Intestinal Permeability, Vitamin A Absorption and Serum Alpha-Tocopherol in Gastrointestinal Stromal Tumor Patients Treated with Imatinib

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Summary Administration of imatinib is the therapy of choice in patients with advanced (inoperable) or metastatic gastrointestinal stromal tumors (GIST). Gastrointestinal toxicity is one of the most common side effects of anticancer therapy, including imatinib. Measurement of intestinal permeability represents a method of noninvasive laboratory assessment of gastrointestinal toxicity. We have measured intestinal permeability (by determining absorption of lactulose, mannitol and xylose), vitamin A absorption and serum alpha-tocopherol in 16 patients with advanced/metastatic GIST treated with imatinib. Lactulose/mannitol and lactulose/xylose ratios as well as parameters of vitamin A absorption did not change significantly during the treatment, but a significant decrease of alpha-tocopherol was observed. We conclude that, in contrast to most other anticancer agents studied so far, imatinib does not have an effect on intestinal permeability. No effect on vitamin A absorption was observed, but serum alpha-tocopherol decreased significantly during the treatment.

Key Words alpha-tocopherol, gastrointestinal stromal tumor, imatinib, intestinal permeability, vitamin A

Gastrointestinal stromal tumors (GIST) are rare tumors characterized by activating mutations of c-kit, or, in the minority of cases, of platelet-derived growth factor receptor-alpha (1). Radical surgery is the only curative therapy in GIST, but up to more than 50% of patients may eventually relapse (2). Before the advent of targeted therapy the prognosis of patients with metastatic GIST was dismal. Based on the results of prospective trials demonstrating objective response in more than 50% of the patients (3, 4), imatinib, a tyrosine kinase inhibitor, became rapidly the therapy of choice in patients with metastatic inoperable GIST. It is increasingly evident that the introduction of imatinib has changed the natural history of metastatic GIST (5). Spectacular effect (6), even pathological complete response (7), has been observed in individual patients.

Gastrointestinal toxicity is one of the most common side effects of anticancer drugs, including imatinib (8). In general, any part of the gastrointestinal tract from the oral cavity to the anus may be damaged by anticancer agents. Depending on the part of gastrointestinal tract affected, gastrointestinal toxicity may manifest as sore mouth, dysphagia, dyspepsia, diarrhea, abdominal cramping, or rectal bleeding. In the case of imatinib, the most common gastrointestinal side effect is diarrhea, suggesting dysfunction of intestinal mucosa induced by the drug, but little is known about the mechanisms of intestinal toxicity of imatinib.

Mucosal damage can be easily assessed by direct inspection only in the oral cavity. The mucosal damage in other parts of the gastrointestinal tract can be evaluated directly by endoscopic methods, but, for obvious reasons, endoscopy is difficult to practice in cancer patients experiencing acute gastrointestinal toxicity, and the diagnosis and the assessment of severity of intestinal mucosal damage are therefore still based on anamnestic data. Methods for objective assessment of gastrointestinal toxicity are needed. Measurement of intestinal permeability is used to study the disorders of gut mucosa in benign disorders, including inflammatory bowel disease and gluten enteropathy (9, 10). The term intestinal permeability reflects the barrier function of bowel mucosa separating the internal milieu from the outside environment both in an immunologic and a metabolic sense (11). The disorders of this barrier function are defined by altered permeability to different substances. A variety of molecules have been used to assess intestinal permeability, including radiopharmaceuti-
cals, macromolecules or non-metabolizable sugars (9, 10). This approach was also tested in patients treated by cytotoxic agents, and aberrations of intestinal permeability similar to those found in patients with benign intestinal disorders have been found (12), but little is known about alterations of intestinal permeability in cancer patients treated with targeted agents.

Along with intestinal permeability, the absorptive function of the bowel is also disturbed in patients treated with chemotherapy or radiation. Recently, we have demonstrated that alterations of intestinal permeability in patients with chemotherapy-induced diarrhea are accompanied by a decrease in postprandial concentrations of retinyl esters (13). The method used for the determination of vitamin A metabolites also measures alpha-tocopherol in a single run (14, 15). The processing in the gastrointestinal tract of vitamins A and E is similar, but the absorption of vitamin E is less efficient compared to vitamin A (16). Because of this less efficient absorption, vitamin E absorption may be more sensitive to disturbances of gut function, and a decrease of alpha-tocopherol could manifest itself even if vitamin A absorption is within the normal range.

