Locally advanced rectal cancer

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Years of preparation.
Months of waiting.
Days to remember.
Hours until sunrise.
Minutes of terror.
Seconds of glory.
Big time is here.

David Carson

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General introduction

Colorectal cancer is the third most common invasive tumor in the Netherlands. In 1997, 8600 new colorectal cancer patients were registered, of whom 25 % had rectal cancer. The mortality of these patients is about 900 per year. The 5 years survival is around 50 % and is largely dependent on tumor stage at diagnosis.

The group of patients studied in this thesis have presented themselves with a locally advanced rectal tumor not amenable for primary resection. Surgical resection of a rectal tumor is the treatment of choice and obtaining tumor-free margins is essential for a potentially curative treatment. Therefore attempts are being made to reduce the tumor size by using radiotherapy alone or in combination with chemotherapy (neo-adjuvant treatment). If successful, this strategy leads to resectability.

Firstly this thesis presents a review of the literature concerning the developments in staging techniques and neo-adjuvant radio(chemo)therapy of primary irresectable rectal tumors. Furthermore it describes the study results regarding prognosis after downstaging and downsizing of rectal tumors after neo-adjuvant radiochemotherapy. Another chapter discusses the relation between survival after radiochemotherapy and the value of molecular markers, in the primary tumor. Chapter 4 reports on a phase I study performed in the University Hospital in Groningen concerning the optimization of neo-adjuvant therapy by the addition of oxaliplatin to the standard radiochemotherapy regime. A study into the implications of an inborn genetic error resulting in an increased toxicity profile with 5FU chemotherapy is described in chapter 5. Finally we report on the fate of patients with locally relapsed rectal cancer, who were referred by their surgeons for salvage treatment.

Chapter 1

Developments in treatment of primary irresectable rectal cancer.

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Colorectal disease, in press

Abstract

The treatment options for primary irresectable rectal cancers are discussed. Assessment of tumor stage is the first step for an appropriate choice of treatment. Following a diagnosis of rectal cancer, a vast array of diagnostic procedures is available to determine its stage, and thereby its best treatment options. From the many (new) diagnostic options the merits and demerits are discussed.

If a diagnosis of irresectability is made, further treatment options should include radiotherapy in most cases, some aspects of timing and application i.e. intraoperative treatment are discussed. Chemotherapy options are manifold, the results are discussed and some new options are explored.

Introduction

Colorectal cancer is a major public health problem in the Western world and ranks as the third leading cause of death in both males and females. In 1997, 8600 new colorectal cancer patients were registered in the Netherlands, 25% of these tumors were located in the rectum (1). In the treatment of patients with rectal cancer the mainstay is surgery. Obtaining free circumferential resection margins is essential for a potentially curative resection (2;3). Locally advanced tumors are those tumors reaching to and beyond the endopelvic fascia (extensive T3 and T4 tumors). These tumors may be extirpable but are not curatively resectable using Total Mesorectal Excision (TME) since achievement of a free circumferential resection margin (CRM) is unlikely, even with a well performed TME. Wide en bloc resection of adjacent organs has been described as treatment but failure rates remain high with 5 year survival rates of only 19-33 percent with surgery alone (4).

Patients with persisting rectal tumor or local relapse often present with severe disabling symptoms like, pain, tenesmi, bleeding and ulcerating perineal wounds. Because of this morbidity and mortality optimizing the neo-adjuvant treatment of the primary irresectable rectal tumor aiming for free CRM resectability is of paramount importance.

The decision on the choice of treatment strategy in rectal cancer is based on preoperative staging. The curative treatment of patients with resectable rectal tumors differs greatly from that of patients with a primary irresectable tumor.

This review addresses the preoperative staging techniques for patients with rectal cancer and the neo-adjuvant treatment options for patients with primary rectal cancer.

Differentiation between resectable and irresectable rectal cancer

The accuracy of any diagnostic technique has to be validated against a gold standard. In resectable rectal cancer the pathological specimen can serve this purpose.

Therefore information on sensitivity and specificity of various staging techniques originate from observations in the resected tumors that usually have a low tumor (T) stage. The diagnosis of irresectability for a rectal tumor requires especially information on the endopelvic fascia with its relation to the primary tumor and less of the regional nodes. In choosing an optimal staging technique it may be appropriate to concentrate on the accuracy to detect extensive T3 and T4 tumors. Most studies of imaging techniques report only on the accuracy of T and N stage. As a result of this the differentiation between a limited and extensive T3 tumor is not possible (figure 1). The importance of this lies in the relation between the distance of the primary tumor to the endopelvic fascia and the rate of local recurrence. A distance less than 5 mm is considered inadequate (5). In this section we report on historical studies investigating preoperative T stage (irresectable) rectal tumors realizing that more recent studies focus on prediction of the circumferential resection margin.

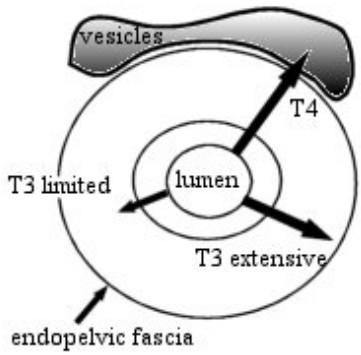

Figure 1. Extent of tumor in relation to the endopelvic fascia.

Digital rectal examination (DRE)

Originally digital rectal examination was the most important and often only technique available. Patient history and physical examination can be of value in determining the local extension of

the tumor. The involvement of surrounding structures may provoke symptoms such as hematuria, pneumaturia, vaginal bleeding and radicular pain. The presence of lower extremity edema is an ominous sign of venous or lymphatic outflow obstruction due to spread of tumor. DRE can assess local tumor spread with an accuracy ranging from 44 % to 83%, as shown in a study by Nicholls (6). In this study a panel of 10 clinicians investigated the limitations and reproducibility of DRE in 70 patients with palpable rectal tumors. Understaging was a particular problem in this study. Tumors penetrating through the rectal wall were assessed as confined in 2-16 % of the cases. This broad range of "accuracy" was mainly due to the difference in experience of the clinicians. Another limitation of digital rectal examination is that in 30 % of rectal cancers, the tumor is too proximal (≥ 10 cm) (7). Not reproducible terms like 'thetered' or 'fixed' where used to indicate irresectability.

Laparotomy and Laparoscopy

In the past a 'staging' laparotomy was performed on patients with a clinical suspicion of an irresectable rectal tumor (8;9). The concept of this laparotomy comprises the possibility to assess the extent of a rectal tumor bimanually, to perform an inspection of the abdominal cavity and to construct an end colostomy to ensure bowel passage. The results of this staging laparotomy could be embedded in the diagnostic work up. More recently the role of laparoscopy as a staging modality in locally advanced disease was studied (10). This procedure allows the inspection of the abdominal cavity to identify patients with unsuspected peritoneal disease and also the construction of a colostomy. The distal rectosigmoïd can be left in the pelvic cavity to act as a biological spacer, facilitating radiotherapy without damaging the small bowel. Since it is important not to open the peritoneum of the pelvic cavity, accurate assessment of the endopelvic fascia or ingrowth in other organs below the pelvic reflection in abdominal staging is restricted to the liver, peritoneal surface and retroperitoneal lymph nodes.
The introduction of endorectal ultrasonography, CT and MRI made a less invasive and reasonable accurate staging possible.

Endorectal ultrasonography (EUS)

Endorectal ultrasonography is very accurate in determining the infiltration of the tumor in the rectal wall, mainly for stages T1 and T3 with a sensitivity of 82 % and 92 %, a specificity of 99 % and 84 % and an accuracy of 92 % and 85 % respectively. The sensitivity of 82 % is high compared to other staging techniques like CT, MRI and MRI with an endorectal coil. These

data are from a review by Kwok et al. (11) who reviewed 83 studies including 4897 patients determining the wall penetration of rectal tumors. Investigated staging techniques were CT, endorectal ultrasound, MRI and MRI with endorectal coil. The results of pathological T4 stage compared to preoperative ultrasound were derived from a group of 1852 patients, from 31 studies. The assessment of T4 lesions is reasonably accurate when comparing with other staging techniques (sensitivity: 85%, specificity: 98 %, accuracy: 97%). The modest sensitivity is the result of limits in the resolution or depth of penetration depending on the ultrasound frequency used (12). A particular problem with endoluminal diagnosis occurs when the tumor obstructs the rectal lumen. This occurs in 17 % of endoluminally staged rectal tumors (12). This problem could be solved by using three-dimensional endorectal ultrasonography. In this case scan planes can be chosen deliberately within the scanned volume. Hunerbein found an accuracy of 78 % in the staging of these obstructing rectal tumors (13). The assessment of T-stage with EUS is reasonable accurate but the visualization of the endopelvic fascia with EUS is not possible. Therefore this technique is not useful in the differentiation between a limited and an extensive T3 tumor.

Computed tomography

The accuracy of computed tomography is also stage dependent. In the review by Kwok 135 patients were preoperatively staged, with CT using the TNM classification. In staging T1-2 tumors 25 of the 40 patients with a pT1-2 tumor were correctly staged, sensitivity of 63 % (specificity 93 %, accuracy 84 %). For T3-4 tumors 83 patients out of 95 were staged correctly (sensitivity 87 %, specificity 50 %, accuracy 76 %) (11). The poor sensitivity of CT in the staging of T1-2 tumors in patients with rectal cancer is mainly related to the inability to demonstrate the single layers of the rectal wall.

New developments in computed tomography show promising results in diagnostic accuracy. Matsuoka compared multi-slice spiral computed tomography to conventional CT (14).

They found in a group of 20 patients, a prediction of T3-4 tumor stage (n=15) with a sensitivity of 100 % compared to 80 % in conventional CT. Conventional CT did not detect three T3 tumors. Spiral CT scan has the advantages of fast volume scanning, absence of artifacts related to motion, absence of missed slices, and availability of reformations in multiple planes and three-dimensional reconstruction. Also the assessment of distant metastases with one spiral CT scan of lungs, liver and retroperitoneum is possible. This is the so-called ‘one stop shop’. Previously not detected metastatic disease is now early visible.

Magnetic Resonance Imaging

MRI studies on staging of rectal cancer can be divided into two groups; one using external surface coils, the other using endorectal coils. The use of an endorectal coil results in an increased signal-to-noise ratio compared with use of a surface coil; higher-resolution images can be obtained because the field of view is decreased. Kwok (11) found an overall sensitivity of 86 % (specificity 77 %, accuracy 82 %) in a group of 521 patients from 18 different studies on the detection of rectal wall penetration in MRI studies with surface coils. In endorectal coil studies a median sensitivity of 89 % (specificity 79 %, accuracy 84 %) was reported in 169 patients from 6 studies. Surface coil MRI studies reporting on the assessment of T4 tumor stage (8 studies, including 246 patients) found a sensitivity of 78 % (specificity 99 %, accuracy 98 %). In a recent MRI study by Beets-Tan, using an external (phased array) coil, with 2 different observers a sensitivity of 75 % and 100 % was found on predicting T4 tumors (5). Kwok reported from 4 studies, including 124 patients, a sensitivity of 83 % (specificity 100 %, accuracy 99 %) using an endorectal coil (11). Studies comparing endorectal ultrasonography with endorectal coil MRI showed a difference in overall tumor stage accuracy between the 0 and 10 % favoring MRI (15-19). One study by Meyenberger (n=32) found an 84 % accuracy in assessment of transmural tumor infiltration compared to 80 % with endorectal coil MRI (20). There are a limited number of reports comparing MRI and computer tomography in the assessment of rectal cancer stage. In the detection of tumor infiltration into the perirectal fat, Cova (n=22) favored CT with a sensitivity of 100 % to 91 % in MRI (surface coils) (specificity: 45 % and 50 % respectively) (21). Guinet on the other hand showed, in a study with 19 patients, a better sensitivity for MRI (74 %) compared to 68 % for CT (22). These results were not statistically significantly different.

Positron Emission Tomography

PET has not been extensively explored in the preoperative staging of rectal cancer. Abdel-Nabi correlated PET and CT findings in 48 patients with colorectal cancer (23). They found a higher sensitivity in PET imaging in detecting carcinomas (100%), but also a specificity of 43 % due to inflammatory conditions. By means of PET imaging it was not possible to determine an accurate T stage. Using PET in the follow up to detect local recurrences and distant metastases seems feasible (24-26). New techniques like combined PET-CT imaging might localize

neoplastic lesions more precisely (27). So far no studies on preoperative staging of rectal cancer, with this technique have been performed.

Predicting the CRM

Recent imaging studies have shown that high-resolution MRI techniques can clearly visualize the endopelvic fascia. The study by Beets-Tan showed that MRI with external coil could predict the CRM more accurate than the T stage (5). Two other studies, one by Brown and another by Bisset both showed that depth of extramural tumor infiltration could accurately be predicted with MRI (28;29). Despite the potential of newer generation spiral CT scans, to date its role in the determination of the CRM has never been investigated. It can be expected that high-resolution multislice spiral CT will compete with high resolution MRI for the determination of the endopelvic fascia and CRM.

The endopelvic fascia is difficult to identify on ultrasonography because of the limited soft tissue contrast resolution and limited field of view; prediction of the CRM with this method is therefore difficult.

Conclusion on preoperative staging of irresectable rectal cancer

After a review of the literature in search of the optimal staging technique to a T4 tumor we found no significant differences between various techniques (table 1).

Table 1. Calculated percentages on preoperative staging of T4 rectal tumors (11).

	CT	***EUS***	***MRI***	***endo-MRI***
Sensitivity:	78%	85%	78%	83%
Specificity:	97%	98%	99%	100%
Accuracy:	94%	97%	98%	99%

When comparing the sensitivity of the different staging techniques there is no significant difference. After calculating the possible differences in sensitivity by means of a Chi-square test the lowest p-value found is 0.49.

The ability to differentiate between normal tissue and tumor growth in adjacent organs or structures predicts the sensitivity of a screening. All studied techniques fail to some degree in that respect. Due to this moderate sensitivity some patients will incorrectly be diagnosed as

having resectable tumors. Recognition of tumor tissue in a normal surrounding might in future be helped by PET scanning or labeling techniques.

Endorectal ultrasound (EUS) is operator dependent and the interpretation of images other then by the operator is difficult, making it less practical in clinical use. EUS and MRI using an endorectal coil can be of great discomfort for a patient with a locally advanced rectal tumor.

In conclusion, the most attractive way to assess the resectability of a rectal tumor in respect to patient, surgeon and radiotherapist requirements seems to be MRI with an external body coil or spiral CT. Still, more studies should be done, investigating accuracy in predicting the CRM with new techniques like spiral CT and comparing them with established techniques.

Treatment options for irresectable rectal cancer

Preoperative radiotherapy

The aim of preoperative radiotherapy in rectal cancer is to decrease the tumor burden in the irradiated area. In resectable cancer this should lead to a decreased incidence of local relapse, an irresectable tumor might be downsized and downstaged so far as to permit a resection with tumor free surgical margins.

Although a dose-response relationship exists in favor of higher doses of radiation (> 55 Gy) (30;31), there is still no consensus about an optimal dose. Measurement of the response in irresectable rectal tumors is mostly clinical. Wong reviewed the results of different studies on the response to radiotherapy in recurrent, residual and irresectable rectal tumors (32). The endpoints used in these studies were improvement of symptoms or clinical assessment of tumor volume regression. There was a suggestion for a more favorable response with doses above the 45-50 Gy. The subjectivity of these endpoints limits the value of these results. Little is known about the histopathological response to radiotherapy alone in rectal cancer. Bouzourene introduced a standardized assessment of a tumor regression grade, which can predict clinical outcome to preoperative treatment (33). They reported a pathological dose-response relationship (downstaging) in 43 % of 103 patients with cT3-4 rectal tumors treated with a biologically effective dose (BED) of 45 Gy.

