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Molecular Detection of gene Leptin receptor promoter (Lepr) in female rats treated with Drug

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Abstract:

The aim of the research is the molecular detection that metformin and propylthiouracil induce on the leptin gene .The research was conducted on female rats with an average weight ranging from 150-180 grams and an age group ranging from 4.5-5.5 months, was divided into four groups and all groups were given water and food throughout the trial period, the results of the current study showed that metformin and propyl thiouracil in terms of mutation rate, as the combination therapy resulted in higher values in the percentage of mutations and deletions

Introduction

The leptin gene is located on the human chromosome 7q31. 3, while in the mouse it is located on chromosome 6 , the similarity between leptin precursors in humans, mouse and rat is very high, that is, the similarity of Mouse and rat is 96% and the similarity of Mouse and human is 83%, the DNA of the leptin gene contains more than 15,000 base pairs and consists of three exons and two introns[1]. Researchers from Rockefeller University in New York announced in 1994 the first successful cloning of complementary DNA (cDNA) of leptin using the method of local cloning, leptin was identified as the gene of the mouse Psammomys Obsus ob / ob in genetic obesity syndromes, where he has strong control over food intake, body weight and energy expenditure [2] The name leptin is derived from the Greek wordleptos, which means thin or light Thin" [3]. The LEP gene is encoded to produce a 16K / Dalton glycoprotein known as leptin, which contains 761 amino acids[4]. Leptin is involved in the regulation of body weight as fat cells in the body release the hormone leptin in proportion to the size of the body and with the accumulation of fat in the cells, more leptin is produced, leptin binds to a protein called the leptin receptor and is active as it is proportional to the receptor such as lock and key and the leptin receptor protein is found on the surface of cells in many The organs and tissues of the body, including the hypothalamus hypothalamus, where the function of leptin in the body is related to regulating the balance between food intake and energy expenditure, as the

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primary role of leptin is to serve as a marker of long-term energy stores of the central nervous system[5] The leptin receptor is a glycoprotein belonging to the cytokine family of Class 1 that is significantly associated with the signal transduction component qp130 of the interleukin-6 receptor (IL-6) and is a granulocyte colony-stimulating factor (G-CSF) and a leukemia inhibitory receptor factor (LIF), the leptin receptor contains at least six similar forms with a difference in inclusion (Ob,Ra,b,c,d,e,f) [6] .Metformin hydrochloride (1-1 dimethyl biguanide hydrochloride) its chemical formula is C4H11N5, it is in the form of a white crystalline powder soluble in water and slightly soluble in ethanol, but insoluble in acetone, ether and chloroform[7]. Metformin is the first-line drug of choice for the treatment of Type II diabetes mellitus Especially in overweight and obese people who have normal kidney function, metformin is from the Biguanide class and is sold under the trade name Glucophage[8]. Propylthiouracil (PTU) is classified from the pharmaceutical family thiamide, an organic compound chemically synthesized from ethyl 3-oxohexanoate and thiourea, its chemical name is 6-propyl-2thiouracil, its chemical formula is C7H10N2OS, as it is in the form of a white crystalline solid that can be found in the form of crystals or powders it dissolves in organic solvents such as ethanol, ether and acetic acid[9].

Key words: Metformin, Propylthiouracil, Leptin gene

Materials and Methods:

Experimental animals

This study was conducted for the period(2023\11\20 to 2024\1\18) at the Animal House of the Faculty of Veterinary Medicine-Tikrit University, and this study used female albino rats, numbering (28) rats, the animals were placed in pre-prepared clean plastic cages and the necessary cages were prepared and sterilized by (ethanol alcohol concentration of 99%) and were divided into (4) groups, each group included (7) animals of similar weights these animals were exposed to laboratory conditions, and of which 12 hours of light and water 12 hours of darkness, The temperature was set to (23-25) degrees Celsius and the animals were left for two weeks to settle and adapt in their new place after making sure they were free of diseases and given water and food continuously throughout the experiment .

