Modulation of Irinotecan-induced Diarrhea by Cotreatment with Neomycin in Cancer Patients

Diederik F. S. Kehrer, Alex Sparreboom, Jaap Verweij, Peter de Bruijn, Corine A. Nierop, Jacqueline van de Schraaf, Elisabeth J. Ruijgrok, and Maja J. A. de Jonge

Department of Medical Oncology, Rotterdam Cancer Institute (Daniel den Hoed Kliniek) and University Hospital Rotterdam, 3075 EA Rotterdam [D. F. S. K., A. S., J. V., P. d. B., C. A. N., I. v. d. S., M. J. A. d. J], and Department of Pharmacy, University Hospital Rotterdam, 3015 GD Rotterdam [E. J. R.], the Netherlands

Abstract

This study was designed to evaluate irinotecan (CPT-11) disposition and pharmacodynamics in the presence and absence of the broad-spectrum antibiotic neomycin. Seven evaluable cancer patients experiencing diarrhea graded ≥2 after receiving CPT-11 alone (350 mg/m² i.v. once every 3 weeks) received the same dose combined with oral neomycin at 1000 mg three times per day (days –2 to 5) in the second course. Neomycin had no effect on the systemic exposure of CPT-11 and its major metabolites (P ≥ 0.22). However, it changed fecal β-glucuronidase activity from 7.03 ± 1.76 µg/h/mg (phenolphthalein assay) to undetectable levels and decreased fecal concentrations of the pharmacologically active metabolite SN-38. Although neomycin had no significant effect on hematological toxicity (P > 0.05), diarrhea ameliorated in six of seven patients (P = 0.033). Our findings indicate that bacterial β-glucuronidase plays a crucial role in CPT-11-induced diarrhea without affecting entero-cycling and systemic SN-38 levels.

Introduction

CPT-11 is an inhibitor of topoisomerase I, an enzyme responsible for variations in the topological form of DNA during replication and transcription. Unlike other clinically used camptothecin analogues, CPT-11 is a produg with very little inherent antitumor activity that needs to be hydrolyzed by a carboxylesterase to form the active metabolite SN-38 (Ref. 1; Fig. 1). SN-38 in its turn is efficiently metabolized by UDP glucuronosyltransferase 1A1 to form the inactive SN-38G (2).

Myelosuppression and diarrhea are among the most common side effects of CPT-11 (3), regardless of the schedule of administration. Delayed-type diarrhea is defined as diarrhea occurring >24 h after CPT-11 administration and contrasts with the early-onset diarrhea that is acetylcholine mediated and can be prevented by atropine (4). CPT-11-induced delayed-type diarrhea has been reported to be severe (NCI-CTC grade 3–4) in ~25% of the patients (5). Moreover, even less severe diarrhea might influence continuation of therapy. The median onset of delayed-type diarrhea is day 5 after start of CPT-11 administration, and the median duration is 5 days. Delayed-type diarrhea necessitated hospitalization in 9% of the cycles for i.v. rehydration. There is no generally accepted prophylactic treatment for the delayed-type diarrhea. However, once diarrhea has occurred, a high-dose loperamide regimen renders this side effect manageable (6).

Many pharmacokinetic analyses in humans have been performed to predict the incidence of delayed-type diarrhea, with conflicting results. Some studies reported a correlation between late-onset diarrhea and biliary secretion of the active metabolite SN-38, as determined by the extent of SN-38G measured in plasma (7). Recently, it was suggested from animal models that β-glucuronidases produced by microflora in the large bowel may play a major role in the development of CPT-11-induced diarrhea by mediating hydrolysis of SN-38G to form the active SN-38 (8). Data obtained in rats have indicated that penicillin combined with streptomycin inhibited the β-glucuronidase activity from the intestinal microflora, thereby decreasing the luminal SN-38 concentration and subsequently reducing cecal damage and ameliorating diarrhea. This antibiotic treatment did not alter plasma pharmacokinetics of CPT-11 or SN-38 in the rat model (9).