The main limiting factor to higher doses is the normal tissue tolerance. The small bowel is the most radiosensitive organ located in the radiation area of the rectal tumor. Radiation induced small bowel complications are described more often in the post- than the preoperatively irradiated patients (n=422; 11% vs. 5%, p=<0.01) (34). This is caused by a descent of small bowel into an emptied pelvis and a decreased mobility of the small bowel due to adhesions after surgery. It is of importance that the volume of small bowel that is irradiated correlates with the amount of acute and late toxicity. Gallagher found that volumes greater than 394 cc receiving a radiation dose above 45 Gy may causes a grade III late toxicity (35). Coia reported in a review of the literature a rapidly increasing major small bowel toxicity with doses above 50-55 Gy when substantial small bowel (no volume stated), but less than whole abdominal, radiation is administered (36). A number of studies have been done to minimize the irradiated volume of the organs at risk. Among these are surgical procedures and radiotherapy adjustments. Surgical procedures included the application of a spacer in the small pelvis, organic (37;38) or anorganic(39;40). There are no comparative studies available but all of these measures led to a reduction of irradiated small bowel volume. The radiotherapy related adjustments include the

adaptation of a radiation technique from antero-posterior postero-anterior fields to a 3-4 beam technique, which results in a decrease of the irradiated small bowel volume and less acute and late toxicity. (41;42). With a 3-4 beam technique a more homogeneous dose distribution is reached with a better sparing of the organs at risk. In combination with the use of small bowel contrast the portal fields can be adjusted by multileaf setting to the optimal treatment volume. Gallagher has shown that a patient lying in prone position with a compressed abdomen has a reduced small bowel volume in the irradiation fields compared to a supine position (37). A disadvantage of the prone position is the diminished reproducibility of the accuracy of patient positioning. However with the introduction of the belly board and an electronic portal imaging device an accurate treatment is ensured with a daily setup variation of < 3 mm (43). These techniques permit the administration of a higher radiation dose (> 50 Gy). New radiation techniques like Intensity Modulated Radiotherapy have not yet shown its benefit in the preoperative treatment of rectal cancer but studies are ongoing (44).

The degree of downsizing and downstaging that can be reached does not only depend on total radiation dose, fraction size and overall radiation treatment time but also on the interval between the end of radiotherapy and surgery. At a relatively low dose of 25 Gy in 5 Gy per fraction (biological effective dose 37.5 Gy) downstaging has been described (45), but only after an interval longer than 10 days between start of radiotherapy and surgery. Marijen demonstrated that no downstaging occurred in the Dutch rectal cancer trial as the overall treatment time did not exceed the 10 day interval (46). The Lyon R90-01 trial demonstrated a significant difference in pathological downstaging for a 6-8 week interval compared to a 2 week interval after the end of irradiation (p=0.005)(47). In a retrospective study by Berger a benefit was also reported in pathological downstaging for a longer interval (> 4 weeks) (48). These results indicate that for an optimal pathological downstaging an interval of 4-8 weeks is needed.

Radiosensitization of tumor cells is another approach to achieve a better response. The response of cells to radiation is strongly dependent upon oxygen and hypoxia in tumors negatively influences the radiation response (49). Radiosensitizing the hypoxic cells either chemically by using nitromidazoles or by hyperthermia have been studied. The rationale for the use of nitromidazoles is the radiosensitization of distant hypoxic cells by diffusion of these drugs out of the tumor blood supply (50). A meta-analysis evaluating 50 randomized clinical trials showed an improvement of the loco-regional tumor control and overall survival rate after radiotherapy (51). The treatment benefit could mostly be related to the response in head and neck and bladder carcinoma. No trials are available on colorectal cancer.

Combining hyperthermia and radiation directly increases radiosensitivity and reduces the repair of damage (52). Furthermore, in a hypoxic environment the hyperthermic damage is enhanced because of direct heat killing of cells. One randomized clinical trial investigated the role of the addition of hyperthermia to radiotherapy in locally advanced pelvic tumors. They found a benefit in response rate for cervical cancer but not for rectal cancer (53).

Peri-operative radiochemotherapy

The rationale of combining radiotherapy and chemotherapy is the expectation of additional or even synergistic cell kill of the combination. In vivo and in vitro experiments indeed demonstrated such an enhancement. (54;55). The limitation of combining chemo- and radiotherapy is obviously that the same effect occurs in normal tissue. In clinical studies, tumor selective synergistic or additive cell kill should certainly be translated into better local control and possibly into increased survival. The latter effect would be dependent on an individual effect of chemotherapy on metastatic disease and a role of local control in ultimate survival. A requirement would also be that toxicity of the combination should not exceed that of radiotherapy alone. To what extend have these expectations been fulfilled in clinical experience so far?

Concomitant radiochemotherapy

Three randomized clinical trials investigated the therapeutic gain of concomitant radiochemotherapy in the treatment of rectal cancer (56-58). The first trial was done by Moertel who randomized a group of 65 patients with unresectable colorectal cancer. They found a difference (p=0.05) in overall survival (17 vs 23 months) between the patients treated with 35-40 Gy alone or combined with 5FU (45 mg/kg/3days) (58). Patients treated in the combined modality arm experienced more acute toxicity (diarrhea ("severe but clinically tolerable") 6% vs 13%, leucopenia (< 2 x 10^9/l) 1% vs 36%, thrombocytopenia (<50 x 10^9/l) 0% vs 9%). Two other trials investigating the role of postoperative radio(chemo)therapy where performed by Krook and the Gastointestinal Tumor Study Group (GITSG). In Krooks study 204 patients who underwent a curative resection, were randomized between postoperative radiotherapy alone or combined with chemotherapy (50.4 Gy, 5FU +/- mCCNU) (57). They found a significant benefit in overall survival (p=0.043), and local and distant recurrence free survival (p=0.036 and p=0.011 respectively). Severe acute toxicities were found more often in the combined modality arm (severe diarrhea 5% vs 20%, leucopenia (<2 x 10^9/l) 0% vs 18%) but there were no drug-related deaths. There was no difference in the occurrence of late toxicity (6% vs 7%).

The randomized trial from the GITSG found a benefit (p=0.04) in disease free survival for the postoperative radiochemotherapy (42 Gy, 5FU/mCCNU) arm (n=46) compared to postoperative radiotherapy alone (n=50) (56). Severe radiation enteritis with diarrhea occurred more frequently in patients who received combined therapy (35% vs 16%). Radiation enteritis developed in 4% of the patients treated with radiotherapy alone and 7% of those given combined therapy.

Neo-adjuvant radiochemotherapy

Three randomized trials investigated the benefit of a combined modality therapy in the preoperative treatment of primary irresectable rectal cancer (59-61). They found no significant survival benefit and only Frykholm (59) found a difference in local recurrence free survival (38% vs 66%, p=0.03). The main toxicities were diarrhea, mucositis, leucopenia and skin problems, which were significantly increased in the group of patients who received the combination treatment.

In these 6 studies the drug used to synergize the effect of radiotherapy has been 5FU. This drug can be given either as bolus or as continuous infusion. A randomized study by O'Connell demonstrated the superiority of 5FU continuous infusion to 5FU bolus in terms of time to relapse and overall survival in a postoperative radiochemotherapy regimen (62).

New drugs, preclinical results

Recently new drugs have been introduced in the treatment of disseminated colorectal cancer. Slowly these drugs are also investigated in the adjuvant and neo-adjuvant setting in rectal cancer. Among these are oral 5FU prodrugs (e.g. capecitabine, doxifluridine and tegafur/uracil (UFT)), oxaliplatin and irinotecan. Preclinical studies provided interesting results for the use of these new drugs.

Capecitabine is an oral fluoropyrimidine. It is rapidly and extensively absorbed as an intact molecule. Thereafter it is metabolized to 5FU in three steps. The final step from 5'-DFUR (doxifluridine) to 5FU is catalyzed by thymidine phosphorylase (TP) and takes place to a higher extent in the tumor cells (provided a high level of TP expression in the tumor cells of the patient). Thus capecitabine offers a potential reduction of the systemic exposure to 5FU with an increased 5FU concentration in the tumor tissue (63). Radiation alone also induces TP and might therefore enhance the efficacy of capecitabine (64). Doxifluridine, also a 5FU prodrug depending on TP has also shown antitumor activity. In preclinical studies Ishikawa found a therapeutic gain when this drug was combined with radiation compared to 5FU (65). Experimental studies with UFT also suggested a radiosensitization of tumor cells (66).

A synergistic antitumor activity for oxaliplatin in combination with 5FU was found (67;68). Moreover oxaliplatin can improve the efficacy of radiotherapy as shown in a study by Cividalli et al. (69). They found an increased antitumor effect in the combination of radiotherapy with oxaliplatin.

An in vivo study has shown a potentiation of the antitumor effect when irinotecan was added to 5FU in rats bearing colorectal cancer (70). Potentiation was also found in vitro between irinotecan and radiotherapy (71).

New drugs, clinical results

Two randomized trials compared capecitabine monotherapy with bolus intravenous 5FU/leucovorin in patients with metastatic colorectal cancer (72;73). Capecitabine achieved an equivalent efficacy compared with bolus 5FU/leucovorin and in one trial a benefit in tumor response rate was found (73). Capecitabine led to a lower incidence of stomatitis and neutropenia, but to a significantly more frequent development of grade III hand-foot syndrome (16% vs 0.3%; $p<0.00001$) and hyperbilirubinemia (24% vs 3%; $p<0.0001$).

Capecitabine as mono-drug combined with radiotherapy (50.4 Gy) in the preoperative treatment of rectal cancer produced pathological complete responses in 10-31 % of the patients with an acceptable toxicity (grade III-IV diarrhea 4-17%) but showed in 33% of the patients a grade III-IV hand-foot syndrome (74;75).

In the preoperative chemoradiation treatment of T3N1 or T4 rectal tumors Kim et al. randomized 28 patients between bolus intravenous 5FU or oral doxifluridine (76). No significant differences were found in the pathological complete responses between the 2 treatment arms (doxifluridine 14 %, 5FU i.v. 21 %). Only grade I-II diarrhea were reported, 36 % in the doxifluridine arm and 14 % in the intravenous 5FU arm. Min et al. studied the efficacy of oral doxifluridine compared to bolus intravenous 5FU in the postoperative combination treatment of 166 stage II-III rectal cancer patients (77). Comparable therapeutic effects were found in both arms, but grade III-IV diarrhea was significantly more frequent in the doxifluridine arm (17% vs 0%) and leucopenia in the intravenous arm (0% vs 7%).

Tegafur/uracil (UFT) was studied in 2 randomized studies comparing UFT and oral leucovorin with intravenous 5FU and leucovorin in patients with metastatic colorectal cancer. Both studies produced no differences in overall survival and tumor response and UFT/LV provided a safer and convenient oral alternative to the standard intravenous regime (78;79).

In the preoperative radio- and chemotherapy of rectal cancer with UFT instead of intravenous 5FU pathological complete responses were found in 13-15 % of the patients with grade III-IV diarrhea occurring in 23-43 % of the patients.(80;81).

De Gramont and Giacchetti found in metastatic colon cancer a significant benefit in disease free survival when adding oxaliplatin to the standard chemotherapy (82;83). No difference in overall survival was found however. In the combination arm significantly more grade III-IV diarrhea and (self limiting) sensory neuropathy was found. The combination of oxaliplatin, 5FU and radiotherapy in rectal cancer treatment was studied in 5 phase I and II studies. Results in response and toxicity differ, depending on treatment regimen (total radiation dose, 5FU CI or as bolus). A range of pathological complete responses of 14-25% was found and grade III-IV diarrhea in 3-33% of the patients (84-88).

The efficacy of the combination of irinotecan and 5FU/leucovorin in the treatment of metastatic colorectal carcinoma was also investigated in 2 randomized clinical trials (89;90). Both studies found a benefit in disease free and overall survival in the combination arm. The study by Saltz was criticized because of an unexpectedly high rate of death associated with the use of the identical drug combination in separate studies (91).

With respect to local effects, irinotecan in combination with 5FU (CI) and radiotherapy is a potent drug in the treatment of rectal cancer with a pathological complete response rate discribed in 3 different studies ranging from 25-38 %. Frykholm found a pathological response rate in 12 % of the patients treated with 5FU/LV and radiotherapy (59). But irinotecan also produced a high frequency of grade III-IV diarrhea (28-43%) (92-95).

A randomized phase II trial comparing capecitabine plus irinotecan versus capecitabine plus oxaliplatin in the first line treatment of advanced colorectal cancer showed similar activity. However the 2 toxic deaths of the 28 patients treated in the irinotecan arm require further investigation (96). Other phase II trials combining capecitabine with oxaliplatin or irinotecan both showed response rates of ≥ 50 % with comparable toxicities mainly concerning grade III-IV diarrhea (97-101).

In conclusion, several phase I-II studies have investigated the safety and feasibility of radiochemotherapy with one or a combination of these new drugs in the preoperative treatment of rectal cancer. A comparison of these studies is difficult since the total radiation dose varies per study form 45 Gy to 50.4 Gy and studies differ also in combining bolus or continuous infusion of 5FU with irinotecan or oxaliplatin. The main dose limiting toxicity is diarrhea. Clinical trials are needed to establish a more conclusive role for these drugs.

Intraoperative radiotherapy (IORT)

The rationale for using IORT after the ultimate resection of a previously irresectable rectal tumor is the possibility to irradiate under visual control the persistent tumor in case of a failed resection (R2 resection), eventual microscopically positive resection margins (R1), or the area at risk for tumor relapse. Displacement of the radiosensitive normal structures out of the boost area, makes it possible to administer a higher single dose with greater biological effectiveness. This benefit must be weighed against the increased risk for late complications due to this single dose. For IORT of the rectum the main dose limiting toxicities are ureteral stenosis and peripheral neuropathy (sensory and/or motor).

Determining the benefit of IORT from the literature is difficult due to the heterogeneity of treatment delivery in the various studies. Differences exist mainly in extent of resection (R0-2) and in the administration of neo-adjuvant external beam radiotherapy with or without chemotherapy. There is no agreement on the optimal IORT technique (irradiation with fotons; IORT, with electrons; IOERT or as High Dose Rate Brachytherapy HDR-IORT) or dose (range 7.5-22 Gy). Eligibility to these studies included primary locally advanced but also recurrent rectal cancer. A benefit in local control for IORT was usually found when results were compared to historical data. Elbe described the efficacy of IORT in a group of 63 patients with stage II-III rectal cancer (102). They found a local tumor control of 96.8% compared to historical figures of 66.2%. A retrospective comparison of Mayo clinic patients with primary, locally advanced rectal cancer treated with (n=38) or without IOERT (n=17) showed that the IOERT group had a local failure rate at 3 years of 21% versus 76% in the conventional group, and an improved overall survival (3-year; 51% vs 24%) (103).

IORT does not seem to compensate for R1 or R2 resections: Mannaerts found a significant difference in disease free survival when comparing R0 with R1-2 resections (p=0.0003) in the treatment of patients with locally advanced primary rectal cancer (n=38) (104). Patients with R2 resections still have a local failure rate of 27-62% (2-5 years actuarial) despite IORT (105-107). IORT seems to deserve a chance of demonstrating its activity in a formal randomized study.

Conclusion

The assessment of resectability of a rectal tumor is the first important step for a succesful treatment outcome. For this purpose the value of endorectal procedures is limited due to intraluminal extent of the tumor. MRI with an external coil or spiral CT seem to be the preferred way to diagnose the resectability of a rectal tumor, with a sensitivity of 78 % and a

specificity of 99 %. Neo-adjuvant treatment is needed to enable maximal tumor reduction with a possible resection as a result. Preoperative radiotherapy can lead to an improvement of local recurrence free and overall survival and has become part of the standard treatment. The small bowel is the dose limiting organ and radiation doses to a maximum of 45 Gy and booster dose to 50.4 Gy (fraction size 1.8 Gy) seem to be safe. IORT should be studied further as an adjunct.

Improvement of response to the neo-adjuvant therapy can probably be reached by adding concomitant chemotherapy. The addition of 5FU/LV with radiotherapy has shown to be effective in terms of disease free and local recurrence free survival in some studies. A manageable increase in acute toxicity is also usually found, but late toxicity is not increased.

From these results it may be concluded that prolonged continuous infusion of 5FU is the preferred treatment in the combination with neo-adjuvant radiotherapy for rectal cancer. The investigation of the efficacy of new drugs is currently performed. Results so far indicate that 5FU can probably be substituted with an oral 5FU prodrug. An interesting development is the efficacy shown by beracirumab in metastatic colorectal cancer (108). Its application in the adjuvant setting also of rectal cancer is eagerly awaited.

Combining these oral 5FU prodrugs with oxaliplatin or irinotecan is tempting. In view of the intrinsic diarrhea that can be produced by irinotecan, oxaliplatin seems to be the first choice for further clinical evaluation.

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Chapter 2

A favorable pathological stage after neoadjuvant radiochemotherapy in patients with initially irresectable rectal cancer correlates with favorable prognosis.

