Pteridines
Vol. 12, 2001, pp. 155 - 160

Neopterin Concentrations in Colon Dialysate

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Abstract

Increased production of neopterin in humans is indicative for an activated cellular immune response. The intestinal immune system encounters an enormous amount of infectious agents or injurious substances evoking a cellular immune response. The objective of this pilot study was to measure neopterin levels in colon dialysates of healthy individuals and in comparison with individuals under a starvation diet and with patients. In parallel, urinary and salivary neopterin levels were determined in these subjects. The mean neopterin concentration in colon dialysate was about 10 times higher than in serum. These high neopterin values agree with a great impact of the cellular immune system within the human colon. Common diseases had a strong and prompt effect on the neopterin levels in colon dialysate, e.g. three individuals with acute gastritis had very high neopterin concentrations. In subjects with a starvation diet according to F.X.Mayr, which is characterized by low fiber and low calorie intake, the neopterin levels in colon dialysate were lower. The data may argue for a regeneration of the cellular immune system during fasting.

Introduction

The laboratory diagnostic potential of neopterin concentrations as indicator for activation of the cellular immune system were was examined in almost all body fluids, e.g. serum/plasma, urine, cerebrospinal fluid or saliva (1,2,3,4). Increased neopterin concentrations are common during virus infections, in autoimmune diseases neopterin concentrations correlate to extent and activity, and in malignant diseases and in HIV infections neopterin concentrations predict the course of the disease (5). Urinary neopterin concentrations were applied as marker of immune system activation also in colon diseases, e.g. celiac disease (6), Crohn's disease (7), ulcerative colitis (8), and they were found to predict survival in patients with adenocarcinoma of the colon (9). Neopterin concentrations have also been measured in stool of a small number of cancer patients and of patients with duodenal ulcer (10).

The mucosal surface of the human gastrointestinal tract represents with 200 - 300 m^2 the largest area in contact with the external environment, for comparison the external skin of the human body covers only 2 m^2. The largest number of potentially harmful bacteria (up to 10^{12}/ml) predominantly anaerobes, e.g. Bacteroides, resides within the colon. Therefore, 75% of the cells of the human immune system are present within the intestinal tract and of these 70% are located within the colon. In the mucosal epithelium and in the lamina propria there are various immunocompetent cells including dendritic cells, macrophages and lymphocytes which are able to kill virus-infected cells in experimental animals. The components of the resident human microflora are regulated by a complex cooperation of the antibacterial effects of gastric, pancreatic and biliary juice, by the human immune system and by the microflora itself (11). This microflora also contributes to limit colonisation with enteropathogens by producing antimicrobial substances such as short-chain fatty acids and colicins (12).

We were interested to study local neopterin concentrations in colon dialysate which may reflect the activity of the cellular immunity within the intestinal tract. Healthy human volunteers under their accustomed diet were compared with healthy volunteers under a controlled diet characterized by reduced nutrition with lowered intake of calories, fibres and antigens according to F.X.Mayr, and in a few patients with common diseases. In parallel to colon dialysates, neopterin levels in urine and saliva of subjects were determined.

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Subjects and Methods

Subjects

Thirty controls (17 males, 13 females; mean age ± S.D.: 40.3 ± 13.3 years), 41 healthy individuals during standardized reduced nutrition diet (24 males, 17 females, mean age ± S.D.: 56.0 ± 9.5 years) and 8 patients (4 males, 4 females, mean age ± S.D.: 45.8 ± 14.9 years) were studied. The controls and patients were employees or their relatives of the Institute of Medical Chemistry and Biochemistry or of the Gesundheitszentrum Lanser Hof. All subjects studied gave informed consent.

The participants of the standardized reduced nutrition diet underwent a physical examination, an anamnesis of dietary history and bowel habits, and a medical family history. Twenty hours after the start of the diet and further on daily in the morning, they received an isotonic solution of 4.75 g $MgSO_4$ in 250 ml of warm water in order to stimulate their flow of bile and the fecal excretion comparable to that of non-fasting subjects. When the frequency of defecation increased, this volume was reduced. They also received three times daily 0.35 g of basic mineral salts, 47.9 % $NaHCO_3$, 16.2 % $MgCO_3$, 10.6 % $CaCO_3$, 6% K3citrate, 3 % NaH_2PO_4 and starch and talcum ad 100 % for avoiding the fasting acidosis. They were on a diet of 2 meals daily without fibre and raw components (2 spelt rolls and 375 ml milk, sour milk or yogurt) during 6 days and then they received additionally potato soup and cheese and finally mixed light diet with few raw contents. The participants of this diet were advised to chew extensively: each small bite has to be chewed at least 40 times, then mixed with little milk before swallowing. The subjects reported no modification of the frequency of daily defecation or diarrhea.