Theoretically, modulation of CPT-11-induced delayed-type diarrhea in humans by coadministration of the poorly absorbed aminoglycoside antibiotic neomycin (10) could be advantageous. In this study, we assessed the influence of coadministration of oral neomycin on the metabolic disposition and pharmacodynamics of CPT-11 in a group of cancer patients using a cross-over design.
Patients and Methods

Patients and Treatment. Patients with a histologically or cytologically confirmed diagnosis of colorectal cancer refractory to therapy with 5-fluorouracil were eligible for the present study. Additional eligibility criteria included: ages between 18 and 70 years; Eastern Cooperative Oncology Group performance status ≤1; no previous treatment with antineoplastic agents for at least 4 weeks (or 6 weeks in case of nitrosoureas or mitomycin C); no prior treatment with CPT-11 or other topoisomerase I inhibitors; adequate hematopoietic (WBCs ≥3.0×10^9/liter, absolute neutrophil counts ≥2.0×10^9/liter, and platelet counts ≥100×10^9/liter), renal (serum creatinine ≤135 μM or creatinine clearance ≥60 ml/min), and hepatic function (total serum bilirubin ≤1.25× upper normal limit, and ASAT and ALAT levels ≤3.0× upper normal limits); and no unresolved bowel obstruction or chronic colic diarrhea. The clinical protocol was approved by the Rotterdam Cancer Institute Ethics Board, and all patients signed informed consent before study entry.

Vials that contained 40 or 100 mg of CPT-11 (as a hydrochloride trihydrate form) formulated as a concentrated sterile solution (active drug concentration, 20 mg/ml) in d-sorbitol and a lactic acid-sodium hydroxide buffer system of pH 3.5–4.5 were provided by Aventis (Hoevelaken, the Netherlands). The CPT-11 dose of 350 mg/m² was administered once every 3 weeks as a 90-min i.v. infusion, after dilution of the pharmaceutical preparation in 250 ml of isotonic sodium chloride. In all patients, premedication consisted of 8 mg of ondansetron i.v. combined with 10 mg of dexamethasone i.v., both administered 30 min before the start of CPT-11 infusion. Delayed-type diarrhea (in the first course) was treated with 4 mg of loperamide, followed by 2 mg every 2 h for a 12-h time period after the last stool. In case the patient developed diarrhea of grade 2 or higher in the first course despite loperamide therapy, neomycin (1000 mg daily × 3) was administered p.o. at days −2 to 5 relative to the second CPT-11 administration. All toxicities were graded according to the NCI-CTC.

Sample Collection and Analysis. Blood samples for pharmacokinetic analysis were drawn during the first course and in case of neomycin cotreatment during the second course as

![Schematic representation of CPT-11 metabolism and enterohepatic recirculation. CE, carboxylesterase; hCE1/2, human carboxylesterase isoforms 1 and 2; UGT1A1/7, UDP glucuronosyltransferase isoforms 1A1 and 1A7; CYP3A4/5, cytochrome P450 isoforms 3A4 and 3A5; APC, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin; NPC, 7-ethyl-10-[4-N-(1-piperidino)-1-amino]carbonyloxycamptothecin. * indicates the sites of drug measurement.](https://clincancerres.aacrjournals.org)
well, from a vein in the arm opposite to that used for drug infusion and collected in 10-ml glass tubes containing lithium heparin as anticoagulant. Samples were obtained at the following time points: before drug administration; at 0.5, 1, and 1.5 h during infusion; and 0.17, 0.33, 0.5, 1, 1.5, 2, 4, 5, 8.5, 24, 32, 48, 56, 196, 360, and 504 h after the end of infusion. Blood was immediately processed to plasma by centrifugation for 5 min at 2500 rpm (4°C), which was then stored at −80°C until the time of analysis by high-performance liquid chromatography as described (11, 12). A pretreatment feces sample was collected from all patients 1 day prior to drug administration in a polystyrene container and stored immediately at −80°C. Similarly, stool collections were obtained separately for the duration of hospitalization (~60 h). After thawing, these samples were homogenized individually on ice (0°C) to prevent enzyme degradation in 1 or 2 volumes of a 0.1-M sodium acetate buffer (pH 7.0), depending on the water content of the sample, using an Ultra-Turrax T25 homogenizer (IKA-Labortechnik, Dottingen, Germany). The homogenates were centrifuged for 5 min at 15,000 rpm, and the clear supernatants were diluted 1-fold with 50% glycerol in water (v/v). The dilutions were stored at −80°C until analysis for β-glucuronidase activity by a miniaturized colorimetric assay using phenolphthalein glucuronic acid as an artificial substrate (13). Urine samples were also collected from each patient during the second treatment course to evaluate the extent of neomycin absorption, as measured by a quantitative cylinder-plate microbial assay (lower limit of detection, 1.0 μg/ml).