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Abstract

Initial treatments of locally advanced rectal cancers focus on local control, as local relapse of a rectal cancer is correlated with a high morbidity and mortality. We studied the effect of neoadjuvant radiochemotherapy on advanced rectal cancer patients in relation to downstaging, local relapse and survival. Post-treatment pathological staging, local relapse and survival were analysed in 66 patients from a single institution. 43 patients had irresectable cancer as determined by laparatomy (*n*=42) or rectal examination (*n*=1). These 43 patients received 45–56 Gy preoperatively with 5-fluorouracil (5-FU) and leucovorin (350/20 mg/m^2×5 day (d)) in weeks 1 and 5 during the radiation therapy. 23 patients had primary resectable tumours with a T1-2 stage. Of the initially irresectable tumours 79% became macroscopically resectable, in 74% a R0 resection was performed. In 6 of 34 (18%) surgical specimens, no tumour was found (pT0), 7 patients had small tumour remnants (pT1-2). In these pT0-2 tumours, no local relapses occurred (observation period of median 4.5 years, range 18–87 months). In the 21 patients with pT3-4 tumours 3 local relapses were seen. In the 23 patients with primary resectable T1-2 tumours the relapse rate was 4%. Downstaging of an initially irresectable rectal tumour to pT2 or less results in a local relapse rate and overall survival that correspond with the rates in primary resectable cancer with the same T classification.

Introduction

Surgery is the mainstay of treatment of patients with rectal cancer and obtaining tumour-free margins is essential for a potentially curative operation (1). If, due to extensive growth in, or fixation to adjacent structures, such margins cannot be obtained, the tumour must be considered to be irresectable. This situation is diagnosed in approximately 10% of cases (2). If this condition is recognised early, before definitive surgery, attempts can be made to reduce the tumour size by using radiotherapy alone or in combination with chemotherapy (neo-adjuvant treatment) (3). If successful, this strategy leads to resectability and a pT and N classification can be determined. We have studied if the prognosis, as far as local recurrence and survival is concerned, of these downstaged tumours is comparable to that of tumours that are resectable without neo-adjuvant treatment. For that purpose, clinical endpoints in 43 patients treated with neoadjuvant radiochemotherapy followed by surgery were compared with 23 contemporary patients who underwent primary resection.

Patients and methods

Over a period of 5 years until 1999, 136 patients were treated in our hospital for rectal cancer. 27 underwent palliative procedures because of metastatic disease. Clinical resectability was evident in 33 patients and they were treated with standard treatment: surgery alone or after 30 Gy irradiation (n=10). If resectability was in doubt, because mobility could not be established, patients were staged bi-manually during surgery with the patient in lithotomy position. In 33 patients, resectable disease was found during this procedure, they received standard treatment as above.

In 42 patients, the bimanual examination revealed invasion of the projected surgical margins hence the descending colon was transected to fashion an end colostomy, a hidden colostomy was made in the proximal colon (4). In one additional patient, clinical examination before surgery established invasion in the perisacral fascia. These 43 patients were treated with combined radiochemotherapy before definitive surgery.

Preoperative treatment in these patients consisted of a combination of chemo- and radiotherapy. Radiotherapy was based on a 3 or 4-field technique. The patients were treated daily with megavoltage radiation (6–15 MV) to a volume encompassing the small pelvis. Anterior/posterior fields were custom-shaped with a 1.5 cm margin lateral to the bony pelvic inlet to cover the iliac lymph nodes. The superior border of the field was at the L5/S1 junction and inferiorly, the field was extended to the anal verge for distal cancer or 3.5-cm inferior to the

distal extent of the lesion for proximal cancer. Lateral fields were shaped to include the external iliac lymph nodes with the border anterior to the symphysis, and a 1.5 cm margin posterior to the sacrum. The whole pelvis received a total of 45 Gy, with the dose prescribed to the 95% isodose line using standard fractions of 1.8 Gy/day (d) 5×/week. This was followed by a reduced field encompassing the tumour for an additional 5–15 Gy.

Radiotherapy was accompanied by 5-fluorourcil (5-FU) and leucovorin (350/20 mg/m^2×5d; in weeks 1 and 5 during radiotherapy). Surgery was attempted after 6–8 weeks. 21 (62%) patients underwent abdomino-perineal resection (APR), 11 patients (32%) underwent a Hartmann procedure and two patients underwent a low anterior resection. During this study period, 11 patients were entered in a prevailing protocol, with intra-operative radiotherapy with a dose of 10 Gy. A weekly dose of 5-FU and leucovorin (450/20 mg/m^2) for a period of 12 weeks was given postoperatively in 17 patients.

Endpoints were postoperative pathological stage, local relapse and survival. Survival of the patients was measured from the start of neo-adjuvant therapy. These results were compared with the same parameters in 23 concomitant patients with primarily resectable clinical and pathological T1 and T2 rectal cancers treated by the same team of surgeons.

7 (30%) of these patients underwent abdomino-perineal resection (APR), 8 patients (35%) underwent a Hartmann procedure and 8 (35%) patients underwent a low anterior resection (LAR). Survival was calculated using the Kaplan–Meier method (5). The survival rates between groups were tested for significance using the log-rank test (6).

Results

From the 43 patients with irresectable tumours, 2 patients did not complete the planned treatment, due to the appearance of metastatic disease and terminal deterioration of the clinical condition. For the other 41 patients, neoadjuvant treatment was uneventful.

Only mild haematological and gastrointestinal toxicity was seen, the maximal score was a grade III anaemia (World Health Organization (WHO) common toxicity score, haemoglobin (Hb)=4.1 mmol/l) in 1 patient. Eleven percent of the patients experienced grade II nausea and 11% grade II diarrhoea. Mucositis was minimal, only 5% of the patients had a grade I mucositis.

After preoperative chemo-radiotherapy, 34 (30 male) irresectable rectal tumours became macroscopically resectable (79%). From the 30 male patients, 8 underwent a total exenteration and 2 a partial bladder resection. In 1 female patient, posterior exenteration (uterus and posterior vagina wall) was undertaken and in 2 patients the posterior vaginal wall was excised.

The mean period between operation to discharge from hospital was 23 days (median 15, range 9–90, *n*=34). However, in 13 of the 34 patients this was more than 20 days, with a mean of 40 days (median 32, range 21–90, *n*=13). The long period of hospitalisation in these patients was due to an abscess (*n*=3), fistula (*n*=6), wound dehiscence (*n*=2), infection (central line: *n*=1; pulmonal: *n*=1), bleeding (*n*=1), cerebro-vascular accident (*n*=1). Long-term complications (>1 year after the resection) were erectal dysfunction in the majority of the patients and 2 patients still had fistula.

Thirty-two (74%) of the resected specimens were microscopically radical (R0). In the group of 34 patients, 6 specimens (18%) showed a pathological complete response (pT0). In 7 cases, only small amounts of vital tumour tissue were found (pT1-2). In 21 patients, limited tumour downstaging (pT3-4) was found, but the tumours became resectable. In this group (pT3-4), 9 patients had positive lymph nodes. There was a statistically significant difference in survival between the node-positive (26%) and node-negative patients (*P*=0.0017). None of the patients in the maximally downstaged group (pT0-2) had positive lymph nodes.

During the median observation period of 4.5 years (range 18–87 months), no local recurrences were found in the pT0-2 group (*n*=13). Three patients in the pT3-4 group developed a local recurrence (14%). Distant metastases where found in one patient in the pT0-2 group (liver) and in ten patients of the pT3-4 group (6 liver, 2 lung, 1 intraperitoneal and 1 brain). There was a significant difference in the development of distant metastases between the two groups (*P*=0.014). The overall median survival in the group of patients receiving neo-adjuvant treatment was 76 months (range 1.5–87 months). In the patient group that showed a downstaging towards a pT0-2 tumour after neo-adjuvant radiochemotherapy, the median survival was not reached during the observation period of 4.5 years. The median survival of the patients who showed minimal downstaging (pT3-4) was 76 months (3.5–79 months) and patients with tumour that were still irresectable had a median survival of 12 months (range 1.5–49 months). The survival difference between the pT0-2 and pT3–4 groups was almost significant (*P*=0.055). A significant difference in survival was found between patients with a R0 and R1 (microscopic irradical) resection (*P*=0.0008).

The 23 concomitant patients with primary resectable T1 and T2 rectal cancers treated with surgery only had a median observation time of 3.8 years (range 20–79 months). Local recurrence was found in 1 patient (4%). No distant metastases occurred. The median overall survival was not reached during this observation period. There was a significant difference

(*P*=0.034) in overall survival between the initially irresectable group (*n*=43) and the primary resectable T1-2 group (*n*=23) (Fig. 1.)

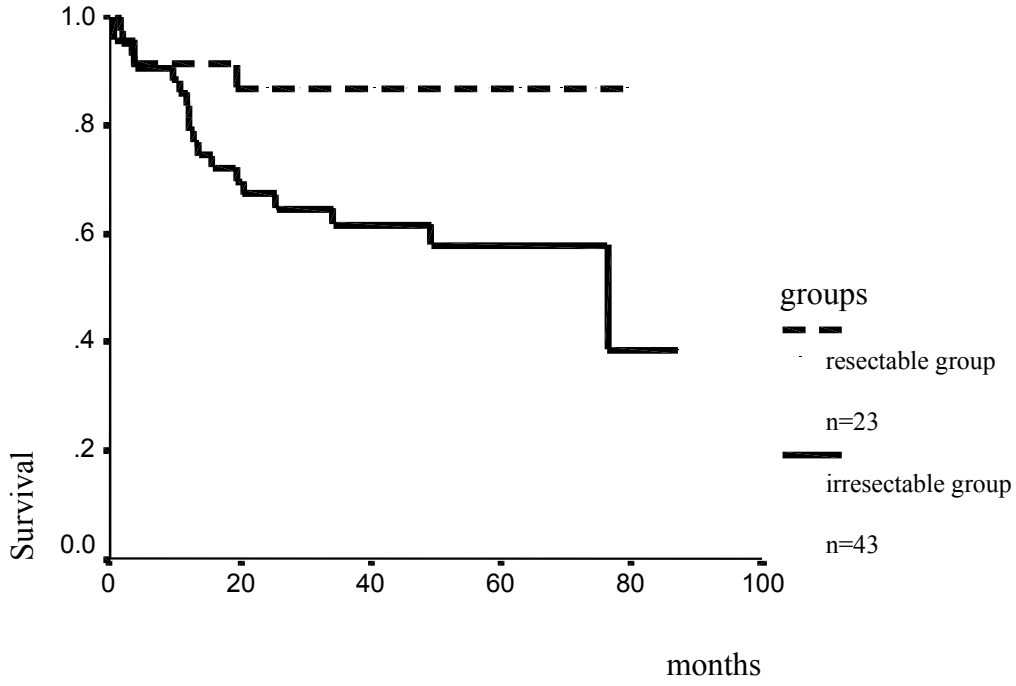

Figure 1. Overall survival of the initially irresectable group and the primary resectable group with T1-2 tumors (p=0.034).

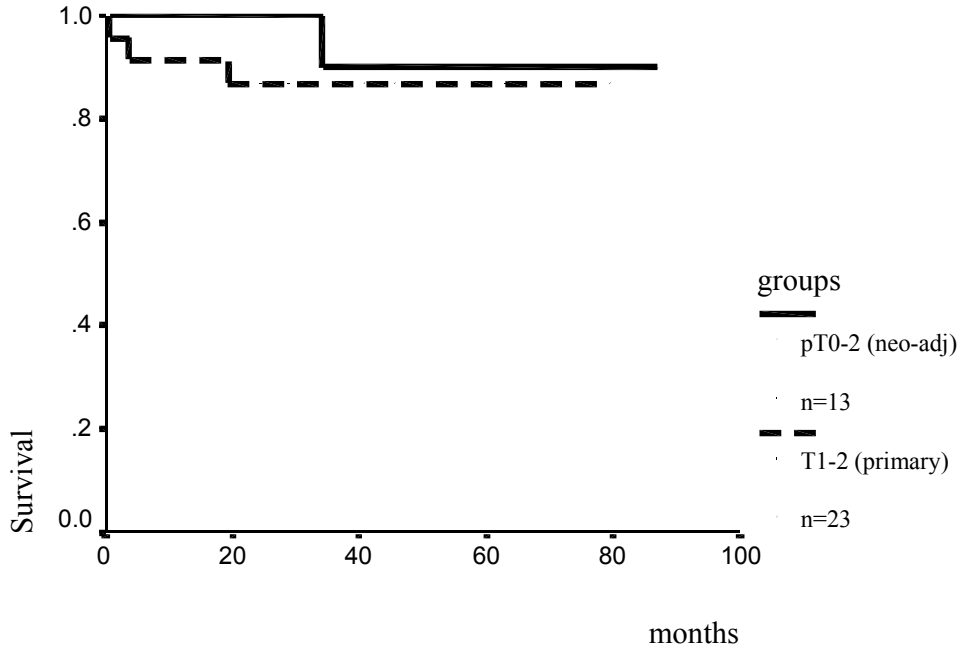

Figure 2. Overall survival of the maximally downstaged group (pT0-2) and the primary resectable group (T1-2).

Discussion

Among the 34 patients undergoing a resection subsequent to neo-adjuvant radiochemotherapy, only 4 were female. This can be explained by the fact that local recurrence often occurs in the anterior plane of the surgical margin. The female patients having three compartments in a relatively large and shallow pelvis, have an advantage when confronted by rectal cancer since fixation to the pelvic sidewalls is less frequent and adequate anterior extension of the resection can be achieved without compromising the bladder or the local radicality while performing a primary resection (7).

In this study, in patients with initially irresectable rectal tumours, neoadjuvant treatment with radio- and chemotherapy leads to a R0 resection in 74% of the patients. Moreover, when there is a downstaging after radiochemotherapy towards a postoperative stage of pT0-2 the survival (5 years overall survival of 90%) is comparable with that in the group of patients with primary resectable (T1-2) rectal tumours as shown in Fig. 2. These results are consistent with the literature (8-12). The incidence of local recurrence in the downstaged group and primary resectable group was not significantly different. So, when an initially irresectable rectal tumour becomes downstaged to a resectable pT0-2 tumour, the final prognosis is the same as in a primary resectable T1-2 tumour. The patients who did not respond to the neo-adjuvant therapy had a significantly worse overall survival compared with responders ($P<0.0001$).

There was a significant difference in the development of distant metastases between the 2 initially irresectable groups (pT0-2 vs. pT3-4, $P=0.014$). Postoperative adjuvant therapy given in 17 patients did not correlate with the occurrence of distant metastases.

In this study, we showed an effect of neoadjuvant radiochemotherapy on initially irresectable rectal tumours. It is not clear to what extent preoperative chemotherapy plays an additional role in the phenomenon of downstaging and improvement in survival. Only a few randomised trials have investigated the role of preoperative radiochemotherapy compared with radiotherapy alone in irresectable rectal cancers (13-15). A slight prolongation of survival was found in one study (13). These studies and several large phase II studies (16), show favourable results following combination treatments. However, until now, evidence for any benefit from neoadjuvant chemotherapy is limited due to the lack of randomised clinical trials investigating the role of neo-adjuvant chemotherapy in combination with radiotherapy for irresectable rectal cancers. Results of the ongoing Nordic trial should help to clarify this.

In our study, in 9 patients (21%) a resection of the tumour was not warranted after neoadjuvant radiochemotherapy because of fixation to the pelvic sidewalls, and these patients had a poorer prognosis.

To predict an insufficient response to neoadjuvant treatment, multiple potential predictive factors have been investigated in several studies, such as p53, BAX, p21, Bcl-2 and Ki-67 (17-20). None of these factors show a sufficient specificity or sensitivity. Identification of a set of genes involved in the sensitivity for radiochemotherapy by the use of DNA microarray techniques might provide an answer in the future (21,22).

The addition of new drugs like oxaliplatin or irinotecan to existing 5-fluorouracil regimens in patients with advanced colorectal tumours improves response rates and the duration of response, and, possibly, overall survival (23-26). These regimens might also increase the level of downstaging in advanced rectal cancers.

In conclusion, downstaging of irresectable rectal cancers results in acceptable local control rates, and a fair prognosis for survival. Optimisation of the neoadjuvant regime, using newly available drugs, might further improve these results.

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Chapter 3

Molecular prognostic factors in locally irresectable rectal cancer treated preoperatively by chemo-radiotherapy.

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Abstract

Background: The aim of this study was to determine the relation between survival and value of molecular markers in the primary tumour in a group of patients with locally irresectable rectal cancer, treated with preoperative chemo- radiotherapy.

