Journal of Physiological Society of Nepal
Vol 1. No 1. Jun 2020

Research Article

JPSN

Dye inhibition of phosphate transport in everted duodenal sacs of mice

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ABSTRACT

Phenol Red has been widely used to test kidney function in man. Using the simple, everted gut sac technique has been observed to inhibit the phosphate transport by phenol red in the mouse intestine. We wanted to see if other similar organic anions are able to inhibit the phosphate transport across the mouse intestine. Both uptake and release of phosphate by the everted duodenal sacs of mice are inhibited by phenol red, bromocresol green and bromophenol blue. At the highest dose all the dyes were able to inhibit both influx and efflux significantly. Loss of phosphate from bathing solution is taken as influx and the gain of phosphate by the solution within the sac is taken efflux. At higher dosages a trend of increase in E/I% was noted. At the highest dose all the dyes were able to increase this parameter significantly over the control. Influx appears to be the primary process to be affected. Possible use of phenol red, on account of its safety in humans, as a hypophosphatemic agent is suggested.

Keywords: duodenal sac, phenol red, phosphate

INTRODUCTION

Phosphate absorption across the intestine occurs in three steps – entry of phosphate from the lumen of the intestine into the enterocyte across the mucosa (influx), intracellular migration from mucosa to serosa and finally release into the interstitial space of by crossing the basolateral membrane (efflux). Using the simple yet time honored everted gut sac technique[1,2] Mary & Rao[3] observed an inhibition of the phosphate transport by phenol red in the mouse intestine. We wanted to see if other similar organic anions are able to inhibit the phosphate transport across the mouse intestine. Along with phenol red (PR), bromocresol green (BCG) and bromo phenol blue (BPB) were used in this study.

MATERIALS AND METHODS

Swiss albino male mice, three months of age were used for the study. Everted duodenal sacs measuring 6 cm were prepared as described by Mary & Rao.3 After filling them with 0.5ml of the phosphate buffer they were suspended in 5ml of the same buffer contained in 10 ml flasks. They were incubated in a shaking water bath for one hour at 37˚C. They were then taken out gently blotted and their contents were emptied into small test tubes and preserved. A sample of the medium from each flask was also collected. Phosphate estimation was carried out by the spectroscopic method of Chen as described.[3] Loss of phosphate from bathing solution is taken as influx and the gain of phosphate by the solution within the sac is taken efflux. The phosphate buffer contained (in mM) NaCl 135, KCl 11, $CaCl_2$ 0.04 dissolved in 2mM phosphate buffer KH_2PO_4 and Na_2HPO_4 adjusted to a pH of 7.4.

RESULTS

As shown below in Table 1, all the dyes at dosages above of 3 microM were able to inhibit both influx and efflux significantly. At these dosages a trend of increase in E/I% was noted. At the highest dose all the dyes were able to increase this parameter significantly over the control.

Dyes Phenol red (PR), Bromocresol green (BCG) and Bromophenol blue (BPB) along with the dose in micromoles per litre are shown in the first column. Influx and efflux are in micromoles per hour. The values are represented as Mean ± SEM of six observations. Values of Influx and Efflux marked (*) are significantly different ($p<0.05$) from the corresponding controls.

Table 1: Comparison of effect of dyes on phosphate transport across duodenal sacs of mice

	Influx	Efflux	E/I as %
Control	9.2±0.2	5.1±0.35	55±3
PR 3	7.9±0.4*	4.8±0.3	52±4
PR 30	4.9±0.2*	3.2±0.5*	65±1*
PR 90	3.2±0.3*	1.9±0.2*	60±3
PR 300	2.2±0.4*	1.5±0.1*	68±3*
BCG 3	8.1±0.4*	4.3±0.1*	53±2
BCG 30	4.8±0.2*	3.3±0.4*	69±7*
BCG 90	3.6±0.3*	2.3±0.1*	64±2*
BCG 300	2.7±0.2*	1.8±0.3*	67±4*
BPB 3	8.6±0.3	4.6±0.2	54±2
BPB 30	5.8±0.2*	3.3±0.2*	57±1
BPB 90	3.9±0.1*	2.3±0.2*	57±1
BPB 300	3.0±0.2*	1.8±0.1*	67±3*

