STUDIES ON THE EFFECT OF PENICILLIN, STREPTOMYCIN AND ISONICOTINIC ACID HYDRAZIDE ON SMALL INTESTINAL OLIGOSACCHARIDASE

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Summary: Studies have been done on the effect of Penicillin, Streptomycin and Isonicotinic Acid hydrazide on small intestinal oligosaccharidase and it was observed that the drug penicillin inhibited the enzyme lactase and sucrase by 62.7% and 34.7% respectively, whereas I.N.H. inhibited the enzyme sucrase and maltase by 57.1% and 56.14% respectively. Streptomycin did not show any inhibitory effect on those enzymes. Lactose tolerance test showed impairment of lactose absorption in case of penicillin. Fasting serum sugar level was diminished both in penicillin and streptomycin and the absorption capacity was increased after oral administration of streptomycin.

Key words: intestinal oligosaccharidase antibiotics Penicillin Streptomycin I.N.H.

INTRODUCTION

It has been reported that administration of Neomycin even in moderate doses (4-8 *gm*) may produce malabsorption syndrome associated with partial atrophy of the villi. It has also been reported that Neomycin inhibits the action of pancreatic lipase, precipitates the bile salts and may reduce disaccharidases activities in the small intestine (3, 5). Inhibition of small intestinal disaccharidases due to oral administration of Neomycin sulphate was also observed by Riener and Patterson (7). Sharma and Majumdar observed that prolonged use of chloramphenicol and chloretetracycline hydrochloride inhibited the enzymes lactase, sucrase and maltase. Whereas oxytetracycline did not show any inhibitory changes on those enzymes (8). Diarrhoea is known to be fairly common side effect of lincomycin therapy. It produces diarrhoea directly by changing the bacterial flora or by damaging the intestinal wall (10). Malabsorption due to subtotal atrophy of small intestinal villi has also been reported after administration of triparanol in rats and two patients receiving the drugs (6). The present problem was undertaken to explore the possibility of such enzyme inhibition after prolonged use of penicillin, streptomycin and isonicotinic acid hydrazide.

MATERIAL AND METHODS

1) Glucostat and chromogen obtained from Worthington Biochemical Laboratory, N.Y., U.S.A., 2) Glucose Oxidase (Sigma Chemical), 3) Horse Radish Peroxidase (Grade D

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Worthington Biochemical Laboratory, Freehold, N.J., U.S.A.), 4) O-Dinisidine, 5) Triton-X-100 (Rohm and Hass Co. Philadelphia, Pa), Tris (Hydroxymethyl-methylamine) obtained from Sigma Chemical Co., St. Louis, Mo., Maleic Acid, Lactose, Sucrose, Maltose, Glucose and Toluene of analytical grade were used. Penicillin (Na), Streptomycine Sulphate and I.N.H. obtained from commercial sources were used for this purpose.

Choice of animals: Male albino rats weighing between 120-150 *gms* and rabbits 1.2-1.5 *kg* obtained from commercial sources were used for this study.

Measurement of enzymes activity—(Effect of Penicillin and Streptomycin *in vitro*):

For the assay of lactose, sucrase and maltase the intestinal mucosal extract was used in dilution of 1/10, 1/80 and 1/120 respectively. The method followed by Dhalquvist, A. for the assay of the pig's small intestinal disacchridase activity was followed here with some modification (1). Each reaction mixture consists of 0.1 *ml* of the enzyme preparation of the dilution stated above, 0.05 *ml* of the substrate (Lactose 2%, Sucrose and Maltose 1% each) and 0.05 *ml* of distilled water or antibiotic solution like penicillin (80 *mcg*) and streptomycin (26 *mcg*). A drop of toluene was added and the tubes were incubated at 37°C for one hour. They were then kept in a boiling water bath for 2 minutes to destroy the enzyme activity. The reaction mixture was diluted with 0.8 *ml* of distilled water to make the volume 1 *ml*, 0.5 *ml* of the diluted reaction mixture was treated, with 3 *ml* of T.G.O. (diluted four folds) (1) and incubated for one hour at 37°C. The reaction was stopped by adding 1 micro-drop of 4 N HCl and the reading was taken in a spectrophotometer (Bausch and Lomb) at 420 μm. Disaccharidase activity was calculated in enzyme units. One unit of activity being equivalent to the hydrolysis of one μ mole of substrate per minute and the results have been described in Table (Ia) for comparison.