Methods and Materials: Immunohistochemistry for p53, p21, bcl-2 and Ki-67 was performed on pre-treatment biopsy specimens of 34 patients with locally irresectable rectal cancer. Preoperative treatment consisted of pelvic irradiation of 45-56 Gy, combined with 5FU and leucovorin (350/20 mg/m² x 5 d; in week 1 and 5 during radiotherapy). The median follow-up was 38 months (range 25-75 months). Endpoints were pathological T-stage and survival after surgery. Eleven patients received intra-operative radiotherapy and 14 patients postoperative adjuvant therapy.

Results: Expression of p21 correlated significantly with survival (p=0.005). Survival and p21 expression also correlated significantly, when adjusted for tumour response (p=0.005, RR=4.8 (1.6-14.7)). No relation was found between p53, bcl-2 or Ki-67 and tumour response or survival. Multi-variate analysis between the different molecular markers showed no significant relation.

Conclusions: Expression of p21 predicts a worse survival in locally irresectable rectal cancer treated with preoperative chemo-radiotherapy. No relationship was found between tumour response in chemo-radiotherapy and p53, bcl-2 or Ki-67.

Introduction

Colorectal cancer is a major public health problem in the Western world and ranks as the third leading cause of death in both males and females. In 1997, 8600 new colorectal cancer patients were registered in the Netherlands, of whom 25% had rectal cancer (1). In early stages a surgical resection is the only curative treatment. Following potentially curative resection however, local recurrence rates vary between 5 and 40% (2-5). Moreover the majority of patients present at an advanced stage. At the time of diagnosis 38% of patients will have regional spread of disease and 25% will already have distant spread (6). (Neo-)adjuvant therapies like pelvic irradiation and chemotherapy, either alone or in combination, have an additional role in these subsets of patients. At present, conventional clinico-pathological parameters cannot entirely identify aggressive tumours that would benefit from (neo-)adjuvant therapy. As for other human malignancies, the development of rectal adenocarcinoma is associated with a series of inherited and/or acquired gene abnormalities that disregulate cell growth and cell death. These genes or their protein products can be measured in tumour tissue.
The aim of this study was to determine the relation between survival after chemo-radiotherapy and the value of molecular markers, in the primary tumour, in a group of patients with locally irresectable rectal cancer treated with preoperative chemo- radiotherapy.

Materials and methods

Patients

Thirty-four patients with locally irresectable rectal cancer treated in the University Hospital Groningen from 1994 to 1998 were studied. Assessment of tumour stage was performed by digital rectal examination, computed tomographic scan and in thirty-three cases by means of a staging laparotomy. All patients received neoadjuvant therapy consisting of preoperative radiotherapy at doses between 45 and 56 Gy administered to the pelvis as described in an earlier paper (7), accompanied by 5-FU and leucovorin (350/20mg/m² x 5d; in week 1 and 5 during radiotherapy). After 4 to 6 weeks patients were subjected to radical surgery with a curative intent. During this study period patients were entered in prevailing protocols, thus intra-operative radiotherapy with a dose of 10 Gy was performed on 11 patients and postoperatively a weekly dose of 5-FU and leucovorin (450/20 mg/m²) for a period of 12 weeks was given at 14 patients. Survival of patients was measured from start of neo-adjuvant therapy.

Immunohistochemical staining

Specimens were fixed in formalin, paraffin embedded and cut into 3 µm thick sections, which were applied to 2-aminopropyltriethoxysilane-coated slides and stretched on a heated plate (30 min at 60°C). Slides were dried overnight in a stove at 37°C. After deparaffinisation of slides, 200 µl blocking solution (2% blocking reagent (Boehringer, Mannheim, Germany) in maleate buffer 0.15 M NaCl, pH 6.0) was added to each slide for antigen retrieval. Slides were heated twice for 10 min at 115°C with 5 min cooling in between and subsequently washed with phosphate buffered saline (8.750 g NaCl, 1.370 g Na_2HPO_4, 0.215 g KH_2PO_4 in 1 L H_2O, pH 7.3 (PBS)). Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in PBS for 30 min.
Different monoclonal antibodies were diluted in 1% bovine serum albumin (BSA) in PBS. For p53-staining, slides were incubated for 1 hour at room temperature with a 1:400 dilution of BP53-12-1 (Biogenex, San Ramon, CA), detecting both wild and mutant type p53. For p21-staining a 1:50 dilution of p21-WAF (Ab-1) (Calbiochem, Oncogene Research Products, Cambridge, UK) and for Ki-67-staining a 1:400 dilution of MIB-1 (Immunotech, Marseille, France) were used with 1 hour incubation at room temperature. For bcl-2 staining, slides were incubated overnight at 4° C with a 1:400 dilution of anti-bcl-2 antibody (Dako, Glostrup, Denmark). After washing with PBS, slides were successively incubated with a 1:50 dilution of peroxidase conjugated rabbit-anti-mouse antibody (RaM^{per}, Dako) and a 1:50 dilution of peroxidase conjugated goat-anti-rabbit antibody (GaR^{per}, Dako) in 1% BSA/PBS and 1% human serum for 30 min each. Peroxidase activity was visualised by incubation with 25 mg diaminobenzidine dissolved in 50 mg imidazol in 50 mL PBS and 50 µL H_2O_2 30%. Counterstaining of the nuclei was performed using Mayer's haematoxylin (Sigma, St. Louis, MO) for 2 min. For p53, a breast carcinoma specimen was taken as a positive control (2+) and for p21, a normal colon specimen was used as positive control (1+). For bcl-2 staining, incubation with an IgG1 antibody (Dako) and subsequently RaM^{per} and GaR^{per} served as negative control (0+) and bcl-2 staining of infiltrative lymphocytes was used as a positive internal control (3+).

Semiquantitative determination of p53, p21 and bcl-2 expression and Ki-67 index

Evaluation of intensity and extension of staining was performed light microscopically by three blinded observers. Staining intensity was graded qualitatively as -, not detectable; +, weak; ++, moderate, +++, strong. The intensity was referred relative to corresponding positive controls.

Ki-67 index was defined as the total number of Ki-67 positive cells per total number of nuclei counted. The results of the immunohistochemistry of p53 and Ki-67 performed on the biopsies were scored positive when a strong staining intensity was found. The overall staining intensity of bcl-2 was less intense then that of other scored markers, for that reason the percentage of stained cells was multiplied times the staining intensity, (weak=1, moderate=2, strong=3). An intensity of 50% or higher was considered positive. The expression of p21 was considered positive when 25% or more nuclear staining was found. The percentage of positive cell staining was categorised as follows in table 1.

Statistical analysis

Associations between p53, p21 bcl-2 and Ki-67 staining and tumour response were determined by the chi-squared test. The logrank test was used for survival analysis regarding staining of p53, p21, bcl-2 and ki-67 respectively,with or without adjustment for other parameters.

Table 1. Distribution of percentages cell staining.

Staining	Non (-)	Weak (+)	Moderate (++)	Strong (+++)
p53	< 5 %	5-25 %	25-50 %	>50 %
p21	< 10 %	10-25 %	25-50 %	50-100 %
Bcl-2	< 50 %	50-100 %	100-200 %	200-300 %
Ki-67	< 25 %	25-50 %	50-75 %	75-100 %

Table 2. Distribution staining intensity of molecular markers.

	Positive	Negative	Total
P53	16	18	34
P21	12	22	34
Bcl-2	14	20	34
Ki-67	29	5	34

Results

The effects of the neo-adjuvant treatment on the downstaging of the rectal tumour in the study have been described before (7). Briefly, 7 of the 34 patients remained irresectable after neo-adjuvant treatment. The median survival of these patients was 12 months (range 1.5 - 49 months). Ten had a postoperative staging of T2 or less in which the overall median survival was not reached in 4.5 years. Seventeen patients had a pT3 or higher with a median survival of 76 months (3.5 - 79 months). Patients receiving IORT (n=11) showed no significant difference in survival compared to the group treated without IORT. No impact was also found from postoperative adjuvant chemotherapy (n=14). As expected an evident relation between lymphnode involvement and survival was found in the resectable group (p=0.009, RR=9.3 (1.7-49.6)).

The distribution of positive or negative scoring of the molecular markers is shown in table 2.

Further analysis of the relationship between the staining intensity and the clinical parameters survival, resectability or downstaging was performed. In this analysis two groups were formed. The first group are the "good" responders, pT0-2. The second group the "bad" responders, pT3-4 and the irresectable.

p53, bcl-2 and Ki-67 expression

Univariate analysis in the expression of p53, bcl-2 and Ki-67 showed no significant difference in survival in the post-treatment irresectable group (n=7) and the resectable group (n=27). There was no relationship between antigen expression and the histological response to preoperative treatment. There were no associations in expression of p53, p21, bcl-2 or Ki-67.

P21 expression

P21 expression correlated with survival in the whole group (p=0.013, RR=4.3 (1.4-13.6)). All irresectable patients died. In the resectable group a positive expression of p21 still correlated significantly with a worse survival (p=0.009, RR=13.3 (1.9-92.2) (Fig. 1). In this group, survival and p21 expression remained correlated, when adjusted for tumour response (p=0.004, RR=15.5 (2.4-99.5)).

Further analysis in the resectable group of p21 and survival adjusted for IORT or adjuvant therapy showed that p21 still was an independent marker (p=0.013, RR=12.2 (1.7-88.4) & p=0.011, RR=12.7 (1.8-89.9) respectively. P21 and lymph node involvement both were

independent predictors for a worse prognosis (p=0.032, RR=9.9 (1.2-79.9) and p=0.025, RR=7.5 (1.3-43.8) respectively).

This significant relation remained when the tumours with less then 10% staining were compared to those with a staining intensity stronger than 25%, p=0.027 RR=3.56 (1.07-11.8).

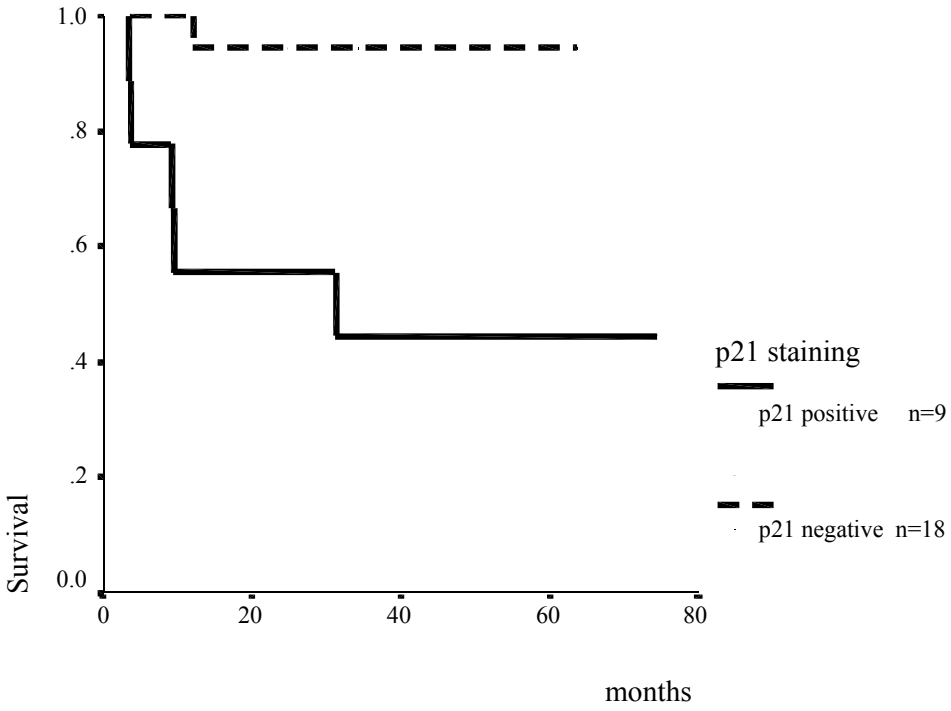

Figure 1. Correlation of staining intensity of p21 and survival in the group which became resectable after neo-adjuvant therapy (p=0.002).

Discussion

In the treatment of locally irresectable rectal cancer some sort of (neo)adjuvant therapy is often applied, usually in the form of a combination of irradiation and chemotherapy, sometimes irradiation alone is used. The results of such schedules can be appreciated at the levels of tumour response, resectability, local relapse and survival. At all levels the number of patients failing treatment is considerable, emphasising the need for treatment alternatives. At present however prediction of treatment outcome is not possible with any standard criterion. The most likely parameters to offer predictive value are cellular proteins that represent pathways engaged in cellular survival or death after anti-tumour therapy and parameters for tumour (re)growth. In this respect the most often studied markers are p53, p21, bcl-2 and Ki-67. Genes in this path have products which play a crucial role in apoptosis, cell proliferation and tumour progression.

The proliferative activity of a given lesion is commonly evaluated by MIB-1, a monoclonal antibody to Ki-67 proliferation antigen, or by counting mitotic figures on histologic sections. In a study of Jansson, Ki-67 expression in 255 human colorectal cancers showed no relation to clinicopathology and prognosis (8). Adell showed in a recent study that Ki-67 stained tumour cells can predict a treatment failure after preoperative radiotherapy of rectal cancer (9).

The tumour suppressor gene p53, localised on chromosome 17, is responsible for the production of a protein that targets among many others the p21 gene. Mutations often lead to excess protein that is unable to function in the appropriate pathway. However mutations not leading to abnormal proteins are not detected by immune histochemistry, as applied in this study. Therefor the incidence of p53 mutation may be underestimated.

Bcl-2 was the first human gene known to encode for an inhibitor of apoptosis. When bcl-2 is expressed at high levels in cells, it forms complexes with bax (a bcl-2 like protein); preventing bax homodimerisation and inhibiting cell death. Schwandner and Leahy, found in two different studies on bcl-2 expression in colorectal cancer respectively no relation to recurrence and better long term prognosis (10,11).

In the literature results of the application of these markers are conflicting. A low level of mutated p53 protein either alone or in combination with other parameters was found to be a favourable factor for tumour response (12-14) as well as for local relapse (15) and more favourable histology (16). In a study also incorporating clinical outcome (relapse) the combination of p53 and bcl-2 markers was of value in patients with colon or rectal cancer (17). However, other studies do not confirm these results, neither at the local response level (18-20) nor at clinical levels of relapse and survival (21).

Therefore these markers are probably insufficient as predictors for prognosis in colo-rectal cancer, in any case they seem of limited value compared with breast cancer (22), lung cancer (23) and ovarian cancer (24). It is conceivable that other intervention factors such as surgical technique have a much more important and variable effect on treatment outcome.

In our study no clinical value in predicting tumour response or survival was found for p53, bcl-2 or Ki-67. We found however a distinct relation between p21 expression and clinical outcome, in patients with p21 positive tumours did worse. This result held true after adjusting for T stage, IORT and adjuvant therapy. Previous experimental and some clinical evidence support these findings. In the context of resistance to irradiation, in a number of cell lines an elevated level of p21 protein is found. Characteristically the level of p21 protein remains high in this situation, and there is no induction of the protein by the irradiation, as is often seen in the p53 induced p21 response to irradiation. Rather than following the cell cycle block to its apoptotic climax, cells survive, giving rise to the phenotype of radiation resistance (25-28). Antisense application in this situation restores sensitivity (28) while p21 mutation also interferes with resistance (27). In these studies the effect of radiotherapy may have influenced survival. However in our patient group we do not find a relation between p21 and the effect of radiotherapy as judged by the T status. Therefore the inverse effect on survival may be caused by mechanisms other than radioresistance. This is supported by observations in patients with colorectal cancer treated surgically (29), patients with prostate cancer treated surgically (30) and patients with breast cancer (31). As in many other respects p21 functions as a negative regulator of growth, progression and metastasis (32,33), further analysis of this intriguing observation seems warranted.

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Chapter 4

The addition of oxaliplatin to (oral) 5FU in concomitant chemo-radio-neo-adjuvant therapy for irresectable rectal cancer, a phase I study.

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Abstract

Purpose: The purpose of this study was to determine the maximum tolerable dose of oxaliplatin in combination with 5FU or capecitabine and radiotherapy in patients with primary irresectable rectal cancer.

Materials and Methods: Eighteen patients with primary irresectable rectal cancer were treated. Fifteen of these patients had primary adenocarcinoma of the rectum, 2 a recurrent adenocarcinoma and 1 a cloacogenic carcinoma. Thirteen patients were treated with intravenous 5FU and five with capecitabine instead of intravenous 5FU. Radiotherapy was given with a 3-4 fields technique, 6-15 MV, 45 Gy in 25 fractions of 1.8 Gy with a boost dose of 5.4 Gy/3 fractions. Chemotherapy consisted of oxaliplatin administered at 2 dose levels: 85 mg/m² and 130 mg/m² synchronously with radiotherapy on day 1 and 29 as a 2h intravenous infusion prior to administration of 5-FU and leucovorin. Two cycles of 5-FU, 350 mg/m² and leucovorin 20 mg/m² (IV bolus injection) were administered on days 1 to 5 and day 29 to 33. Capecitabine was administered orally at a dose of 1000 mg/m^2, 2 x dd., days 1 till 14 and day 25 till 38. The surgical procedure was performed between 4 to 6 weeks after preoperative treatment. The patients were evaluated until one month after operation.