Just before begin of the diet they swallowed dialysis bags (for details see below) and these were found in stool between day 2 and 4 thereafter. So the colon dialysates collected could be investigated during the first few days on starvation diet. The urinary and the salivary samples were obtained at the same time point when the dialysis bags were found. Throughout the starvation diet further dialysates were collected at days 5 - 7 and at days 10 - 13.

The group of 8 patients was heterogeneous, they were selected by occasion when one of the collaborators of this study or relatives felt sick: Three patients had acute nervous gastritis (not gastroscopically verified), 1 mild herpes labialis and 4 had a common cold.

Colon dialysate, saliva and urine specimens were frozen immediately at -20°C until analyses.

Methods

Dialysis tubes were made using seamless cellulose tubes (1.0 cm flat width) filled with 15% aqueous dextran with mean molecular mass of 60,000 D (Sigma Aldrich, Vienna, Austria). Tubes were 4.3 cm long and were prepared according to Wrong et al. (13). They were filled with a solution of dextran and had to be dried at part to be folded and inserted into a size 00 gelatine capsule. Two of these were swallowed under medical supervision and 1 or 2 were found within the next 2-4 portions of stool. If only one tube was found, the other was no longer searched for. Thereafter, tubes contained 0.9-1.3 ml of orange or brown colon dialysate that was used for analyses. In 8 subjects, two tubes were found simlutaneously. In these parallel dialysates the differences of concentrations were within the methodological error(c.v.=2.9 %)

The cellulose membrane used is permeable for soluble substances up to a molecular mass of 2 kD. Neopterin (molecular mass = 253 D), anorganic ions, SCFAs and water diffuse down a concentration gradient from the colonic fluid into the capsule. After 1 hour, the intracapsular concentrations of low molecular mass compounds reaches equilibrium (13). By radiographs it has been shown that dialysis capsules containing $BaSO_4$ suspension reach the colon 3-5 hours after ingestion and were in contact with colonic contents at least for 26 hours. The concentration found in fecal dialysate agree well with the concentrations obtained from filtered homogenates of feces (13).

In parallel, 0.3-0.6 ml saliva specimens were obtained without stimulation.

Urinary neopterin levels were measured by HPLC (14) and neopterin in saliva and colon dialysate by radioimmunoassay (BRAHMS Diagnostika, Berlin, Germany).

Short-chain fatty acids (SCFAs), acetate, propionate, isobutyrate, butyrate, iso-valerate and valerate, were measured by gas chromatography (15) with a Model 3920 gas chromatograph equipped with a flame ionisation detector (Perkin-Elmer Corp., Norwalk, CT). The glass column used was 184 cm x 2 mm (i.d.) packed with 15% SP 1220/1% H_3PO_4 on 100 - 120 mesh chromosorb W, acid-washed (Supelco, Inc., Vienna, Austria). N2 was the carrier gas, and the flow rate was 20 ml/min. The oven, injector part and interface were maintained at 125, 200 and 210°C, respectively. The saliva and colon dialysate specimens were diluted with water 1:3.

Group comparisons were made using Mann Whitney U-test or by two-sided ANOVA.

Results

Neopterin concentrations of healthy individuals in colon dialysate, saliva and urine are presented in Table 1. The absolute concentrations of neopterin in these body fluids differ in their order of magnitude: The saliva has nearly equal values as serum neopterin (14,16), colon dialysate is about 10 times higher and urinary neopterin is 200 times higher (2,14). As with urine, in colon dialysates and saliva a physiologically fluctuating water content has to be taken under consideration. For this purpose, in urinary specimens usually the neopterin per creatinine quotient is calculated (1). To correct at least partly a varying water content, the neopterin concentrations in colon dialysate and saliva were related to total SCFAs (Table 1).

Table 1. Neopterin concentrations (mean ± S.D.) in three different body fluids of healthy individuals (n = number of subjects, SCFAs = total short chain fatty acid content)

Specimen	n	Neopterin concentration	
Colon dialysate	30	70.2 ± 31.6	nmol/L
Colon dialysate (per SCFAs)	30	1.3 ± 0.70	mmol/mol
Saliva	26	7.4 ± 9.9	nmol/L
Saliva (per SCFAs)	26	0.75 ± 0.79	mmol/mol
Urine (per creatinine)	25	132 ± 36.7	µmol/mol

Fig. 1 shows neopterin concentrations in colon dialysate, in saliva and urine measured in healthy individuals during a starvation diet. Compared to the controls, in colon dialysate of the individuals during the starvation diet (day 2 - 4) neopterin levels were lower ($p < 0.01$), also when related to the concentration of total SCFAs (Fig. 2). During the 2 weeks of fasting, the neopterin concentrations remained nearly at the same level (day 2 - 4: 28.51 ± 24.18 nM, n = 41; day 5 - 7: 26.31 ± 23.91 nM, n= 39; day 10-13: 30.36 ± 21.26 nM, n = 32; mean ± S.D., not significant).