1-The first group **(Control)** :was given water and food daily for 50 days.

2- The second group **(Metformin):** was given 1 ml of distilled water for 25 days and from the 26th day metformin (50 mg /kg) of body weight was dosed daily to 50 days.

3- The third group **(Propylthiouracil):** this group was given from the first day to day 25 propylthiouracil at a dose (15 mg/kg) of body weight and then from day 26 to day 50 doses of distilled water.

4- The fourth group **(Propylthiouracil & Metformin)** was given propylthiouracil from the first day to day 25 and then from day 26 to day 50 dosed with metformin .

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Drugs used in the experiment

- The drug propyl thiouracil in tablet form, manufactured by the Italian drug company Recordati, where one tablet had a concentration of 50 mg, dissolved in distilled water and then dosed rats by (1) ml per rat, at a dose of (15 mg /kg) of body weight [10].
- The drug metformin in the form of a white crystalline powder was obtained from the general company for the manufacture of medicines \Samarra and was dissolved in distilled water, then the rats were dosed by (1) ml per rat and at a dose of (50 mg / kg) of body weight [11].

Collect blood samples

After the end of the experiment and the completion of the autopsy of the animals, the blood was drawn from the heart directly by cardiac Puncture and (5) ml of blood was withdrawn and (2) mL was placed in tubes containing anticoagulant tube EDTA and was kept at a temperature of (20-) in preparation for the subsequent DNA extraction process.

Molecular Analysis

Molecular studies were conducted at the Department of central laboratories – presidency of Tikrit University, which included DNA extraction and measurement of its concentration and purity based on the methodblood g DNA Miniprep System Method Relia PrepTM equipped by the American company Promega [12]. The leptin gene was detected using the Thermal Cycler PCR equipped by Applied bio system\Sweden with a final volume of 25 microliters, as well as using specialized primers[13].

Forward primer:´CTCATTTACCCAGGGGGTCT

Reverse primer:´ACACCCAGAGAAGGCAAGTG

Statistical Analysis($P < 0.05$)

For some samples; A4, A5, A7, A9 and A10, SNPs and Windels have statistically significant values indicating a real effect of the therapies; metformin or propylthiouracil on the Leprot gene. Also, chi-squared ($\chi 2$) this value gives the chi-squared test value for suitability for testing genotype frequencies of mentally ill patients against the expected genotype frequencies according to the Hardy-Weinberg scale. Indicates a value greater than 3.84. To one degree of freedom that the observed frequencies differ significantly from the predicted ones; therefore, it is highly likely that the distribution of SNP in mice is driven by drug treatments[14].

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PCR Program

he samples are inserted into the thermal polymerizer device and the program (heat / time) is applied at a temperature of 94 degrees Celsius for the first stage of the metamorphosis for 5 minutes, followed by 35 cycles of the first metamorphosis 94 for 45 seconds and the primer bonding degree 58 degrees Celsius for 45 seconds, then the initial elongation stage at a temperature of 72 for 45 seconds and then the final elongation stage 72 for 7 minutes . After the end of the program, the samples were left to cool the device at a temperature of 4 degrees Celsius and then the electrical relay was carried out .

RESULTS AND DISCUSSION

DNA Extraction:

The DNA genetic material of the control group and the treatment group was extracted with metformin, propylthiouracil and propylthiouracil & metformin and DNA with a concentration of 507.7 to 248.3 ng/mL with a strength ranging from 1.6 to ng/ml 1.9 was obtained as shown in Figure(1).