In the present study, we have evaluated intestinal permeability (using the lactulose/mannitol test), vitamin A absorption and serum alpha-tocopherol in GIST patients during the treatment with imatinib.

**PATIENTS AND METHODS**

Sixteen patients, 12 males and 4 females, aged (mean±SD) 63±12 (range 42–82) y, with histologically verified metastatic or advanced inoperable gastrointestinal stromal tumor treated with imatinib (Glivec, Novartis, Basel, Switzerland; 400 mg daily) were included in the present study. The protocol of the present study was approved by the institutional ethical committee, and the patients gave signed informed consent.

Intestinal permeability was studied by measuring urinary lactulose, xylose and mannitol after oral challenge as described earlier (17). Briefly, after an overnight fast, patients ingested 100 mL of the test solution containing 2 g of mannitol, 2 g of xylose, 10 g of lactulose, and 11 g of glucose in water. The patients then continued fasting for 2 h, and urine was collected for 5 h. Lactulose, xylose and mannitol were determined by capillary gas chromatography, and urinary excretion was expressed as the ratio of lactulose/mannitol and lactulose/xylose (17).

The vitamin A absorption test was performed in 9 patients as described (18). Samples of peripheral blood were drawn after a 12-h overnight fast and they were marked as sample one. These samples were centrifuged (1,600 ×g, 10 min, 16°C) and the serum was separated and stored at −25°C. Subsequently, a single oral dose of vitamin A (Slovakofarma, Holovec, Slovakia; 360,000 IU) was administered to the patients. The second blood sample was collected 5 h after administration of vitamin A and processed by the same procedure. The sample (500 μL) was de-proteinized by cool ethanol denatured with 5% methanol (500 μL, 5 min, 4°C) and then extracted with 2,500 μL n-hexane: toluene: ethanol 8:2 (v/v) mixture for 5 min in a shaker. After centrifugation (1,600 ×g, 10 min, 0°C), the aliquot (2,000 μL) of the extract was separated. Another 2,000 μL of n-hexane: toluene: ethanol 8:2 (v/v) mixture was added to the rest of serum sample for a repetition of the extraction procedure. The extract was then evaporated using a vacuum concentrator. The residue was subsequently dissolved in 400 μL of methanol, and 20 μL of the sample was injected onto the chromatographic column. The analyses were performed using the Perkin Elmer high-performance liquid chromatography set (Norwalk, CT, USA) comprising an LC 200 pump, an LC 200 autosampler, LC Column Oven 101 thermostat and LC 235C Diode Array Detector (DAD) attached to the Perkin Elmer Turbochrom Chromatography Workstation version 4.1. Separation of retinyl palmitate and retinyl stearate was performed using the Chromolith Performance RP-18e, 100×4.6 mm monolithic column (Merck, Darmstadt, Germany). The gradient elution was used at the flow rate 3 mL/min; mobile phase methanol : water 95:5 (v/v) in 0–2.1 min and methanol : 2-propanol 60:40 (v/v) in 2.1–4.9 min. The total time of analysis was 6.0 min. The block heater LC Oven 101 (Perkin Elmer) was utilized to keep the analytical column temperature at 25°C. The injection volume was 20 μL. The DAD detection of retinyl palmitate and retinyl stearate was carried out at 330 nm. Serum alpha-tocopherol was determined on the Perkin Elmer high-performance liquid chromatography set as described (14). Serum total protein and albumin were determined on a MODULAR analyzer (Hoffmann-La Roche, Basel, Switzerland) using commercially available kits.

The laboratory parameters investigated before and during the treatment were compared by the Wilcoxon signed rank test. The decision on statistical significance was based on p=0.05 level. The analyses were performed using NCSS 2001 software (Number Cruncher Statistical Systems, Kaysville, UT, USA).

**RESULTS**

Intestinal permeability, vitamin A absorption and serum alpha-tocopherol were investigated immediately before the start of therapy and on subsequent visits during imatinib administration. No significant changes in the lactulose/mannitol or lactulose/xylose ratios (Table 1) or vitamin A absorption (Table 2) were observed during the treatment. In contrast, serum alpha-tocopherol decreased significantly during the therapy (Table 3). A significant decrease in serum total protein concentrations was observed during the course of imatinib therapy, but the decrease in serum albumin reached statistical significance only at visit 3 (Table 3). At specific time points, measurements were not obtained in one or two patients for technical reasons.