The absolute neopterin concentrations in saliva were higher during starvation ($p < 0.05$) when compared to the controls, but did not differ between groups when related to the levels of total SCFAs. There was no difference of the urinary neopterin concentrations between healthy controls with and without starvation (Fig. 3). The pH of dialysates of the control subjects (7.384 ± 0.697) was lower than that of the individuals during the starvation diet (7.919 ± 0.597; $p < 0.001$).

Neopterin concentrations in colon dialysate, and urine of the 8 patients with various diseases were found significantly higher compared with the controls. Neopterin concentrations in the colonic dialysates were 1.12 ± 1.28 mM (mean ± S.D., $p < 0.01$ or 7.52 ± 6.20 mmol/mol CFAs, $p < 0.001$, urinary neopterin concentrations were 200.5 ± 67.72 µmol/mol creatinine, $p < 0.01$. (Fig. 1).

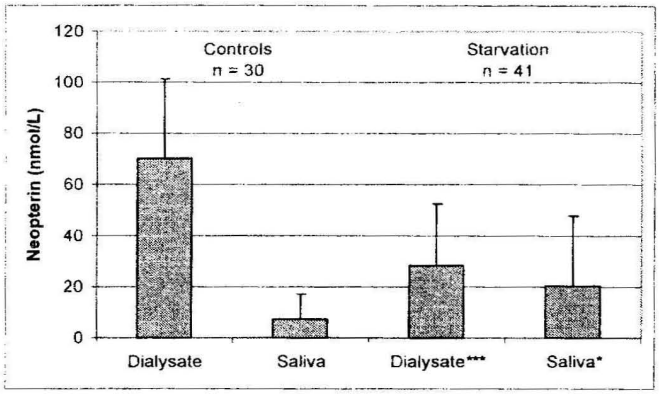

Figure 1. Concentrations of neopterin (mean ± S.D.) in colon dialysate and in saliva of healthy individuals and of healthy subjects during a starvation diet (* $p < 0.05$; *** $p < 0.001$ when compared with controls)

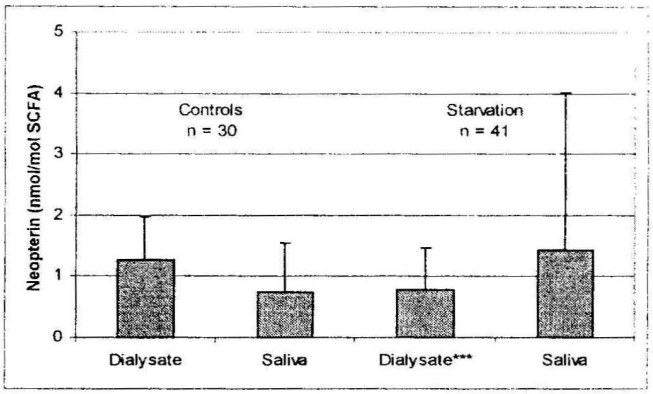

Figure 2. Concentrations of neopterin related to short-chain fatty acids (SCFAs) concentrations (mean ± S.D.) in colon dialysates and salivas of healthy individuals and of healthy individuals during a starvation diet (*** $p < 0.001$ when compared with controls)

Discussion

In the present study, neopterin concentrations in

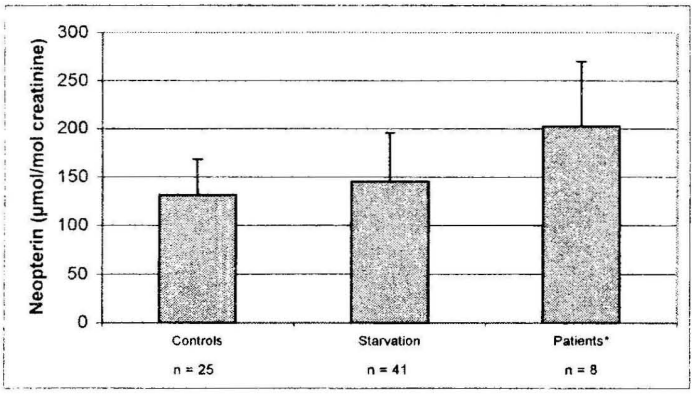

Figure 3. Concentrations of urinary neopterin (µmol/mol creatinine; mean ± S.D.) in healthy individuals and healthy individuals during starvation diet (** $p < 0.05$, when compared with controls)

colon dialysates were measured in healthy individuals and the results were compared with simultaneously determined salivary and urinary neopterin levels. The neopterin concentrations of healthy subjects were about ten times higher compared to concentrations in serum or saliva. Common diseases were associated with a strong increase of the neopterin concentrations in the colon dialysate. During the starvation diet neopterin concentrations in dialysates were lower than those of the control subjects.

