IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 2 Ver. 1 February. (2018), PP 24-26
www.iosrjournals.org

Manipulation of histochemical procedure for detection of uridine Diphospho - galactose 4 – Epimerase enzyme in the tissue of rat

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Abstract : *There are many techniques used for the detection of the activity of the cells. One of these measures is detection of the presence and activity of cell's enzymes using histo-chemical method.*
One of these enzymes is Uridine Diphospho-Galatose 4-Epimerase. It's activity could be estimated using simultaneously coupling method of Diculescu et al method. However this method has technical problem. This problem is the incubation medium is turbid which leads to get tissue slide with a lot of artifact and debris giving false positive result. To overcome this problem, many solvents had been used to have clean incubation medium, without inhibiting the activity of the enzyme, and to get clean and clear tissue slide could be obtained.
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Keywords: *Uridine Diphospho Galactose 4-Epimerase, simultaneous coupling method of deculescu.*

Date of Submission: 16-01-2018 Date of acceptance: 03-02-2018

I. Introduction

Detection of some enzymes activity could reflect the activity of their cells[(1)]. One of these enzymes is Uridine Diphospho-Galastose 4-Epimerase (UDPGE). This enzyme is also called Uridine Diphspho-glucose4-Epimerase. It's E.C number is (E.C.5.1.3.2). It was isolated 1st from fungi, E.coli bacteria, some plants and almost all mammalian tissue cells[(2)]. This enzyme acts through tightly binding of Nicotinamide adenine dinucleotide (NAD), which inturn as a co-factor needed for catalysis of reversible conversion of uridine diphospho-galactose to uridine diphosph-glucose[(3)(4)].
The active binding site of this enzyme with NAD in human being is represented by Tyr157-Gly-Lys-Ser-lys[(4)].
The lactose, the sugar of milk, will be hydrolysed into galactose which then converted into glucose in the liver.
The conversion of UDP galactose to UDP glucose by UDP–galactose–epimerase occurs since the epimerase reaction is freely reversible, glucose can be converted to galactose[(5)].
This enzyme could be evaluated by using histo-chemical technique, using the simultaneously coupling method for UDPGE according to Diculescu *et al* method[(6)].
The mammary gland is the best tissue for demonstration of UDPGE since the lactating mammary gland is usually manufacture lactose (which in disaccharide)[(7)(8)].
UDP-galactose-4-epimerase is highly conserved enzyme that catalyze the interconversion of UDP –galactose into UDP glucose[(5)].
Impairment of this enzyme in human being could result in one of distinct clinical form of epimerase deficiency galactosemia[(9)(10)(11)].
The mammary gland, of the albino rat, had been used for detection of the activity of this enzyme, using simultaneously coupling methods for UDPGE according to Diculescu *et al*. method[(6)]. However, the result obtained showed primarily turbid incubation medium. Secondly, the enzymatic activity in the tissue had been demonstrated as fine dark dots inside the cells with a lot of artifact (debris) outside mammary cells (i.e. false positive reaction could be elicited)[(7)(12)].
Aim of study :
A trial to solve the problem of getting artifact & so minimized the false positive reaction, through getting clear incubation medium and in sequences clean tissue slides without artifact if possible.

II. Material Methods

The experiment animal used in the research was Norway albino rat (Rats Norvegicus). The total no. of the 21 rats used were female rats, aging 8-10 weeks old, weighting about 150-200 gm.

All rats involved in this study received 0.4ml/g fenugreek seeds oil orally for 10 days[(7)(12)]. Then after 10 days one of the mammary gland had been resected under general anesthesia, then frozen using liquid nitrogen. The fresh frozen sectioning had been performed using freezing cryostat. (slee medical equipment ltd. Lanier works, Hillter lane, SEB, London) while temperature was adjusted at 20 c.

The fresh frozen tissue sections (5-6 minutes) had been divided into 3 groups.

- 1st group incubated in incubation medium prescribed by Diculescu *et al* (1968)[(6)] for detection of the activity of UDPGE enzyme.
- 2nd group incubated in a modified incubation medium of diculescu method for detection of UDPGE.
- 3rd group these section incubated in the incubation medium of diculescu for detection UDPGE similar to group (1) with one difference that the medium lack uridine-diphosphate-galactose substance and consider as a -positive control.

