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Bile in the Esophagus—Model for a Bile Acid Biosensor

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Abstract Acid and bile acids form important constituents of the refluxed substances in patients who suffer from gastroesophageal reflux disease. Whilst 24h ambulatory pH monitoring using antimony or glass pH electrodes measures acid levels 5 cm above the gastroesophageal junction, there are no reliable methods of measuring other constituents of duodenal juices such as bile acids. Past studies in detection of bile acids have included esophageal aspiration studies with detection of bile acids with HPLC or indirect methods using fiber-optic bile sensor "Bilitec" to detect bilirubin in the bile. These methods have either been impracticable or unreliable for routine and accurate measurement of bile acid. More recently, impedance technology has been used to define "weakly" acid or alkaline reflux. There are many potential applications of biosensors of various types, and it is envisaged that a biosensor specific for bile acid would be a more practical tool for routine measurement. This paper looks at a model for development of a biosensor for bile acid based on molecular imprinted polymers.

Keywords Bile · Bile acid reflux · Bile acid biosensor · Alkaline reflux · Non acid reflux

Interest in bile acid reflux (Fig. 1—timeline) began with simple infusional studies with demonstration of chemically induced esophagitis in animal models.[1,2] Others demonstrated that metaplastic and carcinogenic changes could be induced by exposing the esophagus of rats to duodenogastric juices with or without a carcinogen.[3,4] There has been growing interest in the molecular effects of bile acid and acid exposure in Barretts cell lines with studies showing changes in gene expression CDx[5] and upregulation of oncogene c-myc.[6] Repeated exposure to acid and bile acids have been shown to increased proliferation through p38 and ERK MAPK pathways[7] and selectively induce colonic phenotype expression in Barretts cell lines.[8] Unconjugates bile acids have been shown to induce COX-2 expression in Barretts esophagus and adenocarcinoma through reactive oxygen species mediation.[9]

While pH probes were successfully developed to accurately monitor acid reflux, measurement of bile reflux has been difficult. Indirect evidence of increased bile in the stomach with aspiration studies[10] or dual pH monitoring[11] showed an association with esophagitis and Barretts. Presence of bile in the esophagus has also been demonstrated by using prolonged esophageal aspiration studies.[12–14] With HPLC separation of bile acids,[14] we have shown reflux of bile acids in concentrations greater than 200 μmol/l in 50% of the patients with severe esophagitis and Barrett's metaplasia. A wide spectrum of bile acids were detected, predominantly glycocholic and taurocholic acid. A significant proportion of the bile acids in patients with extensive mucosal injury were composed of the dehydroxylated taurodeoxycholic acid and the unconjugated cholic and deoxycholic acids. Secondary bile acids by dehydoxylation or deconjugation can occur by bacterial degradation, and these were shown in patients on PPI whose more neutral gastric environment can promote bacterial overgrowth.[15] However, direct aspiration studies are cumbersome and have not been used routinely in a clinical setting.

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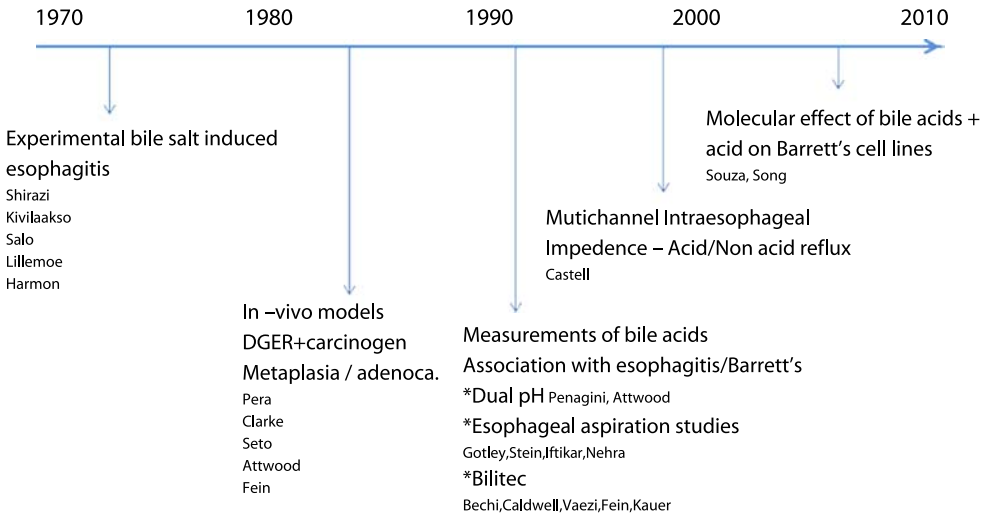
Figure 1 Bile reflux.



