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ORIGINAL ARTICLE

Effect of Metabolism of Carbohydrate in Mammalian Tissues and Tumors on The Level of Enzymes Involved in Direct Oxidative Pathway

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ABSTRACT

Introduction: Literature review has revealed that the distribution of the enzymes of 6-phospo-gluconate dehydrogenase activities of some acetone derived tissues in animal tissue have so far not been investigated systematically. This leaves a gap for further investigation to explore the subject matter deeply.

Method: Barium salts of D-Glucose 6-phosphate (0 6-P), 6-phosphogluconate (6-PO) and D-ribose 5-phosphate (R 5-P) are available and were used in our study. (TNP) triphosphopyridine was prepared and analyzed; its composition was 75% TNP without (DNP) diphosphopyridine nucleotide.

Ice-cold isotone KCL (0-15M-KCL with 8ml, 0-02M-KHCO3) was disintegrated in 09 parts. It is done in a potter glass homogenizer or in a Nelco homogenizer. This is followed by centrifuging and dialysis of the supernatants. Heparinized blood 10ml was used for erythrocyte hemolysis, which was diluted with 10ml of water. 01 part of haemolysate was treated with 9 parts of isotonic KCI.

Spectroscope is used to determine the dehydrogenase activity of dialyzed tissue. The method followed was of Glock and McLean.

Study Design: Quantitative, cross sectional study.

Settings: Institute of Biochemistry, Gulab Devi Educational Complex, Lahore

Duration: 01 Year i.e. 1st July 2020 to 30th June 2021.

Results: Enzymes activities of 6-PG dehydrogenase and Gluco-6- phospo dehydrogenase mentioned in table 1&2 in normal mammalian tissue and mammary glands. The results obtained on the tumour cells are given in table 3. These Values are within the limits in normal tissues whereas it becomes on higher side in lymphomas and sarcomas.

Conclusion: This study shows some limitation that the maximum enzymic activities are determined, whereas in the intact cell other regulatory factors probably limit or control the activity of this pathway.

Keywords: Gluco-6- phospodehydrogenase, 6-PG dehydrogenase, oxidative pathway, Ribose 5-Pentose, mammalian tissue

INTRODUCTION

Literature review have revealed that the distribution of the enzymes of 6-phospo-gluconate dehydrogenase activities of some acetone derived tissues in animal tissue have so far not been investigated systematically. This leaves a gap for further investigation to explore the subject matter deeply. Exploring the quantitative methods of gucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and further exploring the breakdown of ribose 5-phosphate, in different normal mammalian tissues and tumour, the enzyme activity levels of the direct oxidative pathway have been determined.

MATERIAL AND METHODS

Barium salts of D-Glucose 6-phosphate (0 6-P), 6-phosphogluconate (6-PO) and D-ribose 5-phosphate (R 5-P) are available and were used in our study. (TNP) triphosphopyridine was prepared and analyzed, its composition was 75% TNP without (DNP) diphosphopyridine nucleotide.

Ice-cold isotone KCL (0-15M-KCL with 8ml, 0-02M-KHCO3) was disintegrated in 09 parts. It is done in a potter glass homogenizer or in a Nelco homogenizer. This is followed by centrifuging and dialysis of the supernatants. Heparinized blood 10ml was used for erythrocyte hemolysis which was diluted with 10ml of water. 01 part of haemolysate was treated with 9 parts of isotonic KCI.

Spectroscope was used to determine the dehydrogenase activity of dialyzed tissue. The method followed was of Glock and McLean, by using an enzyme solution for reducing TPN, the rate should not exceed 0-015 umole/min. a mixture devoid of TPN is used to start the reaction by adding 0-1 ml, 0.05M substrate to both cells.

A solution 0-1ml of 0.05 M R 5 ribose, 0.5 ml, 0-25 M glycylglycine pH 7.6, 0.5 ml, 1M MgCl, and 0-2 ml of tissue supernatant in a volume of 2-5 ml is heated in a container at 300F, after an interval of 20 minutes, 0.5ml of it is removed and introduced into 4-5 ml, 0-3N HCLO4. Orcinol method was used to determine pentose.