Feces cultures within 2 days prior to therapy were taken to determine the presence of neomycin-resistant microorganism. During the second course, feces cultures were taken daily from days −3 to 3 after CPT-11 infusion and analyzed for the presence of neomycin-resistant microorganism and/or overgrowth with Staphylococci in addition to microorganisms (Salmonella, Shigella, Yersinia, and Campylobacter species) and toxins (Clostridium species) that could provoke diarrhea.

Pharmacological Analysis. Individual plasma concentrations of CPT-11 and its metabolites were fit to a three-compartment model using the Siphar version 4.0 software package (SIMED, Créteil, France), as described (14). The rate constants of the various disposition phases and the AUC (extrapolated to infinity) were estimated with a weighted-least squares method (weighting factor, 1/σ) using the model, whereas the total plasma clearance of CPT-11 was calculated by dividing dose (expressed in mg base equivalents per squared meter of body surface area) and the observed AUC. The C90% values were determined graphically (as observed values) in a log concentration-time scattered plot. Metabolic ratios were calculated as described (15) and included both the relative extent of conversion (REC) of CPT-11 to SN-38 (i.e., \( \frac{AUC_{SN,38}}{AUC_{CPT,11}} \)) and the relative extent of glucuronidation (REG) of SN-38 (i.e., \( \frac{AUC_{SN,38,6g}}{AUC_{SN,38}} \)). The latter was also evaluated as a function of time after drug administration. The systemic SN-38 glucuronidation rate in individual patients was estimated by calculation of the biliary index (B) values (7), expressed as \( \frac{AUC_{CPT,11}}{AUC_{SN,38}} \times (\frac{AUC_{SN,38}}{AUC_{SN,38,6g}}) \). The relative hematological toxicity, i.e., the percentage decrease in WBCs, was defined as: % decrease = [(pretherapy value − nadir value)/pretherapy value] × 100%.

Statistical Considerations. All pharmacokinetic parameters are reported as mean ± SD. Because multiple measurements were performed at different times on the same patients, comparisons between the sets of observations were based on within-subject differences. Therefore, variation between subjects, which is usually considerable, does not affect our ability to distinguish differences between the sets of observations, which here relate to CPT-11 courses given in the absence or presence of neomycin. The effect of neomycin on CPT-11 pharmacokinetic parameters was assessed using a paired Student’s t test and the 95% confidence limits for the mean difference after testing for normality and heteroscedasticity. Similarly, the effect of neomycin on CPT-11-induced diarrhea (scored on a four-point scale according to NCI-CTC and treated as ordered-categorical data) was evaluated using the Wilcoxon-Matched pairs signed rank sum test. All calculations were done using the NCSS 5.0 Series software package (J. L. Hintze, East Kaysville, UT; 1992). Statistical significance was considered to be reached when \( P < 0.05 \), with a two-tailed distribution.