TABLE-I(a): The effect of penicillin and streptomycin on small intestinal oligosaccharidase
Mean value expressed in enzyme unit with ± S. D.

No. of Expts.	*Drugs used in mcg/reaction mixture*	*Enzyme dilution*	*Normal enzyme activities ± S.D.*	*Enzyme activity with antibiotics*	*Mean percentage of inhibition*
6	Penicillin 80 *mcg*	Lactase (1/10)	0.17 ± 0.02	0.065 ± 0.02	62.7%
6	,,	Sucrase (1/80)	4.41 ± 0.48	2.9 ± 0.56	34.2%
6	,,	Maltase (1/120)	11.4 ± 0.31	11.5 ± 0.28	Insignificant
6	Streptomycin 26 *mcg*	Lactase (1/10)	0.17 ± 0.2	0.16 ± 0.03	,,
6	,,	Sucrase (1/80)	2.33 ± 0.41	2.47 ± 0.23	,,
6	,,	Maltase (1/120)	11.1 ± 0.31	11.0 ± 0.28	,,

The above table shows the inhibition of lactase by 62.7% and sucrase by 34.2% by penicillin but maltase did not show any inhibitory change. Streptomycin sulphate did not show any inhibitory effect on lactase, sucrase and maltase.

Effect of I.N.H. on intestinal enzymes (in vivo):

The effect of I.N.H. on intestinal enzyme has been studied *in vivo* on albino rat. Twelve animals were selected for the experiment; six for control and six for test system. Each test animal was fed by I.N.H. (25 *mg*/100 *gm* of body weight) for 21 days. The high dosage of the drug was given due to high metabolic rate and rapid turnover in the animal system. After 21 days each animal was sacrificed and the oligosaccharidases activities have been measured as described by Sharma and Ghosh (9) and the results have been summarised in Table (Ib).

TABLE I(b): The effect of I.N.H. on intestinal oligosaccharidase with ±S.D.

No. of Expts.	*Drug used*	*Enzyme*	*Normal*	*After I.N.H. feeding*	*Mean percentage of inhibition*
6	I.N.H.	Lactase	4.6 ± 1.9	4.8 ± 1.55	Insignificant
6	,,	Sucrase	63.7 ± 15.3	27.2 ± 5.7	57.1%
6	,,	Maltase	171 ± 60.2	75.2 ± 51.5	56.14%

The above table shows the enzyme sucrase and maltase were inhibited by 57.1% and 56.14% respectively after prolonged oral use of isonicotinic acid hydrazide. The enzyme lactase was not inhibited by the drug.

Lactose absorption test on rabbit - (before and after drug administration):

Effect of penicillin and streptomycin on lactose absorption was studied on 12 rabbits devided into two batches. For both batches normal tolerance test was done after taking the fasting blood and feeding lactose of 1 *gm/kg* of body weight. Glucose was estimated by trisglucose oxidase system (1). The two groups of animals were fed by penicillin and streptomycin 15 *mg/kg* of body weight devided into two daily doses fed at 12 hours interval. The feeding was continued for 12 days after which lactose absorption test was repeated on each animal. Each animal was bled at specified hour both under normal and 12 days after feeding drugs. 0.1 *ml* of serum was collected from blood after centrifuging the samples of blood for 15 minutes at 3000 rpm. The serum was then diluted 20 times. 0.25 *ml* of diluted serum was treated with 0.25 *ml* of distilled water and 3 *ml* of T.G.O. (diluted for folds) (1). The reaction mixture was incubated at 37°C for one hour and at the end of the reaction a microdrop of 4 N HCl was added to the reaction mixture and the reading was taken in a spectrophotometer (Bausch and Lomb) at 420 *μm* and the results have been described in Table II for comparison.

