Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(1): 288-293. **OPEN ACCESS**

A comparative study on the effect of erythropoietin and insulin on DNA, RNA and proteins in rat kidney

Abdul Rahman A I Alyahya

College of Applied Medical Sciences Shaqra University, **Saudi Arabia**

***Correspondence: alya7ya@su.edu.sa** **Received 25-12-2019, Revised: 13-02-2020, Accepted: 15-02-2020 e-Published: 16-02-2020**

This study was aimed to determine the effect of erythropoietin and insulin on total DNA, Genomic DNA, RNA contents and protein in rat kidney after administration for a short period of seven days. Two doses of erythropoietin (recombinant human erythropoietin EPO) were administered at two different doses of 100 IU/kg and 250 IU/kg and insulin (Rec Hu insulin) at doses of 0.05IU/kg & 0.125 U/kg were daily injected subcutaneously in rats for one week. The kidneys were isolated for extraction of protein and nucleic acids. The genomic DNA was loaded on 1% agarose and RNA was loaded on agarose 1.3%. Proteins were loaded by SDS / PAGE. The results indicated that genomic DNA from rat kidney was 10 kbp after one-week injection of erythropoietin, which was not different from that obtained with control. The molecular size of genomic DNA from rat kidney after one-week injection was similar in both insulin and control groups, which was more than 10 kbp. Protein patterns on SDS/PAGE revealed the same proteins with the same molecular weight between 14 - 66 kDa in all treated and control groups. Similarly, RNA showed no difference in the molecular size (2.5 kbp) between all treated samples after a short-term period.

Keywords: genomic DNA, recombinant insulin, SDS/PAGE, agarose

INTRODUCTION

The end stage kidney disease is associated with anemia that is treated using recombinant human erythropoietin (rHuEpo). In animals and human beings, erythropoietin is characterized as a hematopoietic cytokine produced in kidney and regulates red blood cell formation (Horng et al., 2015). Erythropoietin is a glycoprotein with 165 amino acids, which can be purified from plasma of anemic sheep and urine of anemic patients (Jelkmann, 2016). Protein intake can stimulate and has stimulatory effect on erythropoiesis by enhancing the erythropoietin hormone in plasma, which binds to the receptors to promote RBC production in bone marrow (Elliott & Sinclair, 2012). It is a part of physiological feedback mechanism that maintains red blood cell number and oxygen supply in the circulatory system at adequate levels (Jelkmann, 2011). Erythropoietin interacts first with a protein receptor on its target cell causing the appearance of a cytoplasmic protein thought to be a mediator, which in turn, causes an increased rate of transcription in the target cell nuclei (Jelkmann &.Lundby, 2011; Liu et al.,2013). The human recombinant erythropoietin has a molecular mass of 21 kDa and due to glycosylation the protein shows 36-40kDa in SDS phage, whereas the sheep erythropoietin is a single chain, has 46kDa molecular mass (Rivera-Cervantes et al., 2019). It has been documented that rHuEPO stimulated gene proliferation in- vitro from cultured bovine glomerular endothelial cells. This was associated with stimulation of RNA-dependent DNA and protein synthesis. Recombinant human erythropoietin dose-dependency stimulated the

proliferation of genes in cell culture (Trost et al., 2013). Recombinant EPO decreased apoptotic cell death of neurons under conditions of hypoxia. Protein mid mRNA for EPO and its receptor are expressed by human.

In end stage of kidney diseases, insulin resistance is a major complication, which is a therapeutic target in chronic stages (Osman et al., 2019). Insulin has been used in the clinical management of diabetes mellitus for over 50 years (Nathan et al., 2009). The effect of Insulin in two therapeutic doses on the genomic DNA, RNA and protein in rat liver for short-term period were performed *in vivo* by Teleb et al., (2004) The aim of this study was to evaluate the effect of erythropoietin on total DNA, genomic DNA, RNA contents and protein in rat kidney after administration for short period.

MATERIALS AND METHODS

Animals

Healthy male albino rats of body weight ranging between 150-200 gm were used. The animals were grown under controlled environmental condition and maintained on a standard diet and free access to water (National Research Council, 2011). The rats were divided into equal groups as follows:

The first group was served as control. The second group was divided into two subgroups for low and high doses of recHuEpo (100 U/kg body weight and 250U/kg body weight). Animals were injected subcutaneously four times per week. The third group divided into two subgroups for the low and high doses of recHu Insulin (0.05 U/kg rat which is equivalent to 100 U/h body weight and 0.125 U/kg rat which is equivalent to 200 U/h body weight respectively), which was injected daily through subcutaneous ruote for one week.

Drugs

Recombinant erythropoietin "EPO" was provided by Cilag Company. It was available in syringes of 1000 U up to 10000 U. Recombinant human Insulin was obtained from Novo-Nordisk, Denmark, in the form of Actrapid 40U/ml.