The optimum pH for glycylglycine is 7.6 and in Tris is approximately 8.0. The variation in enzyme concentration is due to initial reaction rate but it is conditional that

approximately 50% of the ribose 5-pentose is not broken down.

For centrifugation procedure the method used by Glock & McLean was employed, it is concerned with the breakdown of ribose 5-pentose by liver suspensions for intracellular distribution of enzymes.

RESULTS

Enzymes activities of 6-PG dehydrogenase and Gluco-6-phospo dehydrogenase mentioned in table 1&2 in normal mammalian tissue and mammary glands.

Increase levels were found for both dehydrogenases within the adrenal glands, especially within the adrenal cortex, in lymphatic tissue in common (spleen, thymus and lymph nodes), and is entirety rodent embryos within the early stages of advancement. In expansion, the consistently high values found within the livers of grown-up female rats and in lactating mammary organs propose that the levels of action of both dehydrogenases are correlated with female sex hormones. Exceptionally low activities of both proteins were found in skeletal muscle.

The results obtained on the tumor cells are given in table 3. These Values are within the limits in normal tissues whereas it becomes on higher side in lymphomas and sarcomas.

Table 1: CHO metabolism direct oxidative pathway in mammalian tissues and level of enzymes.

			Enzyme activity		
Tissue	Species	G-6-P dehydrogenase	6-PG dehydrogenase		Ribose 5-Pentose breakdown
			pH	pH	
Adrenal gland	Mouse (3)	159±24	349±30	180±10	430±30
Adrenal cortex	Buffalo (1)	729	310	230	285
Adrenal medulla	Buffalo (1)	68	115	68	110
Spleen	Mouse (2)	300±30	95±6	65±4	288±33
Thymus	Mouse (3)	99±1	88±8	50±4	360±15
Lymph node	Mouse (1)	77	77	45	226
Liver	Female Mouse (3)	103±10	291±15	129±11	351±5
Liver	Male Mouse (3)	45±2	145±09	60±9	346±09
Kidney	Rabbit (1)	70±6	63±6	55±2	260±10
Kidney cortex	Rabbit (1)	45	80	66	-
Kidney medulla	Rabbit (1)	30	38	25	-
Ovary	Mouse (1)	29	49	34	110
Testis	Mouse (1)	29±2	45±10	21±3	219±30
Brain	Rabbit (1)	30±2	21±2	10±1	74±2
Pituitary gland	Rabbit (1)	24	93	53	116
Thyroid gland	Rabbit (1)	17	42	29	-
Cardiac muscle	Goat (3)	25±12	35±8	19±2	150±4
Skeletal muscle	Goat(3)	9±1	14±0.4	7±0.2	90±14

Table 2: CHO metabolism direct oxidative pathway in mammalian glands of rat and its effect in pregnancy and lactation.

	Enzyme activity			
		6 PG dehydrogenase		
State of mammary gland	G-6-P-dehydrogenase	pH 9.0	pH 7.6	R 5-P breakdown
15 days pregnancy	90	89	49	200
10 days lactation	189	149	129	349
15 days lactation	1730	669	410	939
19 days lactation	2100	799	450	1079
23 days lactation	5449	1730	879	1301
2 days involution	49	89	44	150

Table 3: CHO metabolism direct oxidative pathway in mammalian tissues and level of enzymes in tumor cells