Figure (1): model of the results of electrophoresis of DNA extraction

Leptin gene detection:

The results of the analysis of the PCR technique showed the receptor of the leptin gene as shown in Figure (2)

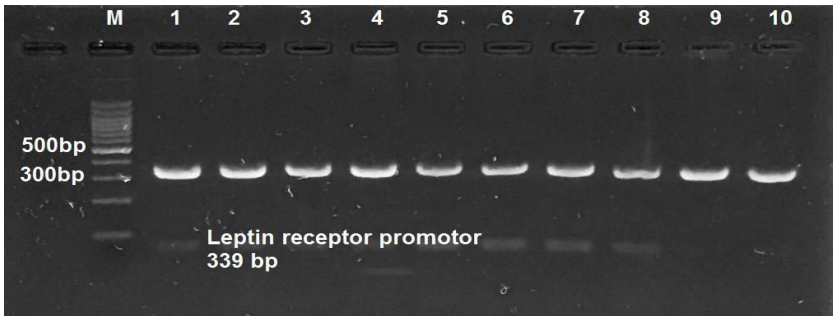

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Figure (2) :electrophoresis of the results of the PCR amplified segment of the leptin gene receptor on acarose gel 2% concentration for the control group and the treatment group with metformin, propylthiouracil, metformin & propylthiouracil in female rats

Table (1): represents the polymorphisms of single nucleotides (SNPs) and insertions and deletions(Indels) in the Leport gene for 12 samples of the study samples, where samples from (1-3) represent the control group, while samples from (4-6) the metformin treatment group, while samples from (7-9) the propylthiouracil treatment group and samples from (10-12) the rubylthiouracil& metformin treatment group .

Sample ID	Position	Mutation Type	Base Change	Type of SNP	Genotype (Based on Sequence Alignment)	Allele Frequency (p/q)	Chi-Square (χ^2)	P-value
A1	758	Transversion	G → T	SNP	Homozygous wild-type (AA)	p = 1	0.5	0.47
A2	818	Transition	C → T	SNP	Homozygous wild-type (AA)	p = 1	0.3	0.58
A3	715	Deletion	-A	Indel	Homozygous wild-type (AA)	p = 1	0.1	0.75
A4	708	Transversion	G → T	SNP	Heterozygous (Aa)	p = 0.75, q = 0.25	4.1	0.042
A5	754	Deletion	-T	Indel	Homozygous mutant (aa)	p = 0.5, q = 0.5	5.2	0.028
A6	977	Transition	T → C	SNP	Homozygous wild-type (AA)	p = 1	0.2	0.65
A7	757	Transversion	T → G	SNP	Homozygous mutant (aa)	p = 0.4, q = 0.6	4.6	0.039
A8	819	Deletion	-T	Indel	Heterozygous (Aa)	p = 0.5, q = 0.5	3.9	0.049
A9	820	Transversion	G → T	SNP	Homozygous mutant (aa)	p = 0.25, q = 0.75	6.2	0.01
A10	977	Transversion	T → G	SNP	Heterozygous (Aa)	p = 0.6, q = 0.4	4.5	0.043
A11	978	Transversion	G → T	SNP	Homozygous wild-type (AA)	p = 1	0.4	0.55
A12	756	Deletion	-G	Indel	Heterozygous (Aa)	p = 0.4, q = 0.6	3.7	0.052

Control group (A1-A3): the control group showed an insignificant number of single nucleotide polymorphisms, with dominant AA alleles. This suggests that the analogue of the Leprot gene

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promotes that under regular physiological functioning, Leprot carries high accuracy which means few or no mutations. The absence of single nucleotide polymorphism in the control group indicates that the Leprot gene in mice shows high preservation and low levels of polymorphism under normal physiological conditions. This result supports the proposed theory that the leptin signaling pathway is involved in metabolic regulation and almost identical populations, with rare evidence of spontaneous mutations in this gene.