Preparation of kidney homogenates

Kidney homogenate was prepared according to the method of Patlolla et al., (2018). The kidney was removed after decapitation and tissue was minced and homogenized for protein. The molecular weight of protein was determined by SDS/PAGE technique in 12.5 % acrylamide gel. The protein content was determined according to Lowry et al., (1951) technique.

Extraction and purification of Genomic DNA and Isolation on Agarose 1 %

Isolation of genomic DNA was performed by Sambrook et al., (1989). The identification of DNA was carried out according to Heilig et al., (1994). The detection of DNA on the gel was performed by ethidium bromide bands containing as little as 1-10 µg of DNA, which were detected by ultraviolet light for obtaining sharp bands (Surzycki, 2000). Determination of DNA purity was carried out according to the method of Surzycki (2012), DNA concentration was determined by studying absorbance at 260 nm and 234 nm.

Extraction and purification of RNA and isolation on Agarose 1.3%

Extraction of RNA was performed by the method of Surzycki (2012). To determine the purity of RNA, absorbance at 260-280 nm and 234 mm was measured and calculated as A 260/ A280 and A260/A234 ratios (Held, 1997). Agarose concentration for isolating RNA was 1.3 % and RNA concentration was 1 ug/lane (Davis et al., 1994).

Statistical analysis of data

The analysis of the data was achieved using the one-way ANOVA followed by Tukey's test. The difference of $P<0.05$ considered to be statistically significant (Snedecor & Cochren, 1967).

RESULTS AND DISCUSSION

In this study, particular attention was focused on the short-term effect of erythropoietin and Rec Hu Insulin on rat kidney function. The study determined the effect of erythropoietin (Rec Hu Epo) and human Insulin (Rec Hu Insulin) at two therapeutic doses administered by injection for a week. There was no significant effect on the protein content of blood and tissue at a low dose of Rec Hu Epo compared to control. The protein content in the blood and wet tissues was significantly increased after administration of high dose of erythropoietin. The Rec Hu insulin had no significant effect on the protein content of the blood and tissues at both doses. It is notable that a low dose of insulin had significant effect on the protein content of the blood and tissues when compared to erythropoietin (Table 1). Recombinant human erythropoietin (rHuEPO) reduces serum insulin levels, increases insulin

sensitivity, and reduces insulin resistance (IR). However, the mechanisms behind these effects are unclear (Pan et al., 2011).

Table 1: Effect of the erythropoietin and RecHu Insulin in two dose levels on total protein contents of rat kidney after one week, (n-6)

Drug	mg protein /ml blood	mg/protein /g tissue
Control	46.46± 1.445	216.67
RecHuEpo (100 U/kg)	43.52±1.392	217.65
RecHuEpo (250 U/kg)	50.52±1.410*	252.58
RecHu Insulin (0.05 U/kg)	50.82± 1.481**	237.15
RecHu Insulin (0.125 U/kg)	47.69±1.326	222.53

All values are mean±SEM, *P<0.05, **P<0.01 compared to control

The effect of erythropoietin and insulin on the DNA concentration and purity were observed. Erythropoietin in two doses exerted no significant increase in the genomic DNA content in rat at the short-term treatment (Table 2). Insulin in two doses also did not produce any significant increase in the genomic DNA content in rat kidney.

RNA concentration and purity of rat kidney under the effect of the erythropoietin and insulin is given in Table 3. EPO increased the RNA concentration by 13.8 and 13% for low and high dose. The RNA concentration and purity has been increased when compared with the control after administration of erythropoietin at two different doses. The low dose of insulin did not show much increase in the RNA concentration whereas, high dose of insulin increased the RNA concentration. The increase in RNA content with erythropoietin is much higher when compared to the insulin.

The ratios of protein / DNA and RNA / DNA in rat kidney after erythropoietin and insulin treatments were studied (Table 4). The RNA/DNA ratio in the kidney of the experimental animals decreased by 43.9% for the low dose and 36.1% for the high dose of erythropoietin. The administration of insulin decreased by 35.3% for the low dose and 37.6% for the high dose in the ratio of protein / DNA and RNA /DNA. The results revealed that protein bands on gel acrylamide were unaffected under the insulin treatment. In the present study there were no additional proteins found in the kidney. At the meantime, all isolated protein bands on the gel have a molecular weight between 14-66 KD. Ladder DNA up to 10 kbp was used against the samples. The results indicated that the genomic DNA from rat kidney has molecular weight much higher than 10 kbp. No differences between treated or untreated samples of isolated DNA were observed. This is genome size, the smallest eukaryotic genomes being less than 10 Mb in length, and the largest over 100,000 Mb. Our results revealed the molecular weight of RNA bands of rat kidney less than 2.5 kbp. Hepatic insulin resistance is a critical component in the development of type 2 diabetes and erythropoietin (EPO) contributes to insulin resistance in fat and muscle (Zhang et al., 2017).

