DIAGNOSTIC METHOD FOR INFLAMMATORY CONDITIONS IN THE INTESTINES

CROSS REFERENCE TO RELATED APPLICATION

This application is a 37 C.F.R. § 1.53(b) divisional of U.S. Application Ser. No. 08/849,282, filed May 30, 1997 now U.S. Pat. No. 6,063,027, which is the national phase under 35 U.S.C. § 371 of PCT Application No. PCT/SE95/01429, having an International filing date of Nov. 29, 1995, which designates the United States of America. The entire contents of both of these applications are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to a new method for the diagnosis of inflammatory conditions in the intestines (i.e. small intestines and large intestines (colon and rectum)). The novelty in the method is based on our discovery that nitric oxide (NO) in intestinal gas is a clinically relevant marker for this type of inflammation.

An inflammatory condition in this part of the gastrointestinal tract may be caused by e.g. inflammatory bowel diseases like ulcerative colitis and Crohn's disease, or food intolerance like coeliac disease or food allergy, or e.g. sepsis.

2. Description of the Related Art

It is known that NO is produced at many sites in the gastrointestinal tract and believed to participate in both physiological and pathological events (Whittle G J R, Physiology of the Gastrointestinal Tract, New York: Raven Press (1994) 267–94).

A pathogenic role of NO in ulcerative colitis has been suggested, and patients with active ulcerative colitis exhibit increased mucosal NO synthesis (Middleton et al., Lancet 341 (1993) 465–66; and Boughton-Smith et al., Lancet 342 (1993) 338–40). However, the cited studies were performed in vitro and used indirect citrulline assays to measure NO-synthase activity in biopsies taken from both colonic and rectal mucosa. The studies did not account for in what layer of the intestinal mucosa the activity was located. It is likely that NO formed in the mucosa would have a tendency to diffuse towards the intestinal lumen, but in order to reach there, NO has to be produced in the superficial layers including epithelial cells, intraepithelial cells and luminal cells like e.g. phagocytes. In case this highly reactive species is produced deeper in the mucosal layer, it will be destroyed during its diffusion route. Results presented herein show for the first time that NO-synthase activity in intestinal inflammatory disease is located in the epithelial cell layer next to the lumen of the colon and rectum.

It is recognized that intestinal bacterias exist that are able to convert small amounts of nitrite to NO (Ji et al., Biochem. Arch. 5 (1989) 61–66).

With respect to the airways, alterations in NO concentrations in exhaled breathing air have been found for inflammatory conditions (Alving et al., WO 9502181; Alving et al., Eur. Resp. J. 6 (1993) 1368–70; Hamid et al., Lancet 342 (1993) 1510–13; Karithonov et al., Lancet 343 (1994) 133–135; and Persson et al., Lancet 343 (1994) 146–147. Increased levels of NO have also been found in regurgitated air (Alving et al., WO 9502181; and Lundberg et al., Gut 35 (1994) 1543–1546). A somewhat different approach for the measurement has lead to suggestions that NO in exhaled

Dreathing air originates from the lung (Gustafsson, WO-A-9305709 and SE-A-91032433).

BRIEF SUMMARY OF THE INVENTION

The objects of the invention include diagnostic procedures for inflammatory conditions in the intestines as defined above and in particular with respect to correlation of NO production to these conditions.

We have now been successful in measuring NO concentrations in luminal gas sampled from colon and/or rectum of patients with active inflammation in the large intestine and rectum. These concentrations have been compared with those obtained from healthy control individuals.

Our finding is that the NO levels are increased in case of inflammatory conditions of the intestine. The expression "healthy individuals" means individuals with uninflamed intestinal mucosa.

Accordingly, the inventive method is characterized by the following steps:

- obtaining a gas sample from the lumen of the intestines of an individual;
- determining its content of nitric oxide (NO);
- comparing the found level with the level obtained for healthy individuals or with the level obtained for the same individual at another occasion.

An increased level relative the level of healthy individuals is an indication of an inflammatory condition in the intestines of the individual from which the sample was obtained. In case the level is compared with a level at another occasion, e.g. an earlier occasion, and found to be increased, this is an indication of a worsening of the inflammatory condition. Correspondingly, a decreased level means improvement. The sampling and measurement techniques should of course be essentially the same for values to be compared.

The sample is preferably obtained after the intestinal lumen concerned has been emptied, but such a procedure may not be necessary. The methods works in spite of the fact that the intestines can never be totally cleared from bacteria. The sample may be taken through catheters placed in e.g. the colon and equipped with syringes for collecting the gas, for instances during colonoscopy (colonoscopes are normally equipped with canals allowing withdrawal of gas samples from the intestinal lumen). Samples may also be taken with the same type of instrument as described by Raab et al. (Am. J. Gastroenterol. 87 (1992) 1453–1459). See also Krog et al., (WO-A-108013). With respect to rectum, gas samples may be directly collected for instance by aspirating rectal gas into a syringe. Samples may also be taken from the lumen of the small intestines, by using e.g. the equipment described by Odlind et al (EP-A-455,368). The methodologies presented by both Raab et al and Krog et al provide the possibility of obtaining samples representative for segments of colon and rectum.

The NO content may be determined/calculated as a concentration value, absolute amount or relative some internal or external standard. The content may be expressed as values normalised against components that natively are present in intestinal gas together with NO, preferably in fairly constant levels (internal standards). The NO content may also be calculated as amount secreted per time unit when taking air flow into account. When using balloon techniques as described by Krog et al., (WO-A-108013) and Odlind et al (EP-A-455,368) the amount found may be taken in relation to exposed mucosal area. The local NO-production may be increased by administering the substrate L-arginine