Enzyme activity				
		6 PG dehydrogenase		
Tumor	G-6-P dehydrogenase	pH 9.0	pH 7.6	R 5-P breakdown
Mouse sarcoma	57±10	59±4	49±1	-
Benzpyrene mouse sarcoma	59±10	100±9	59±6	130±7
Benzpyrene mouse epithelioma	130	140	79	290
Mouse squamous cell sarcoma	70±3	70±2	35±3	119±3
Spontaneous mammary adenocarcinoma C3H mice	110	110	54	491
Mouse lung carcinoma	20	70	39	200
Spontaneous lymphoma C57 mice				
Lymph node	300	141	100	380
Liver	221	152	130	339
Spleen	265	170	109	377
Walker rat carcinoma	64	139	86	249
Spontaneous rat carcinoma	130	200	150	310
A fowl tumor	30±10	62±4	49±4	279±10

Values for R 5-P breakdown, as measured by disappearance of pentose, are moreover included in Tables 1, 2 and 3. With some special cases, all the values are exceptionally much higher than the corresponding G 6-P and 6-PG dehydrogenase exercises, showing that the chemicals included in pentose phosphate digestion system don't control rate-limiting steps in this coordinate oxidative pathway.

DISCUSSION

During lactation, mammary glands and in adrenal cortex, high level of g-6-phosphodehydrogenase and 6-phosphogluconate dehydrogenase, and at the same time low activity of these enzymes in the muscles are suggestive of a very important finding that quantitatively this pathway is of less importance in the tissues, in a study conducted by Anwar-Afghan M and another study conducted by Sassoon HF, Dror Y, Watson JJ, Johnson BC on dietary regulation of glucose-6-phosphodehydrogenase, another study conducted by Chow C, Reddy K, Tappel AL on effect of vitamin E in the diet and the activities of glutathione peroxidase system in rat tissues supplemented by a study Stappenbeck TS, Hooper LV, Manchester JK, Wong MH, Gordon JI in 2002 is support of our study[1-4]. A surge in the activity of g-6-phosphodehydrogenase and 6-phosphogluconate dehydrogenase in mammary glands from the end of pregnancy to the end of lactation with subsequent fall in the level of activity in the concerned glands is not particular to these enzymes in a study conducted in 1993 by Ray S on renal handling of polyamines supports the present study[5].

In mouse after making necessary correction in the milk content the weight of the mammary glands remains constant during lactation, in a study conducted in 2001 by Kim S, Easter R, Hurley W on lactation stage, dietary nutrients, and litter size and another study conducted by Kim S, Hurley W, Han I, Stein H, Easter R on the size of mammary gland is in support of the present study[6,7]. Since the weight of mammary gland is constant the high values of g-6-phosphodehydrogenase and 6-phosphogluconate dehydrogenase activities is somehow concerned with milk secretion rather than with the growth of the gland, a study conducted by Swali A, Wathes D and another study conducted in 2017 by Pathak D, Bansal N is support of the present study[8,9]. These finding are suggestive that their might be a competition of active nature between enzymes of this direct oxidative pathway and some other pathway concerned with synthesis of lactose for glucose, a study conducted in 2016 by Nath A, Verasztó B, Basak S, Koris A, Kovács Z, Vatai G and another study conducted by Lemosquet S, Raggio G, Lobley G, Rulquin H, Guinard-Flament J, Lapierre H is favour of the current study[10,11].

For the synthesis of ribonucleic acid and coenzymes ribose 5-phosphate need to be incorporated in it which is supplied by direct oxidative pathway, thus, playing an important role in the metabolism of tumour and other rapidly diving cells, in a study conducted in 2021 by Faboya OA on the synthesis of Ribose 5'-Phosphate along with a study conducted in 2015 by Benito A, Diaz-Moralli S, Coy JF, Centelles JJ, Cascante M on the possible role of the pentose phosphate pathway in tumour metabolism and another study conducted in 2012 by Riganti C, Gazzano E, Polimeni M, Aldieri E, Ghigo D supports the current study(12-14). The present study indicate that experimentally induced and spontaneous tumour fall within the limit of same activities in normal tissues with regard to the activities of G-6-phophodegydrogenase and 6-phospho-G dehydrogenase activities, in a study conducted by Callea F, Villanacci V, Lorini G, Canini S on Glucose-6-phosphatase deficiency and metastatic lesions of the liver in 1996 and another study conducted in 2004 by Hoyer JD, Allen SL, Beutler E, Kubik K, West C, Fairbanks VF is support of our study[15,16].