Table 2: Determination of DNA concentration & purity of isolated Genomic DNA from rat kidney after treatment by erythropoietin and Hu Rec Insulin in two doses of one week. (n=6)

Drug	A260	A280	DNA Purity A260/A280 Ratio	DNA (µg/ml)	DNA (mg/ml)	DNA (mg/g) Wet tissue
Control	46.46± 1.445	216.67	2.489	1438.7	1.44 ± 0.09	14.4
RecHuEpo (100 U/kg)	43.52±1.392	217.65	2.4	1638	1.638 ± 0.19	16.38
RecHuEpo (250 U/kg)	50.52±1.410	252.58	2.581	1629	1.629±0.16	16.29
Rec Hu Insulin (0.05 U/kg)	50.82± 1.481	237.15	2.3	1571.7	1.572±0.34	15.72
Rec Hu Insulin (0.125 U/kg)	47.69±1.326	222.53	2.6	1841.7	1.842±0.16	18.42

All values are mean±SEM

Table 3; Determination of RNA concentration & purity of isolated RNA from rat kidney after treatment by erythropoietin and Rec Hu Insulin in two doses of one week. (n = 6)

Drug	Conc	A260	A280	RNA Purity A260/A280 Ratio	RNA Conc. µg/ml	RNA Conc. mg/ml	RNA Conc. Mg/g Wet tissue
Control	0.360	0.146	2.489	1438.7	1438.7	1.44 ± 0.09	14.4
RecHuEpo (100 U/kg)	0.409	0.179	2.4	1638	1638	1.638 ± 0.19	16.38
RecHuEpo (250 U/kg)	0.407	0.163	2.581	1629	1629	1.629 ± 0.16	16.29
Rec Hu Insulin (0.05 U/kg)	0.336	0.102	3.260	1345	1345	1.345±0.30	13.45
Rec Hu Insulin (0.125 U/kg)	0.380	0.133	2.907	1520	1520	1.520±0.423	15.20

All values are mean±SEM

Table 4 :Effect of EPO and Rec Hu Insulin on the protein, DNA and RNA ratios.

	Control	Erythropoietin		Rec Hu Insulin	
		(100 U/kg)	(250 U/kg)	(0.05 U/kg)	(0.125 U/kg)
Kidney protein (tng) Mean ± S.D	216.67±1.445	217.65±1.392	252.58±1.410***	237.15±1.481***	222.53±1.326
Kidney RNA (mg) Mean ± S.D	14.70±0.09	16.38±0.19**	16.294±0.16**	13.45±0.30*	15.20± 0.423
Kidney DNA (mg) Mean ± S.D	11.94±0.47	21.6±0.09***	19.06±0.23***	15.72±0.34***	18.42±0.16***
RNA/DNA ratio	1.33	0.76	0.85 -43.9%	0.86	0.83
Protein/DNA	18.15	10.08-27%	13.25 -44.5%	15.10	12.08 -

All values are mean±SEM, *P<0.05, **P<0.01, ***P<0.001 compared to control

The effect of erythropoietin by the two therapeutic doses on the total RNA of rat kidney exerted a highly significant increase, however, no changes in protein patterns of rat kidney were observed. Also, Genomic DNA did not altered under the effect of EPO, hi addition, RNA bands on agarose 1.3% were similar against the untreated samples. The effect of insulin drugs by the two therapeutic doses on the total RNA of rat kidney exerted a highly significant increase under the treatment. No changes in protein patterns of rat kidney under the effect of the insulin were observed. Also, Genomic DNA did not altered under the effect of these drugs, in addition, RNA bands on agarose 1.3% were similar against the untreated samples..

CONCLUSION

Add conclusions of your study here.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

Add acknowledgements here

AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example WEP designed and performleed the experiments and also wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Davis L, Kuchl M, Battcy J, 1994. Basic method in molecular biology. 2nd ed. Appleton & Lange Norwalk, Connecticut. USA.

Elliott S. Sinclair AM, 2012. The effect of erythropoietin on normal and neoplastic cells. Biologics, 6:163–189. DOI:10.2147/BTT.S32281

Held P, 1997. The importance of 240-nm Absorbance measure-merits. The A260/A280ratio just is not enough anymore. Biomed Products, 7:123.

Heilig JS. Lech K, Brent R, 1994. Large-scale preparation of plalsmid DNA. In: Current protocols in Molecular Biology. Vol. 1. Ed. Janssen K. Current protocols publisher.