The incubation medium was consisting of the following materials as prescribed by diculescu *et al*[(6)]:

- 2m M uridine diphospho –galactose (uridine 5- diphospho 1-alpha- D galacto pyronoside UDPG, sodium salt, sigma chemical).
- 5 m M NAD (Nicotinamade adenine dinucleotide C_{21} $H_{27}N_7O_{14}P_2$ Fluka Bachs SG).
- 40m M sodium chloride (Nacl, E. Merk, Darmstadt)
- 10m M nito-BT (nitro-blue tetra zolium chloride $C_{40}H_{30}Cl_2N_{10}O_6$, Fluka, Buchs SG).
- 0.1 m M Tris Hcl buffer.

This buffer was used to keep the PH of the incubation medium at 7.4 .

This incubation medium was used to detect the presence and the activity of UDPGE enzyme inside the cell of the tissue examined, in form of deep blue formazen colored precipitate with in the cell[(3)(7)].

The original incubation medium of diculescu was turbid, unclear and appeared in form of suspension. As shown in fig. 1-A.

To clarify the incubation medium from this turbidity and unclear appearance, many solvent had been tried like methanol, ethanol, dimethyl formamide and glacial acetic acid. These solvent was added, drop by drop, to the incubation medium till the medium became clear. Then the PH of the new incubation medium was adjusted using sodium hydroxide solution till reaching the PH to 7.4 .

III. Results

The UDPGE enzymatic activities had been demonstrated as a distribution of dark coloured spotted precipitates intracellularly. The liver tissue of group III (control group) revealed no enzymatic activity (no precipitate could be detected). While liver tissue of group I revealed uneven distribution of unclear & hazy with a lot of artifacts dark color precipitate intracellularly & extracellularly as shown in Fig. 2-A.

Regarding modified incubation medium, the best solvent that change the suspension into a clear solution without inhibition of the UDPGE enzyme activity was glacial acetic acid. As shown in fig. 1-B ,The mammary tissue of group II animal, which was incubated in the modified incubation medium, revealed clear, clean obvious, well distributed dark colored spot, within the cells of the mammary tissue slide. Besides, no artifacts could be elicited in the tissue slides, as shown in Fig. 2-B

Fig. 1-A Traditional incubation medium Of Diculescu et al method

Fig. 2-B Modified incubation medium of Diculescu et al method

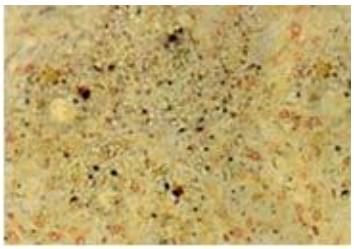

Fig. 2-A UDPGE activity pattern using the traditional histo-chemical method

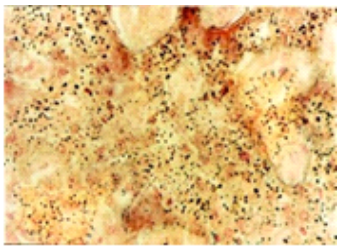

Fig. 2-B UDPGE activity pattern using the modified histo-chemical method

IV. Discussion

The simultaneous coupling method according to Diculescu *et al*[6] was used to demonstrate the presence & activity of UDPGE enzyme. However, fenugreek seeds oil stimulates mammary cell function [7], therefore almost all the enzymes will be activated, so the activity of UDPGE will be more obvious. The traditional histo – chemical method for detection of UDPGE according to Diculescu *et al* revealed an incubation medium in form of unclear suspension . This was occurred since nitro BT salt was not dissolved completely in the buffer solution. Accordingly, the results would be unclear hazy tissue field & filled with artifacts, besides, it could give false positive reaction. To overcome this problem, many solvents had been used to make Nitro BT salt dissolved completely in the buffer & give clear incubation medium.