**DISCUSSION**

Present data indicate a lack of effect of imatinib on...
intestinal permeability and vitamin A absorption. Although the number of patients studied was limited, in earlier reports alterations of intestinal permeability were evident after anticancer therapy in cohorts of patients of similar size. It may therefore be concluded that, in contrast to cytotoxic agents, imatinib has limited effect on intestinal permeability and vitamin A absorption. Increased intestinal permeability has been observed in lymphoma patients treated by standard regimens that included alkylating agents and vincristine (19), breast cancer patients treated by anthracyclines (20), or patients with gastrointestinal tumors treated by 5-fluorouracil-based regimens (21, 22). More recently, increased intestinal permeability has also been described after therapy with geltinib, a targeted drug (18). It has been demonstrated in experimental animals that the morphological changes induced by cytotoxic drugs, including flattening of the villi, necrosis, and inflammatory infiltrate in the submucosa, are accompanied by an increase in intestinal permeability (23, 24). These morphological changes result in increased exposure of the crypts and increased permeability of the intestinal mucosa to disaccharides or other larger molecules. Different methods have been used to assess the intestinal permeability in cancer patients treated by cytotoxic agents or radiation therapy, but most papers report the results of differential urinary excretion of a disaccharide and a monosaccharide.

No significant changes in vitamin A absorption were noted in the present group of patients treated with imatinib. This is consistent with the negative finding regarding intestinal permeability. Vitamin A absorption

| Table 1. Intestinal permeability during the treatment with imatinib. |
|---|
| Visit | 1 | 2 | 3 | 4 | 5 |
| Time from the start of therapy (d) | 0 | 11±1 | 25±4 | 50±6 | 83±11 |
| Lactulose/mannitol ratio | 0.05±0.01 | 0.08±0.02 | 0.05±0.01 | 0.07±0.02 | 0.05±0.01 |
| (n=16) | (n=15) | (n=16) | (n=15) | (n=15) |
| Lactulose/xylose ratio | 0.06±0.01 | 0.07±0.02 | 0.07±0.04 | 0.08±0.04 | 0.04±0.01 |
| (n=16) | (n=16) | (n=16) | (n=16) | (n=15) |

Shown is the mean±SE of the mean (range).

| Table 2. Vitamin A absorption during the therapy with imatinib. |
|---|
| Visit | 1 | 2 | 3 | 4 | 5 |
| Time from the start of therapy (d) | 0 | 11±2 | 27±6 | 50±8 | 84±10 |
| Retinyl palmitate pre-test (μmol/L) | 0.11±0.07 | 0.07±0.05 | 0.01±0.01 | 0.10±0.07 | 0.19±0.18 |
| (n=9) | (n=8) | (n=9) | (n=9) | (n=9) |
| Retinyl stearate pre-test (μmol/L) | 0.34±0.15 | 0.35±0.14 | 0.19±0.08 | 0.22±0.09 | 0.21±0.13 |
| (n=9) | (n=8) | (n=9) | (n=9) | (n=9) |
| Retinyl palmitate post-test (μmol/L) | 22.90±7.23 | 19.35±12.88 | 24.33±6.34 | 23.26±3.53 | 21.37±6.30 |
| (n=9) | (n=9) | (n=9) | (n=9) | (n=9) |
| Retinyl stearate post-test (μmol/L) | 11.07±3.26 | 9.06±1.63 | 12.51±3.09 | 12.70±1.61 | 10.34±2.39 |
| (n=9) | (n=9) | (n=9) | (n=9) | (n=9) |

Shown is the mean±SE of the mean (range).