Results

Toxicity and Pharmacodynamics. Twenty patients entered this study, of which 9 (45%) developed grade 2 diarrhea in the first treatment course and received neomycin as cotreatment in the second course. Two patients in this group were not evaluable for toxicity and pharmacokinetics in the second course; 1 patient went off study after the first course at his own request, and 1 patient was not evaluable because of early death unrelated to treatment. The seven evaluable patients had a median age of 57 years (range, 49–71 years) and an Eastern Cooperative Oncology Group performance status of 0–1, and all patients had normal hematopoietic and liver functions (except ASAT <2 × in two patients) at the time of study entry. The median clinical chemistry values included a total bilirubin level of 9 μmol (range, 7–11 μmol); a serum creatinine level of 99 μmol (range, 72–106 μmol); ASAT levels of 34 units/l (range, 17–83 units/l) and ALAT levels of 22 units/l (range, 6–38 units/l); a total protein concentration of 79 g/dl (range, 69–87 g/dl); and a serum albumin level of 45 g/dl (range, 38–51 g/dl). All seven patients experienced a grade 2 diarrhea with a median duration of 6 days (range, 5–8 days) in their first course. Of these seven (evaluable) patients, five (71%, \( P = 0.0326 \)) did not experience any diarrhea in the second treatment course, one experienced diarrhea grade 1 (3 days), and one experienced diarrhea grade 3 (5 days) in the second course.

The relative hematological toxicity, i.e., the percentage decrease in WBCs, was not significantly different between courses with values of 64.4 ± 26.2% and 43.5 ± 34.7% in the absence and presence of neomycin, respectively (mean difference, 20.9 ± 5.38%; 95% confidence limits for the mean difference, 8.33–33.5; \( P = 0.058 \)). The occurrence and severity of nausea or vomiting during treatment was comparable for both courses (data not shown). Feces cultures taken during the first and the second courses did not reveal neomycin-resistant microorganisms nor any overgrowth of pathogenic microorganisms. Cultures on infectious bacteria and toxins as mentioned above remained negative throughout treatment.
CPT-11 Pharmacokinetics. Plasma pharmacokinetics of CPT-11 and its metabolites were evaluated during both the first and second course (with neomycin) in all seven patients (Table 1). The plasma concentration-time profiles of SN-38 after CPT-11 treatment were very similar for all patients studied (Fig. 1). Administration of neomycin did not significantly alter the AUC of CPT-11 and of the active compound SN-38, as depicted in Fig. 2, A and B, and Table 1. Only the peak levels of SN-38G were significantly lower in the second course (Fig. 2C), but this did not alter the overall exposure to this metabolite. Neomycin levels in urine were very low in all patients (range, 0.72–7.27 μg/ml; interpatient variability, 58 ± 18%), indicating no relevant absorption of the drug.

Fecal β-glucuronidase activity in the first course was significantly higher than in the neomycin cotreatment course (Fig. 3A), compatible with the elimination of the intestinal microflora by neomycin. Consequently, the fecal SN-38G:SN-38 ratio was 3-fold higher in the second course (Fig. 3B).

Discussion

In the current study, we obtained both clinical and pharmacokinetic data in humans that increase our insight into the pathogenesis and prevention of CPT-11-induced delayed-type diarrhea. It has been shown previously that β-glucuronidase activity derived from the intestinal microflora is able to hydrolyze biliary secreted SN-38G to the active compound SN-38. SN-38 can cause histological damage to the colon with minimal damage to the small bowel, as was observed in a rat model and as is believed to be the main course for the occurrence of CPT-11-induced delayed type diarrhea (8). Our present results indicate that β-glucuronidase activity can be inhibited by eliminating the microorganisms by administration of the (poorly absorbed) aminoglycoside antibiotic neomycin, without any signs of negative effects concerning bacterial overgrowth, toxins, or neomycin resistance.