Glacial acetic acid had been found to be the best solvent for nitro BT salt. Besides, it did not affect the activity of the enzyme[13][14]. From the above, It is recommended to use a modified simultaneous coupling method according to Diculescu *et al* for detection of the presence UDPGE & its activity.

V. Conclusion

Since the modified incubation medium, prepared in this study , is absolutely clear and contain no precipitate , that could give false +ve result, one can conclude that this modified method of dealescu revealed better , accurate and imformative results regarding the site and activity of UDPE within the tissue .

Acknowledgements

Special thanks and regard to professor Taghreed. H. Al-Noor and professor M. Al-khalisy for their help to facilitate the equipment needed and tissue slide preparation used in their study in the biochemical and

References

[1]. P.J Stoward. and A.G Pearse.(Eds.), Epimerase enzyme. In: Histochemistry, vol III Churchill Livingstone, 4th ed:610-611. 1991.
[2]. Tsaic., N ,Holmberg. and Ebner K., "Purification, stabilization and properties of bovine mammary UDPG epimerase". Arch. Biochem. Biophys. ; 136:233-244. 1970.
[3]. Liu Y, vanhooke J. L, Frey P.A., UDP-galactose 4 epimerase: NAD+ content and charge-transfer band associated with substrate – induced conformational transition. Biochemistry 35; 23:7615-7620. 1996.
[4]. N ,Daude, T.K.Gallaher, M.Zeschinigk, A.lStarzinski-powite, perty K.G. Haworth I.S., Reichardt J.K.V, Molecular Cloning, Characterization and Mapping of a full-length cDNA encoding human UDP – galactose 4- epimerase. Biochem mol. Med. ; 56:1-7. 1995.
[5]. L.Chi hney wong, sharon , maynie and George M.,Whitesides, Enzyme – catalyzed synthesis of N-acetyllactosamine with insitu regeneration of uridine 5-diphosphate glucose and uridine 5-diphosphate galactose. J. org. chem ; 47 (27) : 5416 – 5418. 1983.
[6]. I.Diculesscu, D.Onicescu, G, Szegli. and A.Dumitrescu,. "The histochemistry of epimerase. I. Uridine diphospho-galactose epimerase". Acta histochem. ; 25:242-250. 1968
[7]. M. H. Alkalisy and H M. Alkhateeb, Possible mode of action of fenugreek seeds in Rats mammary Glands; Histological, Histochemical and Biomedical approach. J. Fac. Med. Baghdad; 44 (1). 2002.
[8]. K.Robert Murray, K Daryl. Granner and W. Victor Rodwell (Eds) Galactose is needed for the synthesis lactose, glycolipid, proteoglycans and Glycoprotein. In: HARPER'S illustrated Biochemistry, 27th edition; chapter 21:181 – 183. 2006.
[9]. A.Alano, S Almashanu, P.Maceratisi, J.Reichardt, S.panny S. and T.M.Cowan, , UDP-galactose 4-epimerase deficiency among African – Americans ; evidence for multiple alleles. J Invest, med. ; 45 : 191. 1997
[10]. W.R Bergen., W.G Ng. and G.N. Donnell. Uridine diphosphate galactose 4-epimerase in human of other mammalian hemolysis . Biochem Biophys acta ; 315 : 464 – 472 . 1973
[11]. B.B Quimby, A.Alano, Almashanu s., AM De sandro, T.M Cowan. and J.L Fridovich – Keil, Characterization of two mutation associated with epimerase – Deficiency galactosemia by use of a yeast expression for human UDP-Galactose 4-epimerase. The American journal of human genetic ; vol. 61(3) : 590 – 598 . 1997.
[12]. M H. Alkhalisy, Induction of Lactogenesis in Rats Mammary Gland; Histological investigation fac. Med.; 43(2). 2001.
[13]. R.Denise ,Ferrier (Ed), Galactose metabolism in: Lippincott's illustrated Review Biochemistry. 6th (ed). Lippicott Williams & wilkins; chapter 12; 140-141. 2014.
[14]. Tsaic, N.Holmberg & Ebner K.; " Purification, stabilization and properties of bovine mammary UDPG epimerase. 1970

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