Metformin Group (A4-A6): metformin-treated samples showed a heterozygous mutation represented by SNPs in sample A4 at position 754 and a deletion represented by A5. And in the presence of a heterozygous Leprot mutation, metformin has the ability to cause genetic instability in the gene, but the effect is moderate, since most mutations are heterozygous and not homozygous. It is understood that metformin alters metabolic actions by reducing weight and fat [15; 18] and functional differences in genes involved in leptin signaling may be related to insulin action and metabolic control. Some mutations identified in the metformin group suggest that metformin alters gene expression by altering epigenetic marks and may cause slight polymorphism of metabolic genes. Leptin is known to play a role in the regulation of food intake and metabolism[16;19] through the Lippert gene encoding the sensitivity of leptin receptor tissues, and there is reason to believe that metformin may have altered the leptin signaling pathway through increased leptin sensitivity in tissues that may have caused the appearance of SNPs in the Lippert gene.

Propylthiouracil group (A7-A9): more severe SNP was recorded specifically in samples treated with propylthiouracil where some samples (A7, A9) showed homozygous mutations (aa). Propylthiouracil may alter transcription and epigenetic differences in the leptin pathway as antithyroid drugs which have been reported to influence thyroid hormone regulation of metabolism[17;20 ;21 ;22] The SNPs identified in the propylthiouracil group correspond to the action of this drug on the thyroid hormone turnover. Their results also proved that propylthiouracil affects thyroid signaling networks along with other metabolic genes with leptin among them. Changes in leptin signaling can be due to differences in thyroid hormone levels which may be the reason for the large number of mutations in the group treated with propylthiouracil.

Propylthiouracil & metformin group (A10-A12): in terms of mutation rate, combination therapy yielded higher values representing mutations and deletions, for example, A10 at position 977. The combination of the use of metformin and propylthiouracil seems to cause more profound changes. The results of the Lippert gene may indicate a synergistic or even an upward effect on the genetic control of this pathway. This may be due to the synergistic effect of variability in metabolic pathways of action, insulin resistance and thyroid function. The two drugs, metformin and propylthiouracil, had a cumulative mutagenic effect compared to the individual effect of the two drugs on the Lippert gene because metformin and propylthiouracil together increase the frequency of SNPs and indels more than the two drugs separately. In both antidiabetic and antithyroid drugs, the severity of the observed metabolic effects can be explained by the disruption of normal leptin signaling. This interaction may impose potential effects on energy balance and metabolic regulation that may be greater than the effects caused by each drug individually

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CONCLUSION:

The results of the current study show that metformin and propylthiouracil, individually and in combination with each other, affect epigenetic changes in the Leprot gene, changes that may affect leptin signaling and metabolic control. The control group showed the importance of this gene under normal conditions and the change as a result of these drugs which may lead to some genetic variability manifested in terms of SNP and indels. The results presented here indicate that both metformin and propylthiouracil affect metabolic genes and pathways involved in the regulation of insulin and thyroid hormone.

References:

1 .Zhao S, Kusminski CM, Scherer PE. Adiponectin, leptin and cardiovascular disorders. Circ Res. 2021;128(1):136-49.

2 .Martins T, Castro-Ribeiro C, Lemos S, Ferreira T, Nascimento-Gonçalves E, Rosa E, et al. Murine models of obesity. Obesities. 2022;2(2):127-47.

3 .Mantzoros CS, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, et al. Leptin in human physiology and pathophysiology. Am J Physiol Endocrinol Metab. 2011;301(4):E567-84.

4 .Waheeb AA, Taher MJ, Fahad A, Sarray TR. Thyroid status effect on leptin hormone and lipid profile in female patients with thyroid dysfunction. J Cardiovasc Dis Res. 2020;11(4):200-4.

5 .Yaghootkar H, Zhang Y, Spracklen CN, Karaderi T, Huang LO, Bradfield J, et al. Genetic studies of leptin concentrations implicate leptin in the regulation of early adiposity. Diabetes. 2020;69(12):2806-18.

6 .Adiga U, Banawalikar N, Mayur S, Bansal R, Ameera N, Rao S. Association of insulin resistance and leptin receptor gene polymorphism in type 2 diabetes mellitus. J Chin Med Assoc. 2021;84(4):383-8.