DISCUSSION AND CONCLUSION

While confirming the inhibitory effect of phenol red on phosphate transport,[3] our study shows that the other two related dyes are also capable of similar inhibition of the transport in dose related manner. While the influx and efflux are significantly reduced and the E/I % elevation at the highest dose employed indicate that the influx is the process primarily affected by the dyes. These organic anions placed on the serosal side of the sac seem to cross the smooth muscle and connective tissue barriers and the basolateral membranes to enter the cytoplasm of the enterocytes where they inhibit the mucosal influx of phosphate. The organic anions used in this study were shown to be capable of using organic anion transporters.[4] Murer et al postulated the presence of one such transporter in

the basolateral membrane of enterocytes.[5] It is difficult to pin point the exact site of action of the dyes. Nicotinamide a vitamin, when injected into the rat peritoneal cavity, has been shown to enter the enterocyte and prevent the intestinal absorption of phosphate by reducing the number of NaPi IIb type of phosphate transporters on the brush border membrane.[6] Since this transporter was found to be absent in the duodena of normal mice,[7] this mechanism is unlikely to be responsible for the inhibitory action of dyes.

Hyperphosphatemia is a well-known consequence of chronic kidney disease (CKD) and nicotinamide is undergoing clinical trials to regulate phosphate levels in CKD.[8,9] Phenol Red has been widely used to test kidney function in man.[10] However, mechanism of the inhibitory action on the intestinal transport of phosphate and the possible role of these dyes as hypophosphatemic agents needs to be explored.

ACKNOWLEDGEMENTS

Financial assistance of the Indian Council of Medical Research is gratefully acknowledged.

CONFLICT OF INTEREST

None

REFERENCES

1. *Wilson TH, Wiseman G J. The use of sacs of everted small intestine for the study of the transference of substances from the mucosa to the serosal surface. Journal of Physiology 1954; 123: 116-125.*
2. *Alam MA, Al-Jenoobi FI, Abdullah M. Al-Mohizea MA. Everted gut sac model as a tool in pharmaceutical research: limitations and applications. Journal of Pharmacy and Pharmacology 2012; 64: 326-336.*
3. *Mary PL and Rao JP. Phenol red inhibits uptake of phosphate by the everted gut sacs of mice. Kobe Journal of Medical Sciences 2002; 48: 59-62.*
4. *Vanverta AL, Gionfriddoa MR, Sweetb DH. Organic anion transporters: discovery, pharmacology, regulation and roles in pathophysiology. Biopharmaceutics & Drug disposition 2010; 31: 1-17.*
5. *Murer H, Biber J, Forster IC, Werner A. Phosphate transport: from micro perfusion to molecular cloning. Pflügers Archiv - European Journal of Physiology 2019; 471: 1–6 .*
6. *Katai K, Tanaka H, Tatsumi S, FukunagaY, Genjida K, Morita K, Kuboyama N, SuzukiT, Akiba T, Miyamoto K, Takeda E. Nicotinamide inhibits sodium dependent phosphate cotransport activity in rat small intestine. Nephrology, Dialysis and Transplantation 1999; 14: 1195-1201.*
7. *Marks J, Srai SK, Biber J, Murer H, Unwin RJ, Debnam ES. Intestinal phosphate absorption and the effect of vitamin D: a comparison of rats with mice. Experimental Physiology 2006; 91: 531-537.*
8. *Ginsberg C, Joachim Ix. Nicotinamide and phosphate homeostasis in chronic kidney disease. Current Opinion in Nephrology and Hypertension 2016; 25: 285-291.*
9. *Fouque D, Verveloet M, Ketteler M. Targeting gastrointestinal transport proteins to control hyperphosphatemia in chronic kidney disease. Drugs 2018; 78: 1171-1186.*
10. *Dunea G and Freedman P. Phenol sulphonpthalein excretion test. JAMA 1968; 204: 621-622*