| Table 3. Alpha-tocopherol during the therapy with imatinib. |
|---|
| Visit | 1 | 2 | 3 | 4 | 5 |
| Time from the start of therapy (d) | 0 | 14±3 | 28±7 | 45±5 | 77±8 |
| (n=16) | (n=16) | (n=16) | (n=15) | (n=14) |
| Albumin (g/L) | 44.1±0.9 | 42.5±1.1 | 42.4±0.7* | 42.7±1.1 | 44.1±0.7 |
| (13.7–52.7) | (12.6–41.5) | (12.9–36.6) | (12.9–28.7) | (12.0–39.0) |
| Total protein (g/L) | 75.0±1.4 | 72.8±1.2* | 72.3±1.4* | 70.5±1.9** | 71.6±1.6* |
| (n=16) | (n=16) | (n=16) | (n=16) | (n=16) |
| Alpha-tocopherol (μmol/L) | 30.1±2.9 | 24.5±2.2** | 24.6±1.9* | 22.9±1.8** | 23.7±2.2** |
| (n=16) | (n=16) | (n=16) | (n=16) | (n=16) |

Shown is the mean±SE of the mean (range).

*p<0.05 compared to baseline; **p<0.01 compared to baseline.
is a multi-step process that involves the hydrolysis of ingested retinyl esters by pancreatic and intestinal brush border lipases, uptake and re-esterification of free retinol by enterocytes, incorporation of retinyl esters into chylomicrons, and the release into circulation (25). As a result, only a relatively minor increase of retinol concentrations is evident after oral administration while serum concentrations of retinyl esters rise markedly. It has been demonstrated that the absorption of vitamin A is impaired in patients with benign disorders of small bowel mucosa, and the rise of serum concentrations of retinyl esters is impaired in these patients (26), but less is known about the changes of vitamin A absorption in cancer patients during systemic chemotherapy or radiation. In the present study, the number of patients was limited and the range of postprandial retinyl ester concentrations was quite wide, making a definitive interpretation of the negative results more difficult.

In contrast to the absence of significant changes of intestinal permeability or vitamin A absorption, administration of imatinib was associated with a significant decrease of serum alpha-tocopherol. This decrease may be caused by decreased absorption or oxidative stress. Vitamins A and E are processed similarly in the gastrointestinal tract, but the absorption of vitamin E is less efficient (16). Moreover, the transport of alpha-tocopherol, but not retinol, is mediated by Niemann-Pick C1-like 1 (NPC1L1) (27). Consequently, a decrease of alpha-tocopherol could manifest even if vitamin A absorption is not altered. Alpha-tocopherol is a major serum antioxidant (28). Serum alpha-tocopherol concentrations are decreased in patients with advanced cancer (29–31). In general, serum alpha-tocopherol decreases during cytotoxic therapy (32–35), and a decrease has also been described in association with targeted therapy (18). There are currently no data on the effect of imatinib on NPC1L1 activity. The decrease of serum alpha-tocopherol was accompanied by decreased serum total protein and albumin concentrations. Vitamin E is a crucial component in the protection against lipid peroxidation. In the present study, serum cholesterol concentrations were not determined simultaneously to alpha-tocopherol, but changes in alpha-tocopherol/cholesterol ratio during imatinib treatment should also be studied in future investigations.

Because imatinib therapy is frequently accompanied by edema (3), weight measurement is not reliable for assessment of nutritional status in this patient population. A decrease in nutritional intake is reflected in lower serum albumin concentrations. The association between serum albumin and alpha-tocopherol that reflects the nutritional status is well characterized (36), and could be invoked as one of the factors responsible for the decrease of alpha-tocopherol observed in this study. However, while a significant decrease in total protein concentrations was evident throughout the course of imatinib therapy, the decrease of serum albumin was less pronounced, and it is unlikely that nutritional factors are solely responsible for the decrease of alpha-tocopherol. Moreover, edema may also affect serum protein concentrations due to dilutional phenomena.

With the median survival in patients with metastatic GIST treated with imatinib currently being around 5 y (37), competing causes of mortality may be important in this patient population. Complications of atherosclerosis are the most common competing cause of death in cancer patients. Atherogenic potential of serum lipids depends on lipid oxidation (38) and is affected by liposoluble antioxidants (39). The administration of antioxidants, including alpha-tocopherol, may delay atherosclerosis (40). The observation of decreased alpha-tocopherol in patients treated with imatinib could be of significance for the long-term health in cancer survivors and warrants further study.

In conclusion, no alteration of intestinal permeability or vitamin A absorption was observed during the treatment with imatinib, but serum alpha-tocopherol decreased significantly during the therapy. The long-term significance of decreased alpha-tocopherol in patients with advanced/metastatic GIST treated with imatinib requires further study.

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