Results: In the first 6 patients treated in the 85 mg/m² dose level one episode of grade 3 diarrhea and one of elevated liver transaminases occurred. Seven patients received 130 mg/m² oxaliplatin, 1 patient went off study because of a DPD deficiency. The last patient treated at this dose level died of neutropenic fever probably due to an urosepsis. In the 5 patients treated with capecitabine 3 patients experienced a grade III diarrhea and 1 a grade III neurotoxicity. Forteen patients underwent resection, in 13 patients surgical margins were free of tumor, 2 patients were found to have progressive disease. A pT0-2 stage was found in 9 of the 14 resected tumors (64 %), two of these were pathological complete responses both treated in the 85 mg/m^2 group.

Conclusion: The addition of oxaliplatin to a neo-adjuvant treatment of primary irresectable rectal cancer in a combination of radiotherapy and 5FU/LV or capecitabine is feasible at a dose level of 85 mg/m² on day 1 and 29 of radiotherapy.

Introduction

The preoperative treatment of irresectable rectal cancer aims for a reduction of the tumor size by using radiotherapy alone or in combination with chemotherapy (neo-adjuvant treatment) (1). If successful, this strategy leads to resectability, resulting in a better local control rate and a better prognosis for survival. However this combination treatment is not yet optimal, because

some patients remain incurable after neo-adjuvant treatment with a median survival of compromised quality, of only 12 months (2) and toxicity of this strategy may be considerable.
Oxaliplatin, a new platinum analog shows promising results in the treatment of cancer. When added to the treatment regime with 5FU and leucovorin in advanced colorectal cancer two phase III studies showed significant differences in progression free survival and response rate with acceptable tolerability (3;4). Other studies showed a synergistic antitumor activity also with 5FU (5;6). Moreover oxaliplatin can act as a radiosensitiser as shown in a study by Cividalli et al. (7). They found an increased antitumor effect in the combination of radiotherapy with oxaliplatin. The addition of oxaliplatin might improve the response rate of preoperative radiochemotherapy treatment for irresectable rectal cancer.
During our study intravenous 5FU was replaced by capecitabine, an oral 5FU. The reason for this change was the convenience of oral administration, combined with an efficacy and tolerability comparable with intravenous 5FU (8-12).
We tested the feasibility of the addition of oxaliplatin to (oral) 5FU and radiotherapy in the neo-adjuvant treatment of irresectable rectal cancer.

Patients and methods

Eligibility Criteria

Patients with histologically proven malignancy of the rectum with a clinical stage T3-4 N0-3 (TNM UICC 1992, staging classification of colorectal cancer), not amenable to primary radical surgery with a tumor free circumferential margin were eligible. The assessment of the tumor stage was performed by means of digital rectal examination, CT scan and by laparotomy.

Patients were included after giving written informed consent. Their performance status of 0-1 (ECOG). Clinical laboratory assessment included the following criteria to be met: White Bloodcell Count (WBC) > 3.0 x 10^9/L, platelets > 100,000 x 10^9/L, bilirubin and creatinine < 1.5 x institutional upper limit of normal (IULN), calculated creatinin clearance > 50 ml/min.
Patients were excluded from the study when they received prior anticancer treatment (radio- and/or chemotherapy) or had another malignancy in the past 10 years, except adequately treated in situ carcinoma of the cervix or non-melanomic skin cancer. Furthermore pregnant or lactating women were excluded and patients with severe cardiac or lung failure, uncontrolled hypertension or angina pectoris. Patients with clinical signs of CNS metastases or a sensory neuropathy of NCI grade 1 also were excluded.

Study design

In this study oxaliplatin was added to an existing treatment strategy given with a curative intent. The additional study medication should not compromise this regimen, therefore, in addition to any grade 4 toxicity we considered toxicity that would lead to adaptation of the dose or timing of the existing regimen, as dose limiting. In our previous experience in 43 patients, dose reduction of 5-FU/LV or radiotherapy was never required (2), neither has surgery been postponed for reasons of toxicity of this neo-adjuvant treatment regimen. After the approval of the use of capecitabine an additional step was added to the study testing the substitution of intravenous 5FU by the oral compound capecitabine in equivalent dose in combination with the advisory dose of oxaliplatin.

When 3 patients had not required dose modification in the first dose step (85 mg/m²), the second dose step (130 mg/m²) was to be initiated. Six patients in the final dose step should be treated without compromising radiotherapy, surgery or 5FU dose. If dose limiting events occurred in the final step, 3 additional patients were to be treated in the first dose level.

Radiotherapy

Preoperative radiotherapy was delivered by an isocentric three or four field technique, using megavoltage radiation produced by a linear accelerator with an energy ≥ 6 MV. Patients were positioned either in supine or in prone position with a full bladder to decrease the irradiated volume of the small bowel. All fields were treated on a daily basis. The radiation field extended superiorly to the L5/S1 junction and covered the obturator foramina inferiorly. The minimal inferior extend was 4-5 cm below the tumor. With carcinoma of the lower one-third of the rectum, the perineum was encompassed in the treatment field. The width of the anterior posterior portal covered the lateral pelvic inlet with a margin of 1.5 cm. The entire sacrum was included with a dorsal margin of 1.5 cm. Anteriorly the lateral fields encompassed the tumor as determined by barium enema and pelvic CT scan and positioned along the posterior border of the pubic bone. If there was clinical evidence of involvement of the bladder, the prostate, the cervix or the uterine body, not only the internal iliac nodes but also the external iliac nodes were included in the radiation field. In that case the anterior border of the lateral field was positioned along the upper border of the pubic bone. Patients received 25 daily fractions of 1.8 Gy up to a total of 45 Gy. After 45 Gy at the locoregional treatment volume, the radiotherapy was continued with a boost dose of 3 fractions of 1.8 Gy each only encompassing the gross tumor volume with a 2-cm margin determined by barium enema and CT scan. The dose

distribution was specified according to the rules of the ICRU 50 report. The dose homogeneity in the target volume was within 5% related to the dose specification point.

Chemotherapy

5-FU and leucovorin were administered during two cycles, on days 1 to 5 and days 29 to 33 as a 350 mg/m^2 bolus (intravenous injection over ≤ 20 min) immediately after the leucovorin bolus of 20 mg/m^2. Oxaliplatin was given at escalating doses (85 mg/m², 130 mg/m²) on day 1 and day 29 as a 2h intravenous infusion prior to administration of 5-FU and leucovorin. Oxaliplatin and 5-FU were not combined in the same infusion bag, and the line was flushed between the administration of oxaliplatin and that of folinic acid.

Capecitabine was administered orally at a dose of 1000 mg/m^2, 2 x dd., day 1 till 14 and 25 till 38.

Surgery

The surgical procedure was performed between 4 to 6 weeks after preoperative treatment. The resection was carried out using sharp dissection to encompass the circumference of the mesorectum. The operation started with the transection of the inferior mesenteric vessels below the left colic artery and continued in the avascular plane between the mesentery and the parietal structures thus preserving the pelvic plexus. In male patients the anterior dissection was carried out in front of “Denonvilliers” fascia. The dissection was carried out in the so-called “holy plane” and both the rectum and the mesentery were transected at least 2 cm below the tumor. When a safe distal margin could be obtained without the need of a perineal phase, and the residual rectal stump was too short to warrant continence with a colorectal anastomosis, the rectal stump was either closed or left open. This in fact is a modified Hartmann procedure. Invaded contiguous structures on the primary assessment were resected en bloc with the rectum. If required, the posterior vaginal wall and/or uterus were excised.

Patient evaluation

We performed a weekly evaluation of toxicity and symptoms during neo-adjuvant treatment, with patient history, clinical examination and a full hematological and blood chemistry assessment. The evaluation of toxicity was performed till one month after the operation. The surgical specimen was examined by the pathologist on resection margin and pathological tumor stage (TNM UICC 1992, staging classification of colorectal cancer).

Results

Between December 2000 and May 2003, 18 patients were treated in this study.

Thirteen patients were treated with intravenous 5FU, eleven of these had a primary irresectable rectal adenocarcinoma and one a recurrence 4 years after a low anterior resection for a pT3N0 tumor. One patient had a cT2N0 cloacal carcinoma. This patient was treated with a higher radiotherapy dose of 61.2 Gy in 29 fractions. No surgery was performed after this treatment schedule, because of results published by Mitchell et al (13) where is shown that radiochemotherapy alone is a sufficient treatment for this type of tumor. One of the patients with rectal adenocarcinoma had 2 liver metastases at the start of the treatment. Resection of the liver metastases took place during the same operation as the abdominoperineal resection. Five patients were treated with capecitabine instead of intravenous 5FU. Four of these patients had a primary rectal adenocarcinoma and one a recurrence 3 years after a low anterior resection for a pT3N1 tumor. Median age of the patients was 60 years (range 30 – 69 yr.). Eleven patients were male and 7 were female.

Toxicities in the first cohort of 3 patients at a dose level of 85 mg/m² oxaliplatin consisted of a diarrhea grade III, elevated liver transaminases gr III, mild leucopenia and anemia (Table 1). The dose was therefore escalated to 130 mg/m². At this dose level 7 patients were treated. One patient discontinued the treatment after 1 chemotherapy cycle and 27 Gy radiotherapy, because of grade 4 mucositis, diarrhea and leucopenia. Subsequently this was found to be due to a dihydropyrimidine dehydrogenase (DPD) deficiency, a germ line mutation known to intensify toxicity to 5FU (14). The DPD activity in this patient was 21 % of the normally expected activity in controls. As this patient was considered not to be evaluable a replacement patient was treated. However the sixth evaluable patient treated in the oxaliplatin dose group of 130 mg/m², experienced a grade IV toxicity with a leukocyte count of 0.10 x 10^9/l and died probably of septicemia related to urosepsis. DNA analysis excluded common DPD deficiencies.

After this treatment related death 8 additional patients were treated in the regime with 85 mg/m² oxaliplatin without any dose limiting toxicity of whom 5 received capecitabine instead of intravenous 5FU. Table 1 lists the toxicities of the patients treated in both oxaliplatin dose schedules. One patient of this group was hospitalized for 11 days because of dehydration due to vomiting and diarrhea grade III.

Table 1. Number of patients with specified toxicity. (NCI CTC Toxicity Criteria)

	oxaliplatin 85 mg/m² & 5FU/LV(iv) (n=6)				***oxaliplatin 130 mg/m² & 5FU/LV(iv) (n=6)***				***oxaliplatin 85 mg/m² and capecitabine (n=5)***			
	grade				grade				grade			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
Hematological												
Leucopenia	1	2			2			1	1	2	1	
Thrombocytopenia					1		1		1			
Anemia	1				1				2	2		
Liver transaminases			1									
Gastrointestinal												
Oral					3				1			
Nausea/vomiting	3	3			1	3			1	3	1	
Diarrhea		5	1			5	1			2	3	
Neurotoxicity												
Peripheral	3	1			3	3			1	3	1	

The median period between the last day of radiotherapy and surgery was 5.5 weeks (range 3.5 – 21.5 weeks). The median duration of admission after surgery was 17 days (range 9 days – 7.5 months). Surgery performed after neo-adjuvant treatment consisted of 6 low anterior resections, 7 abdominoperineal resections and 2 excenterations. Two patients treated with 85 mg/m^2 oxaliplatin were found to have progressive disease at laparotomy. One of these patients underwent a palliative tumor resection and in the other patient no resection was performed.

The pathological tumor stage in the patients treated in the 85 mg/m² group showed 3 patients with stage pT3 tumors, 2 patients with stage pT2 and one patient with no microscopic tumor (pT0) in the resected specimen. In the group treated with 130 mg/m² oxaliplatin 2 patients had a stage pT3 tumor, 1 patient with stage pT2 and 1 patient with a pT1 stage. One patient had a pT0 stage but still showed tumorcells in 2 lymph nodes. In the 4 patients undergoing resection after 85 mg/m^2 oxaliplatin and capecitabine there was 1 patient with a pT3 stage, 2 patients with pT1 and 1 with a pT0 stage. Microscopic radical resection (R0) was performed in twelve of the thirteen operated patients. A summary of these results are listed in table 2.

Table 2. Surgery and pathology results of patients per treatment group.

	Resection after neo-adjuvant treatment	*Microscopic radical resection (R0)*	*pT0-2*
oxaliplatin 85 mg/m² & 5FU/LV(iv) (n=6)	6	5	3
oxaliplatin 130 mg/m² & 5FU/LV(iv) (n=6)	4	4	3
oxaliplatin 85 mg/m² and capecitabine (n=5)	4	4	3
Total (n=17)	14	13	9

Postoperative complications within 1 month consisted of the need for a suprapubic catheter in 5 patients for a maximum period of 4 weeks. Operation techniques in these patients were 3 abdominoperineal resection and 2 low anterior resections. In three patients a second laparotomy was needed to reconstruct a dehiscence of the fascia. One patient developed an ileus, which was treated conservatively. Pelvic exenteration in one patient, treated in the 85 mg/m² dose level was complicated during and after operation by severe bleeding episodes.

Discussion

In our study the addition of oxaliplatin to the radiochemotherapy treatment regime with 5FU and leucovorin in primary irresectable rectal cancer was safe in the 85 mg/m² group. In the next dose step (130 mg/m²) one patient experienced a dose limiting toxicity, a grade 4 leucopenia of 0.1 x 10^9/l and died. Because of this DLT the advisory dose to treat patients in this regime is 85 mg/m² oxaliplatin. The gastrointestinal toxicity in the combination treatment with 5FU and oxaliplatin was comparable to the toxicity in the treatment with 5FU/LV and radiotherapy alone (15). The replacement of 5FU by capecitabine resulted in a more frequent but manageble grade III diarrhea (3/5 patients). The hematological toxicity was higher, mainly leucopenia and thrombocytopenia, this might be attributed to the addition of oxaliplatin. In other studies with a higher cumulative dose of oxaliplatin neurosensory toxicity is the main dose limiting toxicity (16). Neurosensory toxicity grade III was found in 1 patient and was reversible.

Two earlier clinical studies investigated the addition of oxaliplatin to 5FU/LV and radiotherapy. Freyer et al. performed a phase I study treating 17 patients with radiotherapy, 5FU/LV and

oxaliplatin in escalating dose steps (80, 100 to 130 mg/m²) (17). This study used a treatment regimen of oxaliplatin with folinic acid 100 mg/m² intravenous bolus and 350 mg/m² per day 5FU in a continuous infusion on days 1 to 5 in weeks 1 and 5. Radiotherapy consisted of a lower total dose of 45 Gy administered on a smaller volume than we performed in our study (external iliac lymph nodes were not irradiated). In the study by Freyer 1 of the 17 patients experienced grade IV diarrhea and a grade III vomiting in the first dose step of 80 mg/m². No grade III or IV toxicity was found in the higher dosage groups, therefore the 130 mg/m² oxaliplatin dose was recommended. Continuous infusion of 5FU and a smaller irradiated volume might explain the acceptable toxicity in the 130 mg/m² dose step in this study (18).

The second study, performed by Carraro et al. (19) a phase II study, used a different treatment regime. Oxaliplatin dosages of 25 mg/m² given on 4 days during weeks 1, 5 and 10 followed by a bolus of 20 mg/m² LV and a bolus of 375 mg/m² 5FU and on the third week of radiotherapy (50.4 Gy) a single dose oxaliplatin of 50 mg/m². This treatment schedule showed a 27 % (6/22) grade III diarrhea toxicity (RTOG).

The patients in our study were treated for primary irresectable rectal cancer. Of the 18 patients treated 1 went off study, due to a DPD deficiency, 1 was not operated on because of a cloacal carcinoma (and had a clinical complete response) and 1 died during the neo-adjuvant treatment. Two patients had progressive disease at laparotomy 5 weeks after neo-adjuvant treatment. From thirteen patients who underwent a tumor resection, 12 had a microscopic radical resection (R0). Downstaging towards a pT0-2 stage was found in 9/13 patients (70 %), 2 of these patients had a pathologic complete response.

