INVESTIGATION OF GUT PERMEABILITY

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Introduction

Changes in the permeability of the small intestine can result in *under-absorption* of nutrients or the converse, *over-absorption* of the intestinal contents. Either condition can be described as *malabsorption*, although this term is more commonly used in the case of under-absorption of nutrients. A number of investigators have shown that over-absorption syndromes (increased gut permeability) are very widespread but significantly under-diagnosed. This is important, for example, in food intolerance, where derivatives of mal-digested food may be absorbed through the gut wall and into the circulation, resulting in characteristic hypersensitivity symptoms [1,2].

The small intestine is required for digestion and for absorption of nutrients from food. It is also responsible for keeping unwanted substances out of the body and is therefore a part of the immune system. Each intestinal villus is covered by absorptive epithelial cells (enterocytes) and the spaces between them, which hold the enterocytes together, are called the *tight junctions*. Enterocytes transport molecules into the hepatic portal blood supply by one of two mechanisms:

1) *Transcellular* absorption, where the molecule is absorbed into the cell from the gastro-intestinal lumen and then passed into the blood supply; most nutrients are taken into the body by this means.

2) *Paracellular* absorption, where molecules pass through the tight junctions, which are 1.5–2.0 nm in diameter and permit the passage of ions and small molecules (up to a molecular weight of about 1000 daltons). If the villus or its enterocytes is damaged or irritated, the gaps between the cells become disrupted, increasing the size of the molecules that can be absorbed and resulting in what is termed "leaky gut" syndrome.

The recent discovery that secretion of zonulin (pre-haptoglobin 2) is the normal physiological modulator of the tight junctions has greatly increased understanding of the mechanisms that regulate intestinal paracellular absorption [3] and has provided the link between increased gut permeability and the many pathophysiological states in which it is observed. For example, zonulin is over-expressed in the tissues and sera of subjects affected by autoimmune diseases, including coeliac disease. Gliadin affects intestinal barrier function by provoking release of zonulin and this effect is polarized, i.e. gliadin increases intestinal permeability only on the luminal side of intestinal tissue. Among the other intestinal luminal stimuli to zonulin release, exposure to pathogenic bacteria is a powerful trigger. Zonulin secretion has also been shown to be up-regulated in type I diabetes mellitus, multiple sclerosis, rheumatoid arthritis and various gastro-intestinal cancers [3].

The Biolab gut permeability profile is a sensitive test for the diagnosis of tight junction activity and hyper-absorption states across the molecular weight range of absorbable molecules. It also provides a means for the monitoring of treatment and the recovery of gastrointestinal function.

Indications

The use of PEG 400 as a probe for the investigation of intestinal permeability was first proposed by Chadwick, Philips and Hoffman in 1977 [4]. The rationale was that PEG (polyethylene glycol) contains a mixture of inert, water-soluble molecules of different sizes, whose absorption is independent of dosage,

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displaying decreasing mucosal transport with increasing molecular size. PEG 400 is also nontoxic, not degraded by intestinal bacteria, not metabolised by tissues, and rapidly excreted in the urine. PEG is polymerised ethylene oxide and is not the substance - ethylene glycol – which is found in anti-freeze.

The Biolab gut permeability profile shows that there is decreasing absorption of PEG fractions as the molecular weight increases. This can be explained on the basis of the hydrogen bonding capacity of PEG molecules and it is thought that PEGs pass through the intestinal cell membrane by a mechanism involving passive diffusion [5]. Other factors that may influence the urinary excretion of PEG include its space of distribution in the body, the permeability of the renal glomerular membrane [6] and the luminal flow rate in the intestine [5].

Safety of PEG as an intestinal probe

The Biolab gut permeability profile uses a low dose of PEG, well below the amount administered pharmaceutically and that normally present in many manufactured foods. PEG is widely used in the food industry and has also been used to improve the pharmacokinetic properties of a number of drugs (PEGylation) since the approval of PEG-bovine adenosine deaminase by the US FDA in 1990 [7]. Concern was expressed about the toxicological effect and fate of PEG administered in PEGylated drug form, but the data shows that PEG itself is toxic only at very high parenteral doses. The usual target organ is the kidney, which is the route for the excretion of unchanged PEG. An approximately 600-fold window exists between the maximum PEG burden from a currently used biological agent and the dose of PEG associated with human toxicity. The dose of PEG used to assess gut permeability (in which the PEG is taken orally, not parenterally) is thus only a fraction of a percent of the dose associated with its toxicity.