Horng LY, Hsu PL, Chen LW, Tseng WZ, Has, TH, Wu CL, Wu RT, 2015. Activating mitochondrial function and haemoglobin expression with EH-201, an inducer of erythropoietin in neuronal cells, reverses memory impairment. Br J Pharmacol 172(19):4741–4756. DOI:10.1111/bph.13248

Jelkmann W. 2011. Regulation of erythropoietin production. J Physiol 589(Pt 6):1251–1258. DOI:10.1113/jphysiol.2010.195057

Jelkmann W. 2016. Erythropoietin. Lanfranco F, Strasburger CJ (eds): Sports Endocrinology. Front Horm Res. Basel, Karger, 2016, pp 115-127. DOI: 10.1159/000445174

Jelkmann WI, Lundby C, 2011. Blood doping and its detection. Blood, 118(9):2395-404. DOI: 10.1182/blood-2011-02-303271.

Liu S, Ren, J, Hong Z, Yan D, Gu G, Han G, Wang G, Ren H, Chen J Li J, 2013. Efficacy of erythropoietin combined with enteral nutrition for the treatment of anemia in Crohn's disease: a prospective cohort study. Nutrition in clinical practice: official publication of the American Society for Parenteral and Enteral Nutrition 28 (1): 120–7. DOI:10.1177/0884533612462744

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with the Folin phenol reagent. J Biol Chem, 193: 265

Nathan DM, Buse JB, Davidson MB, Ferrannani Ele, Holman RR, Sherwin R, Zinman B, 2009. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetes Care, 32(1):193–203. DOI:10.2337/dc08-9025

National Research Council (US), 2011. Committee for the Update of the Guide for the Care and Use of Laboratory Animals. Washington (DC): National Academies Press (US)

Osman HM, Khamis OA, Elfeky MS, El Amin Ali AM, Abdelwahed MY, 2017. Effect of Short-term Erythropoietin Therapy on Insulin Resistance and Serum Levels of Leptin and Neuropeptide Y in Hemodialysis Patients. Indian J Endocrinol Metab 21(5):724–730. DOI:10.4103/ijem.IJEM_462_16

Patlolla AK, Kumari SA, Madhusudhanachary P, Turner T, Tchounwou PB, 2018. Biochemical and histopathological evaluation of Al_2O_3 nanomaterials in kidney of Wistar rats. Curr Top Biochem Res 19:1–12.

Pan Y, Shu JL, Gu HF, Zhou DC, Liu XL, Qiao QY, Fu SK, Gao FH,. Jin HM, 2011.Erythropoietin improves insulin resistance via the regulation of its receptor-mediated signaling pathway in 3T3L1 adipocytes. Mol. Cell. Endocrinol., 367:116–123. DOI:10.1016/j.mce.2012.12.027

Rivera-Cervantes MC, Jarero-Basulto JJ, Murguía-Castillo J, Marín-López AG, Gasca-Martínez Y, Cornelio-Martínez S, Beas-Zárate C, 2019. The recombinant human erythropoietin administered in neonatal rats after excitotoxic damage induces molecular changes in the hippocampus. Front Neurosci, 13:118. DOI: 10.3389/fnins.2019.00118

Trost N, Stepisnik T, Berne S, Pucer A, Pean T, Komel R, Debeljak N, 2013. Recombinant human erythropoietin alters gene expression and stimulates proliferation of MCF-7 breast cancer cells. Radiol Oncol 47(4):382–389. DOI:10.2478/raon-2013-0056

Teleb ZA, Maamon KM, Ahmed MM, Nermen Zafcaria A, 2004. Effect of some recombinant

DNA products, erythropoietin, Growth hormone and insulin on the nucleic acid and proteins isolated from rat liver. Egypt J Biomed Sci 15:259-284.

Sambrook J, Fritsch EF, Maniatis T, 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

Snedecor GW, Cochren GC, 1967. In "Statistical Methods" Iowa slate university press, Iowa, U.S.A.

Surzycki S, 2000. Basic techniques in Molecular Biology Chapt. 1 "general aspects of DNA isolation and purification and Chapt. 2 "preparation of Genomic DNA from animal cells. Chapt. 8. Agarose gel electrophoresis of DNA. Springer-Verlag., Berlin Heidelberg, Germany.

Surzycki S, 2012. Basic techniques in Molecular Biology Chapt. 6 "Isolation and purification RNA" and Chapt. 12 "Northern transfer and hybridization Springer-Verlag, Berlin, Heidelberg, Germany.

Zhang H, Ge Z, Tang S, Meng R, Bi Y, Zhu D, 2017. Erythropoietin ameliorates PA-induced insulin resistance through the IRS/AKT/FOXO1 and GSK-3β signaling pathway, and inhibits the inflammatory response in HepG2 cells. Mol. Med. Rep., 16:2295-2301. DOI. https://doi.org/10.3892/mmr.2017.6810