To optimize the neo-adjuvant regimen a protracted infusion of 5FU might improve the effect and lessen the toxicity of our schedule (20). However this schedule is more difficult to administer on an outpatient basis. Therefore a treatment regime with radiotherapy, oxaliplatin and an oral 5FU (e.g. capecitabine), which mimics a protracted infusion of 5FU (21) might be the optimal regimen. After conclusion of the ongoing phase II trial a multicentered randomised clinical trial is planned comparing neo-adjuvant radiochemotherapy with radiotherapy alone.

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Chapter 5

5FU and oxaliplatin containing chemotherapy in two dihydropyrimidine dehydrogenase deficient patients

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Abstract

Patients with a germline mutation leading to a deficiency for the dihydropyrimidine dehydrogenase (DPD) enzyme are at risk from developing severe toxicity due to the administration of 5FU containing chemotherapy. We report on the implications of this inborn genetic error in 2 patients who received 5FU and oxaliplatin. A possible co-medication effect of oxaliplatin is discussed and the consequences of screening for DPD deficiency.

Introduction

Mutations in the gene that encodes for the 5FU metabolizing enzyme DPD lead to severe toxicity in individuals exposed to 5FU or its analogs. From all patients with severe 5FU induced toxicity 30-57 % was found to be due to this deficiency (1;2).

The genetic basis of DPD deficiency has been analyzed intensively in recent years resulting in a greatly increased understanding of the pathology and epidemiology of the syndrome.

Basically these mutations lead to a decreased metabolism of 5FU, resulting in accumulation of toxic compounds. Accordingly the end results in an afflicted patient exposed to 5FU or analogs are the symptoms of an overdose of FU both in normal tissues and in the tumor.

In two patients who developed severe toxicity after 5FU and oxaliplatin containing chemotherapy, we found DPD deficiency. The short and long term sequelae of this exposition are described. We further discuss a possible relation with the oxaliplatin medication given in both of these patients.

Case histories

The first patient, a 68 year old woman was diagnosed with an irresectable obstructing rectal carcinoma. Treatment was started with the construction of a deviating colostoma. One month later chemo-radiation was started, radiotherapy was given in a total dose of 50.4 Gy in 28 fractions of 1.8 Gy, chemotherapy consisted of 5FU 350 mg/m^2, leucovorin 20 mg/m^2 and oxaliplatin 130 mg/m^2. On day 21 of this treatment (after 1 cycle of chemotherapy and 15 fractions of 1.8 Gy) she was hospitalized due to diarrhea, mucositis, leucopenic fever and dehydration. She was hospitalized for 142 days of which 10 on the intensive care unit (ICU). Leucopenia resolved rapidly, within one week, however the diarrhea took a prolonged course over four months during this period biopsies were taken during colono- and gastroduodenoscopy. Biopsies showed non specific inflammation and villous atrophy. Clinically there were no signs of toxicity due to oxaliplatin (e.g. sensory neuropathy). One month after discharge patient underwent an abdomino-perineal resection en block with the

posterior vaginal wall, without further preoperative chemo-or radiotherapy. Pathological TN-stage of the specimen was pT3N0 with a microscopic tumor free radical resection margin. One year after surgery patient was doing well, she had normal blood chemistry and adequate bowel function with a colostomy.

The second patient, a woman of 58 years of age had a curative resection of a Dukes B sigmoid colon carcinoma at the age of 52. Four years later, she presented with liver metastases for which she underwent a right hemihepatectomy. After a period of 18 months lung metastases developed and palliative chemotherapy was started with biweekly 5FU (2600 mg/m2, 24h infusion), leucovorin (200 mg/m2 1h bolus) and oxaliplatin (85 mg/m2, 2h bolus). Fifteen days after the first treatment patient developed oral mucositis, diarrhea grade II and leucopenia grade III (WBC 1.0 x 10^9/l). After recovery the second course was given after a 10 day delay. Sixteen days later she was hospitalized for 5 days because of the development of fever, leucopenia grade III (WBC 1.1 x 10^9/l), anemia grade II (Hb 5.6 mmol/l) and mild cerebellar ataxia. After a period of 2 weeks blood counts normalized and cerebellar ataxia improved gradually until full recovery after 5 months. Assessment of tumor response after the first chemotherapy cycle showed stable disease and 2 weeks after the second cycle there was progression of disease. After DPD deficiency was established, no 5FU based treatment was given, she received oxaliplatin monotherapy with minor toxicity, without response. Finally irinotecan was given, but also without response. Seventeen months after the first treatment with 5FU, the patient was alive with slowly progressive disease without signs of bowel or cerebellar dysfunction. Determination of the DPD activity in peripheral blood mononuclear (PBM) cells was performed according to methods previously described (2). The DPD activity in the patients was obtained during the toxicity crisis and repeated after full recovery. A DPD activity of 2.1 nmol/mg/h was detected in the PBM cells of the first patient, 21% when compared with that observed in controls (10.0 ± 3.4 nmol/mg/h; n = 22) and 0.5 nmol/mg/h (5 %) after recovery. DPD activity in the second patient was 3.7 nmol/mg/h (37 %) during cytopenia and 4.2 nmol/mg/h (42 %) after 5 months.

Discussion

The toxicity most often encountered in patients with a low DPD activity receiving 5FU is a grade III-IV neutropenia (2), Milano described in addition especially an increased incidence of severe neurotoxicity in 7 of the 19 DPD deficient patients (confusion, cerebellar syndrome or coma) (1). Other toxicities such as mucositis, gastro-intestinal toxicity, especially diarrhea and

cardiotoxicity are also in line with the spectrum of 5FU side effects in patients with a normal DPD activity. Consistent with the findings in our patients the intensity of toxicity is excessive, but the spectrum of symptoms is recognizable. In consequence a fraction of the severe toxicities occurring during regular treatment with 5FU can be ascribed to the prevalence of DPD deficiency in the population.

The incidence of grade IV hematological toxicity, mucositis and diarrhea was 2.5 % (3) in a meta-analysis of patients treated with 5FU for colorectal cancer. This is close to the estimated 3 % incidence of relevant DPD deficiency with activity below 70 % (1), suggesting that the majority of toxic events on 5FU could be caused by this genetic defect.

Also the overall mortality after 5FU, estimated to be 0.5 % (3), could be explained with the approximate mortality of 10 % among patients with DPD deficiency related 5FU toxicity (1)

Some observations suggest that the risk of 5FU induced toxicity might be somewhat higher than expected in women (4). This could be due to a gender effect, as suggested by Milano (1), but this could not be confirmed by Kuilenburg (2). Alternatively co-medication might play a role, as breast cancer patients often receive agents in addition to 5FU. In this respect the co-medication in our patients might be of interest, in both this consisted of the new platinum analogue oxaliplatin. Kim investigated the mechanism of antitumor activity in combination treatment of 5FU and cisplatinum in human gastric cell lines (5). They found that the DPD activity and 5FU concentration were not changed by treatment with cisplatinum.

Other data however suggest that metabolism of 5FU may be altered by platinum analogs. Fischel analyzed the intracellular determinants of the combination of 5FU and oxaliplatin in a human colon cancer cell line (6). They found a reduction of the 5FU catabolism due to the addition of oxaliplatin. A pharmacokinetic study by Boisdron-Celle showed a decreased plasma clearance of 5FU after the addition of oxaliplatin in a group of 29 patients with colorectal cancer (7). These findings were not linked to a DPD inhibition.

The high financial costs of treatment for the complications encountered in our patients underscores the potential importance of screening for DPD deficiency. A requirement for a screening test would in addition to specificity and sensitivity be its rapid availability preferably without exposition of the patient to 5FU.

Determination of the DPD activity in PBM cells is possible by an analysis using reverse phase high performance liquid chromatography as used in our patients (8). Alternatives are mutation analysis in the DPD gene after PCR amplification of the coding exons (9).

Maring et al. described measurement of 5FU clearance after an initial supposedly non-toxic chemotherapy dose to identify patients with a low DPD activity (10).

Some estimates concerning cost benefit relation of screening for DPD deficiency can be made; assuming that for some time to come the most common indication for 5FU or analog will be Dukes C colon cancer.

In 1997, 8600 new colorectal cancer patients were registered in the Netherlands (11), of whom 30 % with a Dukes C stage (n=2580) (12). Probably at least half of them will receive chemotherapy. With the given incidence of DPD deficiency approximately 30 hospitalizations and 3 deaths might be prevented, the cost benefit ratio could be improved if fewer controls for the non-risk patients could be scheduled as result of screening.

Furthermore there are a growing number of studies being performed with oral 5FU prodrugs (e.g. capecitabine, doxifluridine and tegafur) also for other indications than colon cancer. With an increase of the use of these drugs the incidence of severe 5FU related toxicity will also increase. DPD deficient patients might be selected for alternative treatment modalities containing novel non-fluoropyrimidine compounds or raltitrexed. Irinotecan and oxaliplatin have been shown to possess anti-neoplastic activity in colorectal cancer and these agents have been safely applied in the treatment of a patient suffering from a partial DPD deficiency (13).

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Chapter 6

Treatment of locally recurrent rectal cancer, results and prognostic factors

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Abstract

Purpose: Assessment of the results and prognostic factors in patients with locally recurrent rectal cancer treated with curative intent.

Patients and methods: Forty patients with an isolated pelvic recurrence of rectal cancer were studied. The treatment consisted of radiotherapy alone or combined with surgery. Radiotherapy was given with a 3-4 fields technique (6-15 MV), 5 times a week. The median radiation dose was 50 Gy (range 25-66.6 Gy). Twenty-five patients underwent salvage surgery. Five patients were treated with concomitant chemotherapy (5FU/LV) during the 1st and 5th week of radiotherapy.

Results: The local recurrence free survival after 3 and 5 years respectively was 50 % and 40 %. Factors that significantly correlated with a failure of local control were male gender, APR as primary surgery technique, distant metastases, R2 resection and a Tr 4-5 tumor stage. The 3 and 5 year overall survival of the total group was 36 % and 18 % respectively, with a median survival of 26 months.

Conclusion: In a careful selection of patients in the treatment of locally recurrent rectal cancer valuable local palliation if not cure, can be reached. A multi modality approach seems to offer the best chances in this threatening situation.

Introduction

Local relapse in the absence of metastatic disease after previous surgery for rectal cancer is a challenge in the treatment of digestive tract cancer. Although prognosis is usually considered to be grim, strong incentives argue for active intervention in this situation. Uncontrolled local progression is disastrous for the quality of the remaining life and in the absence of life threatening metastases this can mean prolonged suffering. Any treatment that could lead to a remission or stabilization of the relapse might well be worthwhile. On the other hand although somewhat limited by the mode of treatment of the primary tumor, the treatment options that are potentially curative in rectal cancer: surgery, radiotherapy and chemotherapy still are applicable after relapse. This could mean that local cure and even prolonged survival might be within reach for some patients.

In the literature considerable variation in the effect of treatment for local relapse is reported. Long term survival varies between nil and 60 % (1;2). This variety is explained by patient selection and possibly by treatment related factors. Further data on prognosis in defined groups of treated patients might be helpful in determining realistic perspectives in this condition.

We report on the fate of patients with locally relapsed rectal cancer, who were referred by their surgeons for high dose radiotherapy, and or in whom surgery was an option, all were considered to be possible candidates for curative intervention.

Patients and methods

Forty patients with an isolated pelvic recurrence of rectal cancer were studied. The treatment for the primary tumor had been surgery alone. Patients were selected for this analysis when the treatment for the local recurrence was with an intention to cure. Patient characteristics are given in table 1.

Table 1. Patient Characteristics

Number of patients	40
Median age	66 yr. (31-83)
Gender (male : female)	22:18
Time to local recurrence	17 months (5-74)
Type of primary surgery	
APR	8
LAR	24
transanal excision	8
Primary T and N stage	
T1-2 N0	16
T1-2 N1, T3 N0	16
T1-2 N2, T3 N1, T4 N0	4
T3 N2, T4 N1-2	4

APR: abdomino-perineal resection; LAR: low anterior resection. Primary T and N stage categorized according to Gunderson (3).

Radiotherapy

Radiotherapy was given with a 3-4 fields technique (6-15 MV), 5 times a week. The median radiation dose was 50 Gy (range 25-66.6 Gy). Radiotherapy was given preoperatively in 13 and postoperatively in 12 patients. In 5 of the patients receiving preoperative radiotherapy a shorter treatment schedule was given with higher daily fraction doses (e.g. 3 Gy daily to 30 Gy or 5 Gy daily to 25 Gy). Other radiation schedules used a fraction size of 1.8-2 Gy with a minimum dose of 45 Gy.

Salvage surgery

Twenty-five patients underwent salvage surgery. The type of resection performed was an abdomino-perineal resection in 12 patients, a low anterior resection in 7 patients, 1 transcoccygeal resection and 2 tumor resections en bloc with hysterectomy and posterior

vaginal wall excision. Local tumor resections were performed in 2 patients, 1 from the perineum and 1 from the posterior vaginal wall. One debulking operation was performed leaving a tumor infiltrated prostate in situ. The stages of the resected tumors were classified according to the Wanebo system (4) (table 2).

Chemotherapy

Five patients were treated with concomitant chemotherapy (5FU/LV) during the 1st and 5th week of radiotherapy. This radiochemotherapy was followed by resection in 3 patients.

Statistical methods

For the calculation of overall and local recurrence free survival the interval between the start of treatment and death or incidence of recurrence was used. The assessment of the second local recurrence or local progression in case of a R2 resection was performed by CT scan, biopsy and or digital rectal/vaginal examination. Survival and local recurrence free survival of the whole group was calculated with the Kaplan Meier method. For the uni- and multivariate analysis of prognostic factors the Cox proportional hazards model was used.

Results

In this group of 40 patients, no attempt at surgery was finally made in 12 patients: co-morbidity was the non tumor related cause in one patient, definite signs of irresectability, clinically or on CT scanning, was the cause in 8 patients. In 3 patients significant metastatic disease became obvious before surgery. In 28 patients surgery was attempted, however three patients undergoing laparotomy after radiotherapy still showed irresectable tumors. Characteristics of the resected tumors are given in table 2.

Table 2. Characteristics of resected tumors

Radical resection (R0)		17
Non-radical resection (R1-2)		8
T-stage (Wanebo classification (4))		
Tr1-2	Invasion in bowel wall (subserosal)	12
Tr3	Invasion in perirectal fat	7
Tr4	Invasion in anterior urogenital organs	4
Tr5	Invasion in sacrum or pelvic side walls	2
N-status (N0:N+:unknown)		14:5:6

The median follow up was 81 months (range 20-134 months). The local recurrence free survival after 3 and 5 years respectively was 49 % and 39 %. The diagnosis of recurrence was made in 8 patients by digital rectal and or vaginal examination, in 8 patients by CT scan and one patient by coloscopy. Biopsies were taken in combination with coloscopy, during CT in one patient and at clinical examination in 2 patients. Factors that significantly correlated with the development of a second local recurrence were male gender (p=0.032; hazard ratio (HR)=3.3) APR as primary surgery technique (p=0.006; HR=4.4), distant metastases (p=0.006; HR=4.0), R1-2 resection (p=0.014; HR=2.0) and a Tr 4-5 tumor stage (p=0.009; HR=1.9). In a multivariate analyses for the development of a second local recurrence 1 factor remained independent. This was male gender (p=0.028; (HR)=3.5).

The 3 and 5 year overall survival of the total group was 36 % and 19 % respectively, with a median survival of 26 months. Factors associated with a worse survival where high primary tumor stage (p=0.025;) HR=1.6), when categorized as proposed by Gunderson (3) (table 1.) and the presence of distant metastases (p=0.042; HR=2.2). Distant metastases free survival after 3 and 5 years was 65 % and 56 % respectively. Distant metastases occurred in one or more sites after the treatment of the local recurrence (liver n=8, lung n=5, bone n=3, subcutaneous n=1, peritoneal n=2 and brain n=4). In the group of patients treated with radiotherapy and surgery a Tr 4-5 stage and an R1-2 resection also correlated with a worse survival (p=0.018; HR=1.5, p=0.005; HR=1.8 respectively). In a multivariate analysis no independent predictive factors for survival remained.

There was no difference in overall, disease free or local recurrence free survival when a specimen contained positive (n=5) or negative (n=14) lymph nodes.

Discussion

The goal for the treatment of patients with an isolated local recurrence should be local tumor control as this will determine the quality of the remaining life (5). Local recurrence free survival in this study after resp. 1, 3 and 5 years was 76 %, 50 % and 40 %.