Because SN-38 is considered the active compound of CPT-11 treatment, it is of the utmost importance that plasma SN-38 pharmacokinetics are not altered by cotreatment with neomycin. Because SN-38 is subject to enterohepatic recirculation, suppressing intestinal β-glucuronidase activity could potentially influence plasma pharmacokinetics. In this study, we have shown that influencing β-glucuronidase activity by co-treatment with neomycin in patients does not alter the plasma SN-38 pharmacokinetics nor CPT-11 plasma disposition. The paradox between the enterohepatic recirculation of SN-38 and the lack of relationship of fecal β-glucuronidase activity with SN-38 pharmacokinetics is presumably caused by the lack of enzyme activity in the luminal contents of the entire small intestine, where reabsorption of drug is most likely to occur (13). However, we did find a minor but significant decline in the peak levels of SN-38G. Although the total CPT-11 metabolism has still not been completely elucidated, this particular alteration might be explained by an up-regulation of serum β-glucuronidase activity after repeated exposure to CPT-11, as has been described earlier in a rat model (16), rather than by the coadministration of neomycin. The earlier reported correlation between systemic glucuronidation and the incidence of diarrhea as expressed in the biliary index (7) could not be found in our patient population nor in a larger previous study (17). In contrast, we found unaltered biliary indices with and without neomycin cotreatment (Table 1), with complete disappearance of diarrhea after neomycin coadministration in five of seven patients. This is in keeping with the postulated concept regarding the mechanism of CPT-11-induced diarrhea, being a direct local

Table 1 Pharmacokinetic variables of 350 mg/m² i.v. CPT-11 for seven patients in the absence (course 1) and presence (course 2) of oral neomycinα

<table>
<thead>
<tr>
<th></th>
<th>Course 1</th>
<th>Course 2</th>
<th>95% C.L. (d)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPT-11</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (μM)</td>
<td>7.67 ± 1.90</td>
<td>7.62 ± 1.72</td>
<td>-1.07 to 1.19</td>
<td>0.90</td>
</tr>
<tr>
<td>T1/2(z) (h)</td>
<td>12.9 ± 3.40</td>
<td>13.4 ± 5.34</td>
<td>-5.99 to 4.92</td>
<td>0.81</td>
</tr>
<tr>
<td>AUC0→ss (μM \· h)</td>
<td>41.0 ± 15.6</td>
<td>45.1 ± 20.3</td>
<td>-11.8 to 3.52</td>
<td>0.22</td>
</tr>
<tr>
<td>CL (l/h/m²)</td>
<td>15.8 ± 3.79</td>
<td>14.8 ± 4.42</td>
<td>-1.88 to 3.74</td>
<td>0.44</td>
</tr>
<tr>
<td>Vz (l/m²)</td>
<td>137 ± 36.4</td>
<td>141 ± 48.9</td>
<td>-31.8 to 22.5</td>
<td>0.68</td>
</tr>
<tr>
<td>MRT (h)</td>
<td>9.70 ± 2.38</td>
<td>10.6 ± 2.76</td>
<td>-2.79 to 1.02</td>
<td>0.29</td>
</tr>
<tr>
<td>SN-38</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (μM)</td>
<td>0.223 ± 0.168</td>
<td>0.161 ± 0.093</td>
<td>-0.081 to 0.161</td>
<td>0.43</td>
</tr>
<tr>
<td>T1/2(z) (h)</td>
<td>46.7 ± 6.72</td>
<td>41.9 ± 3.86</td>
<td>-4.92 to 14.4</td>
<td>0.25</td>
</tr>
<tr>
<td>AUC0→ss (μM \· h)</td>
<td>1.65 ± 1.24</td>
<td>1.64 ± 1.20</td>
<td>-0.143 to 0.183</td>
<td>0.75</td>
</tr>
<tr>
<td>REC (× 10³)</td>
<td>2.98 ± 2.73</td>
<td>2.58 ± 2.03</td>
<td>-0.782 to 1.70</td>
<td>0.36</td>
</tr>
<tr>
<td>SN-38G</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cmax (μM)</td>
<td>0.414 ± 0.128</td>
<td>0.290 ± 0.072</td>
<td>0.035 to 0.211</td>
<td>0.016</td>
</tr>
<tr>
<td>T1/2(z) (h)</td>
<td>33.5 ± 9.11</td>
<td>36.9 ± 7.69</td>
<td>-10.1 to 3.29</td>
<td>0.23</td>
</tr>
<tr>
<td>AUC0→ss (μM \· h)</td>
<td>8.92 ± 4.45</td>
<td>8.66 ± 3.38</td>
<td>-1.39 to 1.90</td>
<td>0.69</td>
</tr>
<tr>
<td>BI</td>
<td>3390 ± 2360</td>
<td>3880 ± 2830</td>
<td>-1260 to 295</td>
<td>0.16</td>
</tr>
</tbody>
</table>

α Data are expressed as mean values ± SD.