7 .Ismail AH, Al-Garawi ZS, Al-Shamari K, Salman AT. Metformin compounds: A review on the importance and the possible applications. J Phys Conf Ser. 2021;1853(1):012060.

8 .Haque MSU, Khan UZ, Memon RA. Pharmacological management of diabetes. In: BIDE's Diabetes Desk Book. Elsevier; 2024. p. 71-101.

9 .Negm SH. Effect of soybean, virgin coconut and Moringa oleifera seed oils on the propylthiouracil induced hypothyroidism in rats. Alex Sci Exch J. 2019;40(Oct-Dec):649-59.

ISSN: 0975-3583, 0976-2833 VOL15, ISSUE 11, 2024

10 .Yu W, Wu N, Li L, Wang J, OuYang H, Shen H. Side effects of PTU and MMI in the treatment of hyperthyroidism: a systematic review and meta-analysis. Endocr Pract. 2020;26(2):207-17.

11 .Veiga-Matos J, Remião F, Motales A. Pharmacokinetics and toxicokinetics roles of membrane transporters at kidney level. J Pharm Pharm Sci. 2020;23:333-56.

12 .Nouri MA, Al-Halbosiy MM, Dheeb BI, Hashim AJ. Cytotoxicity and genotoxicity of gliotoxin on human lymphocytes in vitro. J King Saud Univ Sci. 2015;27(3):193-7.

13 .Mohammadi-asl J, Larijani B, Khorgami Z, Tavangar SM, Haghpanah V, Kheirollahi M, et al. Qualitative and quantitative promoter hypermethylation patterns of the P16, TSHR, RASSF1A and RARβ2 genes in papillary thyroid carcinoma. Med Oncol. 2011;28:1123-8.

14 .Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. Methods. 2001;25(4):402-8.

15 .van der Aa MP, Elst MAJ, Van De Garde EMW, Van Mil EGAH, Knibbe CA, van der Vorst MM. Long-term treatment with metformin in obese, insulin-resistant adolescents: results of a randomized double-blinded placebo-controlled trial. Nutr Diabetes. 2016;6(8):e228.

16 .Friedman JM. Leptin and the endocrine control of energy balance. Nat Metab. 2019;1(8):754-64.

17. Jabbar A, Pingitore A, Pearce SHS, Zaman A, Iervasi G, Razvi S. Thyroid hormones and cardiovascular disease. Nat Rev Cardiol. 2017;14:39-55.

18 AL-Samarraie, M. Q., Omar, M. K., Yaseen, A. H., & Mahmood, M. I. (2019). The wide spread of the gene haeomolysin (Hly) and the adhesion factor (Sfa) in the E. coli isolated from UTI. Journal of Pharmaceutical Sciences and Research, 11(4), 1298-1303.

19 Al-Samarraie, M. Q., Yaseen, A. H., & Ibrahim, B. M. (2020). Molecular study of polymorphism for gene TNF-alpha using ARMS-PCR technique for patients with rheumatoid arthritis. Biochem Cell Arch, 19(2), 4285-4290.

20 AL-Samarraie, M. Q., AL-Obaedi, A. I., Hamed, N. M., & AL-Azzawie, A. F. (2021). Molecular Identification of Aspergillus nigar Using Randomly Amplified Polymorphic Deoxyribonucleic Acid Polymerase Chain Reaction Technique. Journal of Drug Delivery Technology, 11(4), 1221-1224.

21 Majeed, H. M., Noomi, B. S., & AL-Samarraie, M. Q. (2020). Detection of some virulence factors and antibiotic sensitivity test of enterococcus faecalis isolated from sheep by multiplex pcr. European Journal of Molecular & Clinical Medicine, 7(09), 68-74.

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22 Al-Sammarraie, H. W. M., Al-Samarraie, M. Q., & Al-Obaedi, A. I. (2023). Detection of Hepatitis B virus (HBV) by using the RT-PCR and Serological Assay for Detecting Surface Antigen HBsAg. Journal of Advanced Zoology, 44.