The fate of patients with local recurrent rectal cancer can to some extent be predicted by circumstances evident at the time of the operation for the primary tumor. Among these are APR as primary surgery and male gender. After abdomino-perineal resection for primary rectal cancer, the relapse tends to occur in a pattern of diffuse pelvic cancer or laterally situated masses invading the pelvic sidewall. Therefore due to irresectability, recurrence after APR has in general a poorer prognosis (4;6-8). Irresectability is also diagnosed or suspected earlier in males than females (9). The smaller anatomical margins diminish the chance of a curative resection (10).

Other factors can be identified in the work up prior to the salvage treatment. The presence of a preoperatively elevated CEA level has been reported as a prognostic factor leading to a decreased overall and disease free survival (4;6). The fact that a local recurrence can present itself without an elevated CEA indicates the limitations of this parameter, and this is supported by a study by Moertel investigating the utility of CEA monitoring for detection of surgically treatable recurrences of colon cancer (11).

Prognosis is best determined by preoperative evaluation by CT or MRI of the involved region.

Valentini et al. found that the extent of the fixation in a pelvic recurrence along the pelvic sidewall, classified according to a modified Suzuki system, predicted a worse local control and overall survival rate (7). They studied 47 patients with locally recurrent rectal cancer receiving radiotherapy with or without surgery and IORT.

In analogy with treatment results of primary rectal cancer (12) the radicality of resection is also an important prognostic factor for local control after relapse. Wiig studied 107 patients with isolated pelvic recurrences receiving preoperative radiotherapy (46-50 Gy) with or without IORT (2). The five year actuarial survival was better in patients who had an R0-1 resection compared to a R2 resection ($p<0.001$). No significant difference was found between R0 or R1 resection in overall and local recurrence free survival. There was no benefit of the addition of IORT. Mannaerts described in a retrospective analysis a local recurrence free (and overall) survival benefit in patients with microscopically radical resected recurrences compared to

positive resection margins (R1-2) (13). Patients undergoing a R0 resection survived significantly longer and developed less second local recurrences.

The results in our patients suggest that these doses of radiotherapy can be applied in recurrent rectal cancer that have a curative intent. This notion is supported in the literature. Wong and Cummings reported on their results of 348 patients with a local recurrence without extrapelvic metastases treated with radiotherapy with or without surgery (8). The local recurrence free survival after 1 and 5 years was 22 % and 8 %. The total radiation dose (ranging from 4.4 Gy to 65 Gy) correlated positively with the local progression free survival. Total radiation dose above 40 Gy corresponded with the lowest chance in developing a second local recurrence. One should cautiously interpret these results because there was also a correlation between patient performance status and total radiation dose applied. Wong and Thomas reviewed the literature to find an optimal radiation dose in the management of local recurrent rectal cancer. Analysis of the retrospective data suggested a more favorable response with higher doses (> 45 Gy) (14).

The applicability of salvage radiotherapy can be influenced by the primary treatment. The addition of (neo-)adjuvant radiotherapy to the primary treatment of rectal cancer has led to a decrease of the incidence of local recurrences in several randomized studies (15). An increasing number of patients will be treated this way. Salvage therapy of a local recurrence in these patients with radiochemotherapy alone or combined with surgery has been studied by Mohiuddin (16). Hundred and three patients underwent reirradiation (median dose 34.8 Gy) and 34 of these also underwent resection. After a median follow up of 2 years they found that radical surgical resection of recurrent carcinoma can be performed after cumulative doses of primary and secondary irradiation (median 85.8 Gy) with reasonable healing. Late toxicity was however seen in 21 % of the patients, including persistent diarrhea (18%), small bowel obstruction (15%) and fistula formation in 4 % of the patients. A median survival of respectively 44 and 14 months was found in the patients undergoing reirradiation before surgical resection or reirradiation alone.

In contrast to the emphasis on reirradiation, others have advocated a surgical approach.

The treatment of second recurrences without unresectable distant metastases has been described by Verrees et al. (17). They underscore the importance of a re-operative approach because they found a reduction of adverse symptoms caused by the recurrences with a median survival of 24 months in 20 patients, without mortality.

Conclusion

Locally recurrent rectal cancer will remain a therapeutical challenge for valuable local palliation if not cure. This study and others in the literature have shown this can be achieved with a careful selection of patients. A multi modality approach seems to offer the best chances in this threatening situation.

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Summary and future perspectives

This thesis gives an overview of the treatment of locally advanced rectal cancer. The main focus is on neo-adjuvant treatment which aims for a decrease of tumor burden resulting in resectability. This should lead to a decreased incidence of local relapse and a more favorable prognosis.

The **first chapter** reports on a review of the literature. Three aspects are discussed: assessment of resectability of advanced rectal cancer, neo-adjuvant radiotherapy alone and combination radiochemotherapy. The most attractive way to assess the resectability of a rectal tumor seems to be MRI with an external body coil or spiral CT. These techniques are able to visualize the endopelvic fascia and its relation to the primary tumor predicting the circumferential resection margin.

To achieve downsizing and downstaging of a primary irresectable rectal tumor a dose ≥ 45 Gy is required with an interval between the end of radiotherapy and surgery of 4-8 weeks. The main limiting factor to higher doses is the normal tissue tolerance. With a 3-4 beam technique a more homogeneous dose distribution can be reached with a better sparing of the organs at risk. The addition of intra-operative radiotherapy after resection of a rectal tumor is an interesting approach but data from randomized clinical trials are lacking.

The expectation of combining radio- and chemotherapy is an additive or even synergistic cell kill. Few randomized clinical trials have investigated and shown a benefit of the addition of chemotherapy (5FU and leucovorin) in the neo-adjuvant treatment of locally advanced rectal cancer. Only one study reported a benefit in local recurrence free survival. New drugs are being investigated in the adjuvant and neo-adjuvant setting in rectal cancer. Among these are oral 5FU prodrugs (e.g. capecitabine, doxifluridine and tegafur/uracil (UFT)), oxaliplatin and irinotecan. (Pre)clinical studies provided interesting results for the use of these new drugs. There is a need for clinical trials to establish more conclusively if there is a role for these drugs.

Chapter 2 describes the results of our study of the effect of neo-adjuvant radiochemotherapy on primary irresectable rectal cancer in relation to downstaging, local relapse and survival. These results were compared to the prognosis of tumors that were resectable without neo-adjuvant treatment. For that purpose clinical endpoints in 43 patients treated with neo-adjuvant radiochemotherapy followed by surgery were compared to 23 contemporary patients who underwent primary resection. We found that 79 % of the initially irresectable tumors became macroscopically resectable, in 74 % a microscopic radical resection (R0) was performed. In 6 of 34 (18 %) surgical specimens no remaining tumor cells were found (pT0), 7 patients had small tumor remnants (pT1-2). After an observation period of 4.5 years there were no local

recurrences in the patients with pT0-2 tumors. In the 21 patients with a pT3-4 tumor 3 local relapses were seen (14 %). In the 23 patients with primary resectable T1-2 tumors the relapse rate was 4 %. No difference in overall survival was found between the downstaged group (pT0-2) and primary resectable group (T1-2). From these data we conclude that downstaging of an initially irresectable rectal tumor to pT2 or less results in a local relapse rate and overall survival that corresponds with the rates in primary resectable cancer with the same T classification.

Chapter 3. Parameters which might identify aggressive tumors that would benefit from neo-adjuvant therapy could be helpful in clinical managment. We investigated the relation between survival and the value of molecular markers, in biopsy material of the primary tumor, in a group of 34 patients with irresectable rectal cancer treated with neo-adjuvant radiochemotherapy. Neo-adjuvant treatment consisted of pelvic irradiation of 45-56 Gy, combined with 5FU and leucovorin (350/20 mg/m² x 5 d; in week 1 and 5 during radiotherapy). The most likely parameters to offer predictive value are cellular proteins that represent pathways engaged in cellular survival or death after anti-tumor therapy and parameters for tumor (re)growth. In this respect the most often studied markers are p53, p21, bcl-2 and Ki-67. Genes in this path have products which play a crucial role in apoptosis, cell proliferation and tumor progression. The intensity of marker staining was compared to the survival after therapy. After a median follow up of 38 months, p21 proved to be an independent marker predicting a worse survival when overexpression was found. The other markers showed no significant correlation with overal survival.

A relation between p21 and radiotherapy effects has been described in the literature. In a number of cell lines which are resistance to irradiation an elevated level of p21 protein is found. Characteristically the level of p21 protein remains high in this situation, and there is no induction of the protein by the irradiation, as is often seen in the p53 induced p21 response to irradiation. Rather than following the cell cycle block to apoptotosis, these cells survive. However in our patient group we do not find a relation between p21 and the effect of radiotherapy as judged by the T status. Therefore the inverse effect on survival may be caused by mechanisms other than radioresistance. Further analysis of this intriguing observation seems warranted.

In **Chapter 4** we report the results of a phase I study determining the maximum tolerable dose of oxaliplatin in combination with (oral) 5FU and radiotherapy in patients with primary irresectable rectal cancer. The addition of oxaliplatin to a standard chemotherapy regime has lead to an improved clinical outcome in metastatic disease. As reported in chapter 2 not all rectal tumors respond to neo-adjuvant radiochemotherapy. To optimize the chemotherapy in this regime oxaliplatin was added. In combination with radiation oxaliplatin can also act as radiosensitizer as described in the literature.

In our study eighteen patients with primary irresectable rectal cancer were treated with oxaliplatin added to the standard neo-adjuvant radiochemotherapy regime. Thirteen patients received intravenous 5FU and five capecitabine (an oral prodrug of 5 FU). Radiotherapy was given with a 3-4 fields technique, 6-15 MV, 45 Gy in 25 fractions of 1.8 Gy with a boost dose of 5.4 Gy in 3 fractions. Chemotherapy consisted of two cycles of 5-FU, 350 mg/m² and leucovorin 20 mg/m² (IV bolus injection) administered on days 1 to 5 and 29 to 33 of radiotherapy. Capecitabine was administered orally at a dose of 1000 mg/m^2, 2 x dd, day 1 till 14 and 25 till 38. Oxaliplatin was administered at 2 dose levels: 85 mg/m² and 130 mg/m² synchronously with radiotherapy days 1 and 29 as a 2h intravenous infusion prior to administration of 5-FU and leucovorin. The surgical procedure was performed between 4 to 6 weeks after preoperative treatment.

The first 3 patients received an oxaliplatin dose of 85 mg/m^2. At this dose level a grade 3 diarrhea, elevated liver transaminases and mild leucopenia and anemia were found. The dose was therefore escalated to 130 mg/m². At this dose level 7 patients were treated. One patient discontinued the treatment because of grade 4 mucositis, diarrhea and leucopenia. Subsequently this was found to be due to a dihydropyrimidine dehydrogenase (DPD) deficiency, a germ line mutation known to intensify toxicity to 5FU (chapter 5). As this patient was considered not to be evaluable a replacement patient was treated. However the sixth evaluable patient treated in the oxaliplatin dose group of 130 mg/m², experienced a grade IV toxicity with a leukocyte count of 0.10 x 10^9/l and died probably of septicemia related to urosepsis. DNA analysis excluded common DPD deficiencies.

After this treatment related death 8 additional patients were treated in the regime with 85 mg/m² oxaliplatin without any dose limiting toxicity (DLT) of whom 5 received capecitabine instead of intravenous 5FU. Because of the DLT in the 130 mg/m2 oxaliplatin dose level the advisory dose to treat patients in this regime is 85 mg/m² oxaliplatin.

Chapter 5 discusses the impact of the administration of 5FU or its analogs in patients with a DPD deficiency. Mutations in the DPD gene leads to a decreased metabolism of 5FU, resulting in accumulation of toxic compounds. The incidence of a DPD activity below the 70 % is estimated at 3 % with a mortality of 10 % among patients with DPD deficiency related 5FU toxicity.

We describe two patients who developed severe toxicity after 5FU and oxaliplatin containing chemotherapy, with DPD deficiency. The first patient was treated for a primary irresectable rectal tumor. After the administration of 5FU she developed severe mucositis, diarrhea and hematological toxicity. She was hospitalized for 142 days of which 10 on the intensive care unit. The second patient received 5FU and oxaliplatin because of disseminated colon carcinoma. She developed oral mucositis, diarrhea grade II, leucopenia grade III, a mild cerebellair ataxia and was hospitalized for 5 days. Both patients recovered fully from their toxicity episode without signs of late toxicity. Some observations suggest that the risk of 5FU induced toxicity might be somewhat higher due to a co-medication effect. The combination of 5FU and oxaliplatin might put patients with lowered DPD activity at a higher risk of developing severe toxicity. It was shown in a pharmacokinetic study that the addition of oxaliplatin to 5FU resulted in a reduction of the 5FU catabolism.

A growing number of studies are being performed with oral 5FU prodrugs, this results in an increased use of these drugs and therefore in an increase of the risk of developing 5FU related toxicity. High risk patients might be selected by screening for DPD deficiency. These patients could then receive alternative treatment modalities containing novel non-fluoropyrimidine compounds.

Chapter 6 reports the results of salvage treatment in care of local recurrence of rectal cancer. A group of 40 patients who received radiotherapy alone or combined with surgery were studied. Radiotherapy was given with a 3-4 fields technique (6-15 MV), 5 times a week. The median radiation dose was 50 Gy (range 25-66.6 Gy). Twenty-five patients underwent salvage surgery. In 5 patients treated with radiotherapy and surgery preoperative radiochemotherapy combination was given. The 3 and 5 year overall survival of the total group was 36 % and 19 % respectively, with a median survival of 26 months. Factors associated with a worse survival where high primary tumor stage and the presence of distant metastases. In the group of patients treated with radiotherapy and surgery an invasion of neighboring organs (Tr 4-5) and a micro- or macroscopic irradical resection (R1-2) also correlated with a worse survival.

The local recurrence free survival 3 and 5 years after treatment for the recurrence respectively was 49 % and 39 %. Factors that significantly correlated with the development of a second local recurrence were male gender, abdominoperineal resection as primary surgery technique, distant metastases, R1-2 resection and a Tr 4-5 tumor stage. Our results and a study of the literature indicate that in a carefully selected group of patients intensive salvage treatment can contribute to a longer survival with a reasonable quality of life.

Future perspectives

Developments in the treatment of rectal cancer are manifold. Firstly, new imaging techniques will select more accurately the patients that will benefit from neo-adjuvant radio(chemo)therapy. The definition of irresectability is changing from 'fixed' or 'tethered' to an extended T3 tumor that should receive neo-adjuvant treatment in order to downsize and downstage to reach curability. Secondly a new staging system was developed based on the evaluation of 50,042 patients with colorectal cancer which stratifies stage III cancer in 3 subsets (stage IIIA: T1-2, N1, stage IIIB: T3-4, N1, stage IIIB: any T, N2) (1). This way patients with stage IIIC can be selected for aggressive therapy as they have an extremely poor prognosis.

In the third place the introduction of new drugs like oxaliplatin, irinotecan and oral 5FU prodrugs in clinical studies have shown interesting results. A multi-centered phase II clinical trial is taking place at this moment in the Netherlands as a result of the phase I trial described in chapter 5. After the conclusion of that trial a phase III randomized clinical trial is planned comparing neo-adjuvant treatment with radiotherapy alone to radiochemotherapy in patients with primary irresectable rectal cancer.

Finally the development of monoclonal antibodies targeting vascular endothelial growth factor (VEGF) and VEGF receptors might improve clinical outcome in the treatment of rectal cancer. Since VEGF is needed for tumor angiogenesis and an increased expression is seen in colorectal cancer and most other tumors this seems to be an intriguing approach. Hurwitz et al. presented interesting results from a randomized trial comparing 5FU/LV and irinotecan with placebo to 5FU/LV and irinotecan with Bevacizumab (VEGF antibody) (2). They found a significant benefit in response rate, progression free and median survival in patients primarily treated for colorectal cancer.

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Nederlandse samenvatting en toekomstperspectieven

Samenvatting

Per jaar krijgen in Nederland tenminste 2150 patiënten een rectum tumor. Vijf jaar na behandeling leeft ongeveer de helft van die patiënten nog. Hierbij is vooral het tumor stadium bij diagnose van belang. Rond de 15 % van de patiënten heeft bij eerste presentatie een gevorderd stadium dat niet meer toegankelijk is voor primaire chirurgie. De behandeling van keuze is een chirurgische resectie van de rectum tumor. Daarbij is het verkrijgen van tumorvrije sneevlakken essentieel voor een curatieve behandeling. Om deze reden wordt er onderzoek gedaan naar mogelijkheden om met radiotherapie alleen of in combinatie met chemotherapie (neo-adjuvante therapie) de tumor kleiner te maken. Het doel hierbij is het reseceren van de tumor met tumorvrije sneevlakken. Dit zou moeten leiden tot een verminderde kans op het krijgen van een lokaal recidief en een betere prognose.