The "bilitec probe" was developed as a useful tool for detection of bilirubin in bile; however, the technology had limitations due to non-clearance of the probe and the requirement of a modified diet during the testing period. The current method of detecting acid and non-acid reflux with the usage of combined pH-impedance catheters has been more promising. The recordings have been successful in differentiating liquid acid reflux from liquid weakly acid or weakly alkaline reflux[16] but lacks in determining the composition of the refluxate.

Biosensors for Bile Acids

A biosensor specific for bile acid would seem to be a more practical tool for routine measurement. The system would be reliable with high specificity and sensitivity. It would have miniaturized components, easily tolerated by the patient and preferably incorporated within the current pH monitoring devices. Such a biosensor could be devised using molecular imprinting technology (MIP) (personal communication with Professor Anthony Turner, Department of Biosciences, Cranfield University, UK). MIPs are robust and inexpensive and possess the desired affinity and specificity for a wide range of target analytes. The commercial application of MIPs to the fields of separation and sensing is particularly promising to bile acid detection and treatment of bile reflux. MIP is a novel technique based on recognition characteristics of polymers that have complimentary size shape and binding site to specific substrates and have been applied to recognize steroids such as cholesterol and to bile acids which share the same four-ring nucleus as the steroid.[17] Three particular features make MIPs the target of this investigation: The striking resemblance of their binding properties (affinity and selectivity) to those of natural receptors; their unique stability, superior to that demonstrated by natural biomolecules; and their ease of preparation and adaptation to different practical applications.

The principle of MIP involves selection of polymer that is capable of forming non-covalent interactions with the template molecules such as glycocholic acid (Fig. 2). A

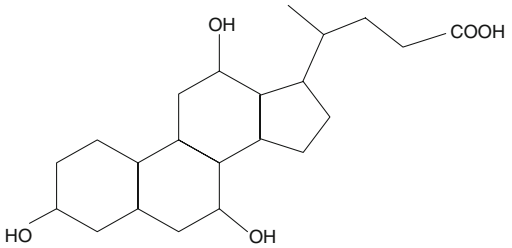


Figure 2 Sodium salt of cholic acid used as a template.[17]

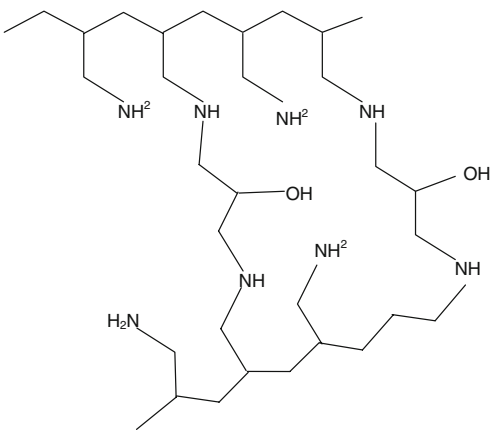


Figure 3 Imprinted polyammonium salt network containing binding sites complimentary to the shape of the bile acid skeleton.[17]

cross-linking agent such as ethylene glycol dimethacrylate is used to obtain imprinted polymer networks. These are subjected to a series of washing cycles to remove the template. The polymer is ground and sieved to particle with a size range of 65–100 μm, and the performance (affinity, specificity, capacity, and stability) is analyzed by chromatographic experiments, using model samples under acidic conditions similar to those existing in the human stomach and esophagus. These cholic imprinted polymer networks (Fig. 3) contain binding sites that mirror the carboxyl group and the shape of the parent steroid molecule. This forms the basis of a detection probe of bile acids. Polymeric sequestrants have been used for treating hypercholesteremia, but MIP has added therapeutic potential in the development of more potent and selective bile acid sequestrants to lower the concentrations of specific bile acids in the esophageal refluxate.

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