CONCLUSION

This study shows some limitation that the maximum enzymic activities are determined, whereas in the intact cell other regulatory factors probably limit or control the activity of this pathway.

REFERNCES

1. Anwar-Afghan M. Comparative values of liver nicotinamide nucleotide coenzymes, endocrine glands, and their relationships with production traits of diallel crossed beef bulls. 1967.
2. Sassoon HF, Dror Y, Watson JJ, Johnson BC. Dietary regulation of liver glucose-6-phosphate dehydrogenase in the rat: starvation and dietary carbohydrate induction. The Journal of nutrition. 1973;103(3):321-35.
3. Chow C, Reddy K, Tappel AL. Effect of dietary vitamin E on the activities of the glutathione peroxidase system in rat tissues. The Journal of nutrition. 1973;103(4):618-24.
4. Stappenbeck TS, Hooper LV, Manchester JK, Wong MH, Gordon JI. [15] Laser capture microdissection of mouse intestine: Characterizing mrna and protein expression, and profiling intermediary metabolism in specified cell populations. 2002.
5. Ray S. Renal handling of polyamines: Memorial University of Newfoundland; 1993.
6. Kim S, Easter R, Hurley W. The regression of unsuckled mammary glands during lactation in sows: the influence of lactation stage, dietary nutrients, and litter size. Journal of animal science. 2001;79(10):2659-68.
7. Kim S, Hurley W, Han I, Stein H, Easter R. Effect of nutrient intake on mammary gland growth in lactating sows. Journal of animal science. 1999;77(12):3304-15.
8. Swali A, Wathes D. Influence of the dam and sire on size at birth and subsequent growth, milk production and fertility in dairy heifers. Theriogenology. 2006;66(5):1173-84.
9. Pathak D, Bansal N. Histoenzymic Studies on the Hypothalamus of Indian Buffalo During Different Reproductive Stages. Indian Vet J. 2017;94(06):38-41.
10. Nath A, Verasztó B, Basak S, Koris A, Kovács Z, Vatai G. Synthesis of lactose-derived nutraceuticals from dairy waste whey—A review. Food and Bioprocess Technology. 2016;9(1):16-48.
11. Lemosquet S, Raggio G, Lobley G, Rulquin H, Guinard-Flament J, Lapierre H. Whole-body glucose metabolism and mammary energetic nutrient metabolism in lactating dairy cows receiving digestive infusions of casein and propionic acid. Journal of Dairy Science. 2009;92(12):6068-82.
12. Faboya OA. Synthesis of Ribose 5'-Phosphate: a Starting Material for the Enzymatic Synthesis of Pseudouridine Monophosphate: Southern Illinois University at Edwardsville; 2021.
13. Benito A, Diaz-Moralli S, Coy JF, Centelles JJ, Cascante M. Role of the pentose phosphate pathway in tumour metabolism. Tumor Cell Metabolism: Springer; 2015. p. 143-63.
14. Riganti C, Gazzano E, Polimeni M, Aldieri E, Ghigo D. The pentose phosphate pathway: an antioxidant defense and a crossroad in tumor cell fate. Free Radical Biology and Medicine. 2012;53(3):421-36.
15. Callea F, Villanacci V, Lorini G, Canini S. Glucose-6-phosphatase deficiency (G-6-PD) and focal lesions of the liver. Pediatric Pathology and Laboratory Medicine. 1996;16(2):348-9.
16. Hoyer JD, Allen SL, Beutler E, Kubik K, West C, Fairbanks VF. Erythrocytosis due to bisphosphoglycerate mutase deficiency with concurrent glucose-6-phosphate dehydrogenase (G-6-PD) deficiency. American journal of hematology. 2004;75(4):205-8.