Increased concentrations of neopterin are related to activation of the human cellular immune system. This type of immune response is characterized by the production of interferon-γ (IFN-γ) preferentially by type 1 T helper (Th 1) cells. In turn, neopterin is released by human monocytes/macrophages (17). Measurement of neopterin concentrations in urine or serum is of practical value in a variety of infections, autoimmune diseases, and malignant diseases and to detect allograft rejection episodes (5,18 - 20). The human intestine contains monocytes, macrophages, dendritic cells and much more lymphocytes than that of rodents. Th-1 type lymphocytes of the intestinal mucosa in lymphoid tissues and in lamina propria are able to secrete IFN-γ and are capable of mounting an intense protective inflammatory reaction in response to infectious agents or injurious substances present in the intestine (21,22). These initiating events may in turn lead to production, secretion and transport of neopterin into the colon. Higher neopterin levels in colon dialysate compared with neopterin in serum would be in line with high density of immunocompetent cells and with a prominent role which the cellular immune system is playing in the human intestine. The intestinal mucosa cooperates with mucous membranes of the lung, tonsils and others as one single immunocompetent organ. Initiating events within one mucosal site can, therefore, contribute to a state of activation of mucosa at other sites (23). Consequently, the activation of the cellular immune system by pathogens and antigenic macro-molecules should be indicated by neopterin in colon dialysate very early and highly sensitively even if pathogens invade another mucosal site. In agreement with this background, the neopterin concentrations in colon dialysate of the patients rose and differed more from those of the control group than the corresponding salivary and urinary neopterin levels. They seem to be also high when the diseased mucosa was situated outside of the gastrointestinal tract. However, there was no difference of urinary neopterin concentrations and also of salivary neopterin per SCFAs concentrations between individuals on starvation diet and controls. Therefore, it will be necessary to study these incidental observations in a larger number of patients. However, the measurement of neopterin concentrations in colon dialysate or probably also in the fecal centrifugate might be important to monitor the gut as a major immune system compartment.

The surprisingly lower neopterin levels of the studied individuals during the starvation diet may likely reflect the diminished intake of antigens and injurious agents leading to lowered activation of Th-1 type lymphocytes. Alternatively, this could be due to a down-regulated Th-1 type immune response by Th-2 cytokines as was observed also by, e.g. histamine, another mediator involved in Th-2 type immune response which down-regulates IFN-γ-induced neopterin formation in monocytes (24). However, the studied subjects were under continuous medical observation during the diet and allergic diseases have not been observed. Third, our observation could be explained by a larger water content of the dialysate caused by more drinking and by the laxative effect of the $MgSO_4$ solution the fasting subjects have received.

Concentrations of SCFAs, which were measured concomitantly with neopterin were also lower than that of the normal controls ($p < 0.001$; data not shown). A quotient of neopterin and SCFAs might represent a means to take into account to some extent a fluctuating water content of saliva or colon dialysate. In colon dialysate, negatively charged SCFAs appear together with their cations and represent the main soluble substances. They are produced by the colonic microflora from carbohydrates at constant rate. SCFAs play a pivotal role in maintaining homeostasis in the colon. The time point of defecation and, thus, the water content of the colon and feces is shortened by high osmolarity, high concentrations of SCFAs and low pH-value, and e.g. butyrate is known to lower colonic pH (25,26). In

our study, pH values were also lower in controls compared with individuals under starvation diet. Since SCFAs are also present in lower but well measurable concentrations in saliva, a quotient of neopterin and SCFAs was chosen for improvement of measurements in saliva and colon dialysate.

During starvation diet the neopterin per SCFAs quotient in saliva was also higher than that in controls but the difference is not significant, mostly due to a large standard deviation. In colon dialysates the difference remained at the same level of significance as compared to absolute concentrations (Fig. 2). Thus, our data support the assumption that the lower neopterin concentrations in colon dialysate rather reflect reduced production of neopterin than increased dilution. The neopterin per SCFAs quotient in saliva did no longer show a difference between groups. Different chewing habits in the advised population of individuals on diet could probably explain the lower concentrations in saliva compared to controls.

In conclusion, the low amount and carefully chewed food without fibers and raw contents of the individuals during the starvation diet may charge their intestinal tract with significantly less antigens, infectious agents and injurious substances and thus may allow to relax the intestinal immune system. Additionally, the better homogenated food without fibers is likely to reduce the load of the colon with undigested carbohydrates and to modify the proliferation and numbers or antigenicity of the colonic microflora. Both effects may relate to decreased production of neopterin and also of SCFAs. The low neopterin levels during the starvation diet suggest a rest and likely perhaps a regeneration of the intestinal immune system and a benefit for the participants due to the starvation diet.

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