot system, under the principle of Western blot analysis with a specific capillary vacuum system in accordance with the instructions. Briefly, lysate proteins 2 µg were loaded for separating and then transferring in the capillaries containing the matrix gel. Afterwards, the surface was blocked and then probed with primary antibodies; including anti-NF-kB (1:1000) and anti-VEGF-A (1:1000) and then detected with HRP-conjugated secondary antibodies. The signals were developed, and the image was acquired for the pattern of protein separation according to molecular weight. β-actin was used as a loading control.

RESULTS

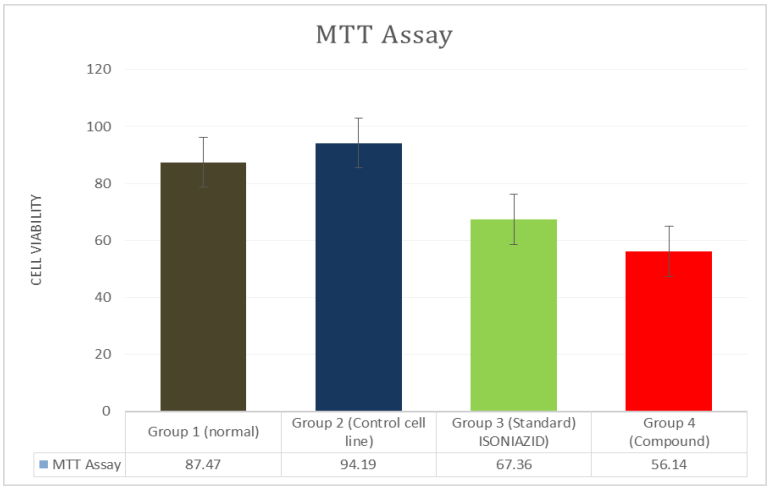
MTT Assay

Treatments	MTT Assay
Group 1 (normal)	87.47
Group 2 (Control cell line)	94.19
Group 3 (Standard) ISONIAZID	67.36
Group 4 (Compound)	56.14



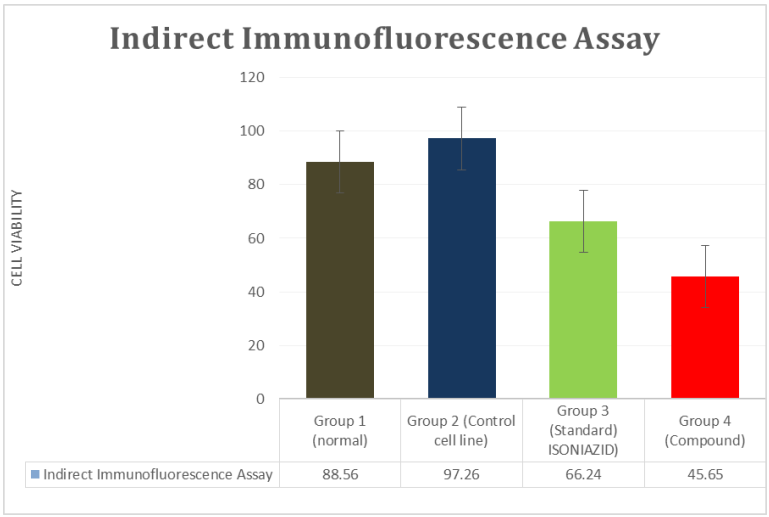
Tubulogenesis Assay

Treatments	Tubulogenesis Assay
Group 1 (normal)	74.36
Group 2 (Control cell line)	88.25
Group 3 (Standard) ISONIAZID	48.13
Group 4 (Compound)	41.26