Patient preparation

The patient should fast for 3 hours before starting the test. Water intake during the first 2 hours of the 6 hour urine collection should be limited to 250 mL. Water consumption during the remainder of the test should be moderate.

The PEG test, which is a measure of mucosal permeability, should not be performed if the patient has gastroenteritis or is suffering from any other cause of intestinal hurry, as this will affect the absorption of PEG from the intestine and invalidate the urinary reference interval for the recovery of PEG.

Interfering substances

The patient should not take medicine containing Movicol (Macrogol), which is a type of PEG given to relieve constipation. This could invalidate the test results and show apparent increased excretion of certain molecular weights of PEG.

Mannitol, or hexan-1,2,3,4,5,6-hexol ($C_6H_8(OH)_6$), is a polyol that is used as an osmotic diuretic agent and a weak renal vasodilator. Mannitol is also found in "Seven Seas zinc plus vitamin C". This substance co-elutes in the GC profile with the lowest molecular weight of PEG, so supplements containing mannitol should be avoided prior to the test. Sorbitol (Mw 182.17), a non-stimulant laxative often used as a sugar substitute and xylitol (Mw 152.15) can cause similar interference in the test results. Both sorbitol and xylitol are found in stone fruits and xylitol occurs naturally in the fibres of many other fruits and vegetables, such as berries, corn husks, oats and mushrooms. Beer (including Guinness) and various soft drinks may also contain similar interfering substances.

Specimen requirements

The sample required for the gut permeability profile is a 6 hour urine collection after a 3 gram oral dose of PEG. A 20 mL aliquot of urine may be sent for analysis if the volume of the total collection can be accurately measured.

Postal samples must reach Biolab within 3 days of collection.

Methodology

PEG fractions are converted to their acetyl derivatives [4,6,8] which are stable on storage and give good chromatographic separation. Extraction of PEG from urine is by ion exchange chromatography, using the procedure described by Sivakumaran et al [8]. PEG is then acetylated with acetic anhydride in the presence of a pyridine catalyst and the acetyl PEGs are separated by capillary GLC.

Turn around time: 4-5 working days.

Synonyms: Gut permeability profile, PEG test, leaky gut test

Interpretation

The Biolab reference interval for recovery of the different molecular weights of PEG in a 6 hour urine collection is displayed graphically. Results above the upper limit of the reference interval are suggestive of hyperpermeability. Results below the lower limit of the reference interval are suggestive of malabsorption, but this is not a recommended test for the diagnosis of malabsorption syndromes.

References

1. Mackie RM. Intestinal permeability and atopic disease. Lancet 1981;I:155.
2. Jackson PG, Lessof MH, Baker RWR and Ferrett J. Intestinal permeability in patients with eczema and food allergy. Lancet 1981;1:1285-1286.
3. Fasano A. Zonulin and Its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity and cancer. Physiol Rev 2011;91:151-175.
4. Chadwick VS, Phillips SF, Hofmann AF. Measurement of intestinal permeability using low molecular weight polyethylene glycols (PEG 400). I. Chemical analysis and biological properties of PEG 400. Gatroenterology 1977;73:241-246.
5. Lloyd JB. Intestinal permeability to polyethyleneglycol and sugars: a re-evaluation. Clin Sci 1998;95:107-110.
6. Blatzinger JG, Rommel K, Ecknauer R. Elimination of low molecular weight polyethylene glycol 400 in the urine following an oral load, as a measure of intestinal permeability. J Clin Chem Clin Biochem. 1981;19:265-266.
7. Webster R, Elliott V, Park BK et al. PEG and PEG conjugates toxicity. In: PEGylated protein drugs: basic science and clinical applications. Veronese FM ed. Pub Birkhauser, Basel 2009, pp 127 – 146.
8. Sivakumaran T, Jenkins RT, Walker WHC et al. Simplified measurement of polyethylene glycol 400 in urine. Clin. Chem. 1982;28:2452-2453.