β Two-tailed paired Student’s t test. 95% C.L. (d), 95% confidence limits for the mean difference; Cmax, peak plasma concentration; T1/2(0→ss), apparent half-life of the terminal disposition phase; AUC0→ss, area under the plasma concentration-time curve extrapolated to infinity; CL, total plasma clearance; Vz, volume of distribution at steady-state; MRT, mean residence time; REC, relative extent of conversion of CPT-11 into SN-38G (i.e., AUC0→ss[CPT-11]/AUC0→ss[REC]); REG, relative extent of glucuronidation of SN-38 into SN-38G (i.e., AUC0→ss[SN-38]/AUC0→ss[REG]); BI, biliary index [i.e., AUC0→ss[CPT-11] × (AUC0→ss[SN-38]/AUC0→ss[SN-38G])].
toxic effect of the active compound SN-38, and sheds light on an important mechanistic aspect of the role of bacterial β-glucuronidases in its etiology.

In contrast to earlier data from Phase III studies reporting on CPT-11-induced delayed-type diarrhea (3), only 45% of the patients enrolled in the current study developed diarrhea in the first course of chemotherapy, and this diarrhea was never worse than grade 2. This low frequency of diarrhea could be either attributable to patient selection or to the small number of patients enrolled in this study. Importantly, however, from the seven patients evaluable for study purposes, five did not experience any diarrhea after coadministration of neomycin, whereas additionally in one other patient the grade of diarrhea reduced from grade 2 to 1, and this diarrhea lasted for only 3 days. Only one patient experienced a more severe diarrhea with coadministration of neomycin.

We selected the poorly absorbed aminoglycoside antibiotic neomycin because of its broad antimicrobial spectrum as well as its local gastrointestinal disposition. Indeed, neomycin concentrations in the urine were very low after its administration, indicating that no substantial drug absorption took place. As with many antibiotics, treatment with this agent can cause diarrhea and malabsorption, which could have been the case in the one patient who experienced grade 3 diarrhea in the second course. This side effect is usually seen only with chronic administration in high (≥12 g/day) dosage regimens (18). Because neomycin can induce diarrhea, largely depending on dosage and duration of treatment, with no data available on β-glucuronidase activity compared with lengths of treatment, these are currently the subject of investigation by us.

In theory, other antibiotics could also have been used in the treatment of CPT-11-induced delayed-type diarrhea, but clearly a nonresorbable drug is to be preferred. It would be even more attractive to use an agent that specifically inhibits the microbial β-glucuronidase activity. Hange-shasin-to (also referred to as TJ14), a herbal medicine that contains the β-glucuronidase inhibitor baicalin, has been described recently to be a potent inhibitor of delayed-type diarrhea caused by CPT-11 in a rat model (19) as well as in humans (20). Unfortunately, there is yet no information on possible changes in plasma β-glucuronidase activity because of this agent. Neither are there data whether and how this agent influences plasma CPT-11 and SN-38 disposition, information of vital importance considering antitumor activity.

In conclusion, cotreatment of CPT-11 with neomycin effectively decreases fecal β-glucuronidase activity and consequently decreases enteral SN-38 concentrations without altering the plasma pharmacokinetics and metabolic profiles of both CPT-11 and SN-38. This therapy could ultimately lead to de-
increased CPT-11-induced delayed-type diarrhea. A large randomized clinical trial of CPT-11 chemotherapy with or without prophylactic neomycin administration to patients is presently being conducted to confirm the present findings.

References
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