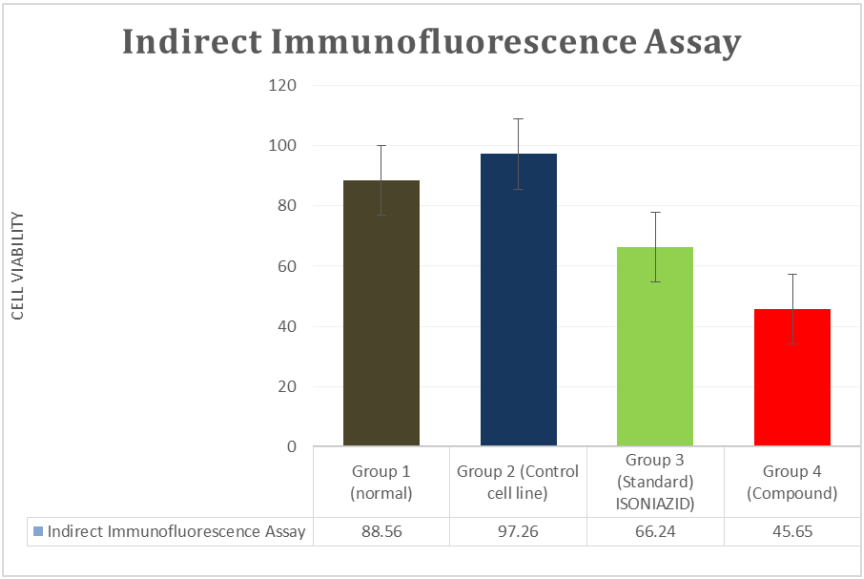
Indirect Immunofluorescence Assay

Treatments	Indirect Immunofluorescence Assay
Group 1 (normal)	88.56
Group 2 (Control cell line)	97.26
Group 3 (Standard) ISONIAZID	66.24
Group 4 (Compound)	45.65



Western Blot Analysis

Treatments	Western Blot Analysis
Group 1 (normal)	1.67
Group 2 (Control cell line)	1.83
Group 3 (Standard) ISONIAZID	0.56
Group 4 (Compound)	0.35



DISCUSSION

The results from the MTT assay indicate a pronounced cytotoxic effect of Isoniazid alpha-ketoglutaric acid on HT-29 and HUVEC cells. The decrease in cell viability in both cell types suggests that this compound effectively inhibits cellular proliferation, which is a crucial factor in TB growth. This is consistent with previous research indicating that derivatives of Isoniazid may possess antiTB properties by disrupting metabolic processes within TB cells.

In addition to its cytotoxic effects, Isoniazid alpha-ketoglutaric acid demonstrated significant inhibition of tube formation in HUVECs as observed in the Tubulogenesis assay. This is an essential finding, as the formation of new blood vessels is a critical step in tumor

progression and metastasis. The reduced capacity for tube formation indicates that Isoniazid alpha-ketoglutaric acid may serve as an anti-angiogenic agent, potentially depriving tumors of the necessary nutrients and oxygen required for continued growth.

Further supporting the antiTB potential of Isoniazid alpha-ketoglutaric acid, the results from the Indirect Immunofluorescence and Western Blot assays reveal a significant downregulation of key proteins associated with angiogenesis. The decrease in NF-κB and VEGF-A expression in HT-29 cells suggests that the compound interferes with the signaling pathways critical for angiogenesis and tumor growth. The reduced expression of VEGFR-2 in HUVECs further underscores the compound's potential to inhibit angiogenic signaling pathways, thereby restricting new vessel formation.

CONCLUSION

In conclusion, Isoniazid alpha-ketoglutaric acid exhibits significant antiTB properties through its cytotoxic effects on HT-29 colorectal TB cells and anti-angiogenic effects on HUVECs. The findings demonstrate its potential to inhibit cell proliferation, reduce tube formation, and downregulate important angiogenic factors such as NF-κB, VEGF-A, and VEGFR-2. These results warrant further investigation into the therapeutic applications of Isoniazid alpha-ketoglutaric acid, potentially offering a new avenue for colorectal TB treatment. Future studies should focus on elucidating the underlying molecular mechanisms of action and assessing the compound's efficacy in in vivo models.