It is evident from the Table II that lactose absorption was diminished after oral administration of penicillin and the fasting serum sugar level was diminished as compared to normal. After streptomycin sulphate feeding serum sugar level was diminished and the rate of absorption

of lactose was doubled but the level of serum glucose, one and half hour after the lactose meal was lower than the normal control animal (here auto control was done).

TABLE II: The effect of penicillin and streptomycin on lactose absorption in rabbit.

No. of Expts.	Drugs	Mean values expressed in mg% with ± S.D.			
		Before drug therapy		After drug therapy	
		Hours	± S.D.	Hours	± S.D.
6	Penicillin (Na)	0 hr	82.9 ± 11.4	0 hr	66.4 ± 9.1
		1 hr	109 ± 14.8	1 hr	72.6 ± 6.5
		1.5 hr	80.3 ± 6.8	1.5 hr	74.6 ± 6.8
6	Streptomycin Sulphate	0 hr	74.1 ± 9.2	0 hr	48.4 ± 9.6
		1 hr	98.7 ± 12.4	1 hr	82.9 ± 19.5
		1.5 hr	96.9 ± 8.1	1.5 hr	67.4 ± 12.0

DISCUSSION

The results of the present study lead to conclusion that continued oral administration of penicillin (Na) caused inhibition of lactase and sucrase activities of rat small intestinal mucosal preparation tested *in vitro*. The lactose absorption test showed impaired absorption of lactose through small intestine. The drug streptomycin sulphate did not show inhibitory effect on intestinal oligosaccharidase *in vitro*.

Continued oral administration of streptomycin sulphate lead to decrease in fasting serum glucose level and markedly increased the rate of absorption of sugar through small intestine.

Prolonged oral administration of isonicotinic acid hydrazide (I.N.H.) caused inhibition of the enzyme sucrase and maltase in albino rat. Sharma and Majumdar (9) showed that continued oral administration of broad spectrum antibiotics like chloramphenicol and chlorete-tracycline hydrochloride inhibited the intestinal oligosaccharidases but oxytetracycline did not show any inhibitory effect on those enzymes. Faloon, W.W. *et al.* (3), Jacobson (4) described sprue like syndrome in neomycin therapy. Reiner and Patterson (7) described malabsorption of lactose after neomycin therapy. Mehta *et al.* observed that bacterial metabolists and some antibiotics are responsible for the inhibition of pancreatic lipase (5). The present study shows the enzymes lactase and sucrase deficiencies caused by prolonged oral penicillin therapy may result in malabsorption of disaccharide and consequent diarrhoeal disorders. The drug I.N.H. causes the inhibition of sucrase and maltase and may cause malabsorption syndrome. Possibily the cachexia produced by pyridoxine deficiency after prolonged I.N.H. therapy is due to intestinal enzyme deficiency and consequent malabsorption syndrome. The drug streptomycin sulphate will not produce any malabsorption syndrome, but there is possibility of hypoglycemia after prolonged oral administration of the drug, and the absorption capacity of the small intestine is greatly increased after the therapy. The present results indicate caution in the use of milk and cane sugar containing food during prolonged oral penicillin therapy which may cause fermentation

type of diarrheal disorders as a result of enzyme deficiencies. The drug isonicotinic acid hydrazide inhibit the enzymes sucrase and maltase and may cause malabsorption syndrome and consequent diarrheal disorders. The enzyme deficiencies are always multiple. So, work will be undertaken to explore the possibility of other enzyme deficiencies produced by the drug penicillin and I.N.H.

ACKNOWLEDGEMENTS

The authors are sincerely grateful to Dr. A.C. Majumdar, former Senior Scientist, Indian Institute of Experimental Medicine, Calcutta-32 and Dr. B. Duttachaudhury, Lecturer, Department of Physiology, University College of Medicine, Calcutta, for their kind help during the course of the study.

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