Het **eerste hoofdstuk** geeft een overzicht van de literatuur. Drie onderwerpen komen hierbij aan de orde: de preoperatieve diagnostiek naar de resectabiliteit van een rectum tumor vervolgens de neo-adjuvante radiotherapie en tenslotte de combinatie radiochemotherapie. De meest aantrekkelijke techniek om resectabiliteit te beoordelen lijkt een MRI met een uitwendige lichaamsspoel of met een multidetector spiraal CT. Deze technieken hebben de mogelijkheid om de endopelviene fascie in beeld te brengen en haar relatie met de primaire tumor. Op deze manier kan het circumferentiële resectie snijvlak worden voorspeld en daarmee kan de juiste behandeling worden gekozen.

Een minimale dosis van 45 Gy en een interval van 4-8 weken tussen het einde van de bestraling en operatie is nodig om een primair niet resectabele rectum tumor kleiner te maken. De belangrijkste beperking voor een hogere dosis radiotherapie is de tolerantie van de normale weefsels binnen het bestralingsgebied. Met een 3-4 velden bestralingstechniek kan er een homogenere dosis verdeling worden gegeven zodat organen beter gespaard worden. Het toevoegen van intraoperatieve radiotherapie na resectie van de tumor lijkt een interessante aanvulling op de behandeling maar er zijn nog geen gerandomiseerde onderzoeken welke de winst aantonen.

De reden om radio- en chemotherapie te combineren is de verwachting dat er een toename van celdood wordt bereikt. Het voordeel van de toevoeging van chemotherapie aan neo-adjuvante radiotherapie is in een aantal gerandomiseerde klinische studies onderzocht maar een eenduidige conclusie kan nog niet worden getrokken. Nieuwe medicijnen die worden onderzocht in de behandeling van het rectumcarcinoom zijn orale 5FU (bijv. capecitabine,

doxifluridine en tegafur/uracil (UFT)), oxaliplatin en irinotecan. (Pre)klinische studies naar het effect van deze nieuwe medicijnen bij de behandeling van colorectale tumoren hebben interessante resultaten opgeleverd. Om een uitspraak te kunnen doen over de rol van deze medicijnen zullen er eerst nog gerandomiseerde studies moeten worden gedaan.

Hoofdstuk 2 beschrijft de resultaten van onze studie naar het effect van neo-adjuvante radiochemotherapie bij de behandeling van het primair irresectabele rectum carcinoom. Er is gekeken in welke mate de tumor kleiner is geworden en naar de relatie met lokaal recidief en overleving. Deze resultaten werden vergeleken met de prognose van rectum tumoren welke resectabel waren zonder neo-adjuvante therapie. Met dit doel werd de prognose van 43 patiënten, behandeld met neo-adjuvante radiochemotherapie en chirurgie, vergeleken met 23 patiënten behandeld in de zelfde periode met chirurgie alleen. Van de 43 patiënten met een primair irresectabel rectum carcinoom kon in 79 % van de gevallen een macroscopisch radicale resectie worden gedaan. In 74 % van alle patiënten was dit een microscopische resectie. In 6 van de 34 (18 %) chirurgische preparaten werden er bij microscopisch pathologisch onderzoek geen rest tumorcellen meer aangetroffen (pT0). Bij 7 patiënten werden alleen nog kleine groepjes tumorcellen gevonden (pT1-2). Na een observatie periode van 4,5 jaar werden er geen lokale recidieven gezien in de patiënten met een pT0-2 tumor. Bij de 21 patiënten met een pT3-4 tumor na neo-adjuvante therapie en resectie werd er bij 3 patiënten een lokaal recidief gevonden (14 %). In de groep van 21 patiënten met een primair resectabele tumor werd in 4 % van de gevallen een lokaal recidief gevonden. Er werd geen verschil gevonden in overleving tussen de groep patiënten waarbij de tumor kleiner was geworden (pT0-2) en de primair resectabele groep (T1-2). We concluderen dat sommige patiënten baat hebben bij de neo-adjuvante procedure.

Hoofdstuk 3. Niet alle tumoren reageren even goed op neo-adjuvante therapie. Parameters die kunnen aangeven welke agressieve tumoren het meeste baat hebben bij neo-adjuvante therapie zouden behulpzaam zijn bij een behandelingskeuze. We hebben de relatie onderzocht van overleving en de aanwezigheid van bepaalde moleculaire merkstoffen bij 34 patiënten met een irresectabel rectum carcinoom behandeld met neo-adjuvante radiochemotherapie. De neo-adjuvante behandeling bestond uit bestraling van het bekken met een dosis van 45-56 Gy, gecombineerd met 5FU en leucovorin. We hebben in het bijzonder gekeken naar eiwitten die een rol spelen ofwel in celoverleving na antitumor behandeling ofwel in tumor (her)groei. P53, p21, bcl-2 en Ki-67 zijn daarvoor de meest bestudeerde merkstoffen. De intensiteit van de kleuring van deze eiwitten in het biopsie materiaal van de primaire tumor werd vergeleken met

de overleving na behandeling. Na een mediane observatietijd van 38 maanden bleek p21 een onafhankelijke parameter te zijn welke een slechtere overleving voorspelde wanneer er overexpressie werd gevonden. De andere parameters lieten geen significant verband zien met overleving. In de literatuur is een relatie beschreven tussen p21 expressie en het effect van radiotherapie. Bij een aantal cellijnen welke radioresistent zijn is een toegenomen expressie van p21 gevonden. Typisch in dit geval is dat de hoeveelheid p21 eiwit hoog blijft en er geen verandering door de bestraling optreed, zoals meestal wordt gezien bij de door p53 geïnduceerde reactie van p21 ten gevolge van bestraling. Deze cellen overleven de bestraling in tegenstelling tot de verwachtte blokkade van de celcyclus resulterend in apoptose (celdood). In onze patiëntengroep hebben wij echter geen relatie gevonden tussen de expressie van p21 en het uiteindelijke T stadium na neo-adjuvante radiotherapie. Waarschijnlijk is er een ander mechanisme dat een rol speelt in de relatie p21 expressie en overleving. Verdere analyse van deze intrigerende bevinding alsmede de prospectieve evaluatie ervan lijkt zinvol.

In **hoofdstuk 4** presenteren we de resultaten van een fase 1 studie ter bepaling van de maximaal tolereerbare dosis van oxaliplatin wanneer deze wordt toegevoegd aan 5FU en radiotherapie in de behandeling van het lokaal gevorderde rectum carcinoom. De toevoeging van oxaliplatin aan een 5FU chemotherapie regime heeft geresulteerd in een verbetering van de behandeling van gemetastaseerd coloncarcinoom. Zoals beschreven in hoofdstuk 2 reageren niet alle rectum tumoren op neo-adjuvante therapie. In een poging om het effect van chemotherapie te verbeteren werd oxaliplatin toegevoegd. Verder blijkt uit de literatuur dat oxaliplatin cellen gevoeliger kan maken voor bestraling.

In onze studie werden 18 patiënten behandeld met oxaliplatin toegevoegd aan de standaard neo-adjuvante radiochemotherapie. Dertien patiënten ontvingen 5FU intraveneus en 5 in de vorm van capecitabine (een orale variant van 5FU). Radiotherapie werd gegeven met behulp van een 3-4 velden techniek, 6-15 MV, 45 Gy in 25 fracties van 1,8 Gy en een boost van 5,4 Gy in 3 fracties. De chemotherapie bestond uit twee cycli van 5FU, 350 mg/m^2 en leucovorin 20 mg/m^2 (intraveneuze bolus injectie) toegediend op dagen 1 t/m 5 and 29 t/m 33 van de radiotherapie. Capecitabine werd voorgeschreven in een dosis van 1 gram/m^2, 2 keer per dag, van dag 1 t/m 14 en dag 25 t/m 38. Oxaliplatin werd toegediend op 2 dosis niveaus: 85 mg/m^2 en 130 mg/m^2 op de eerste en 29ste dag van de radiotherapie als een 2 uur durende infusie voorafgaand aan 5FU en leucovorin. Een chirurgische procedure vond plaats 4 tot 6 weken na beëindiging van deze neo-adjuvante therapie.

De eerste 3 patiënten werden behandeld met een oxaliplatin dosis van 85 mg/m^2. Bij deze dosis werden graad 3 diarree, verhoogde leverenzymen en een milde leucopenie en anemie gezien. Hierna werd de oxaliplatin dosis verhoogd tot 130 mg/m^2. Met deze dosis werden 7 patiënten behandeld. Bij één patiënt werd de behandeling voortijdig gestaakt in verband met een graad 4 mucositis, diarree en leucopenie. Uiteindelijk bleek dit het gevolg te zijn van een deficiëntie van het enzym dihydropyrimidine dehydrogenase (DPD). Dit enzym zet het toxische 5FU om in een niet toxisch product (hoofdstuk 5). Om die reden werd deze patiënt niet meegenomen in de toxiciteit evaluatie van de studie en werd een extra patiënt behandeld met 130 mg/m^2 oxaliplatin. Echter de 6de patiënt behandeld in deze dosisstap ontwikkelde een graad 4 leucopenie en overleed mogelijk ten gevolge van een septische shock gerelateerd aan een urosepsis. Bij DNA analyse werden de meest voorkomende DPD mutaties niet aangetoond.

Na het overlijden van deze patiënt werden er nog 8 patiënten behandeld met 85 mg/m^2 oxaliplatin zonder dosis beperkende toxiciteit. Vijf van deze patiënten ontvingen capecitabine in plaats van 5FU intraveneus. De adviesdosis voor een fase 2 studie van oxaliplatin bij de behandeling van het lokaal gevorderde rectum carcinoom met intraveneus 5FU of oraal capecitabine is 85 mg/m^2.

In **Hoofdstuk 5** wordt het effect van de toediening van 5FU aan patiënten met een DPD deficiëntie besproken. Mutaties in het DPD-gen lijden tot een verminderd metabolisme van 5FU met als gevolg een accumulatie van toxische verbindingen. De incidentie van een DPD activiteit lager dan 70 % wordt geschat op 3 %, waarbij de mortaliteit bij patiënten met een DPD deficiëntie gerelateerde 5FU toxiciteit 10 % is.

In dit hoofdstuk worden 2 patiënten beschreven welke ernstige toxiciteit hadden ontwikkeld na de toediening van 5FU en oxaliplatin bevattende chemotherapie bij een DPD deficiëntie. De eerste patiënt werd behandeld voor een lokaal gevorderd rectumcarcinoom. Na de toediening van 5FU ontwikkelde zij ernstige mucositis, diarree en hematologische toxiciteit. Zij was opgenomen in het ziekenhuis gedurende 142 dagen waarvan 10 op de intensive care. De tweede patiënt kreeg 5FU en oxaliplatin vanwege gemetastaseerd coloncarcinoom. Zij ontwikkelde orale mucositis, diarree graad 2, leucopenie graad 3, milde cerebellaire ataxie en was opgenomen gedurende 5 dagen. Beide patiënten zijn volledig hersteld van hun toxiciteit zonder tekenen van late toxiciteit.

Een aantal observaties suggereren dat het risico op het ontwikkelen van een 5FU geïnduceerde toxiciteit hoger kan zijn ten gevolge van een co-medicatie effect. De combinatie 5FU en oxaliplatin zou het risico op ernstige toxiciteit kunnen verhogen bij patiënten met een verlaagde

DPD activiteit. Het is aangetoond in een farmacokinetische studie dat de toevoeging van oxaliplatin aan 5FU kan resulteren in een vermindering van het 5FU katabolisme.

Een toenemend aantal studies worden verricht met orale 5FU medicijnen, dit leidt tot een toename van het gebruik van deze middelen en daarmee tot een verhoogd risico op het ontwikkelen van 5FU gerelateerde toxiciteit. Patiënten met een verhoogd risico zouden kunnen worden geselecteerd met behulp van screening naar DPD deficiëntie. Deze patiënten zouden dan met andere nieuwe chemotherapeutica zonder fluoropyrimidine verbindingen kunnen worden behandeld.

Hoofdstuk 6 geeft de resultaten van de behandeling van patiënten met een lokaal recidief rectum carcinoom. Een groep van 40 patiënten welke werden behandeld met radiotherapie alleen of gecombineerd met chirurgie werd bestudeerd. Radiotherapie werd gegeven met behulp van een 3-4 velden techniek (6-15 MV), 5 maal per week. De mediane bestralingsdosis was 50 Gy (25-66.6 Gy). Vijfentwintig patiënten ondergingen chirurgie. Bij 5 patiënten behandeld met radiotherapie en chirurgie werd een combinatie van radiochemotherapie gegeven.

De 3 en 5 jaars overleving van de gehele groep was respectievelijk 36 % en 19 %, met een mediane overleving van 26 maanden. Factoren geassocieerd met een slechtere overleving waren een hoog primair tumorstadium en de aanwezigheid van afstandsmetastasen. In de groep patiënten welke werden behandeld met radiotherapie en chirurgie werd een slechtere overleving gevonden wanneer er invasie van omliggende organen was of een micro- dan wel macroscopisch irradicale resectie werd verricht.

De kans op overleving zonder nieuw lokaal recidief 3 en 5 jaar na behandeling van het recidief was respectievelijk 49 % en 39 %. Factoren welke significant correleerden met het ontwikkelen van een lokaal recidief waren mannelijke sekse, abdominoperineale resectie als primaire chirurgische techniek, afstandsmetastasen, resectie resultaat en een tumor stadium. Onze resultaten geven aan dat in een geselecteerde groep patiënten een intensieve behandeling van het lokaal recidief rectum carcinoom kan bijdragen tot een lange overleving met een redelijke kwaliteit van leven, deze opvatting wordt gesteund door recente literatuur.

Toekomst perspectieven

Er zijn verschillende ontwikkelingen gaande in de behandeling van het rectumcarcinoom. Ten eerste zullen nieuwe beeldvormende technieken patiënten nauwkeuriger selecteren welke baat hebben bij een neo-adjuvante radio(chemo)therapie. Hierdoor verandert de definitie van "een niet resectabele rectum tumor" van de algemene termen "gefixeerd" of "vast" naar "een uitgebreide T3 tumor" welke neo-adjuvante behandeling zou moeten ontvangen om een curatieve behandeling mogelijk te maken. Ten tweede is er een nieuw stagerings systeem ontwikkeld, gebaseerd op de evaluatie van 50042 patiënten met een colo-rectaal carcinoom, dat stadium III tumoren stratificeert in 3 groepen (stadium IIIA: T1-2, N1, stadium IIIB: T3-4, N1, stadium IIIC: alle T, N2). Op deze manier kunnen patiënten met een stadium IIIC rectumcarcinoom worden geselecteerd voor een agressievere therapie aangezien zij een slechte prognose hebben. In de derde plaats heeft de introductie van nieuwe chemotherapeutica zoals oxaliplatin, irinotecan en orale 5FU in de behandeling van colo-rectale tumoren geleid tot interessante resultaten. Op dit moment loopt er een fase 2 studie (REX) in verschillende Nederlandse ziekenhuizen wat een direct gevolg is van de resultaten van de fase 1 studie beschreven in hoofdstuk 5. Na afronding van die studie is er een fase 3 studie gepland welke zal randomiseren tussen een neo-adjuvante behandeling met radiotherapie alleen dan wel gecombineerd met chemotherapie in de behandeling van patiënten met een lokaal gevorderd rectumcarcinoom.

Ten slotte zou de ontwikkeling van en de behandeling met monoklonale antilichamen gericht op vasculaire endotheliale groei factor (VEGF) receptoren en VEGF zelf, de prognose van het rectumcarcinoom kunnen verbeteren. Aangezien VEGF nodig is voor de vaatnieuwvorming van tumoren en er een toegenomen expressie wordt gezien in colo-rectale tumoren lijkt dit een intrigerende benadering. Hurwitz en anderen hebben interessante resultaten gepubliceerd van een gerandomiseerd onderzoek waarbij 5FU, leucovorin en irinotecan met een placebo werd vergeleken met 5FU, leucovorin en irinotecan met Bevacizumab (VEGF antilichaam). Zij vonden een significante verbetering van respons, progressie vrije en mediane overleving bij de primaire behandeling van patiënten met een colo-rectale tumor.

Samenvattend geven verschillende ontwikkelingen aanleiding tot de verwachting dat in de komende jaren de prognose ook van het ongunstige stadium rectum carcinoom enigszins zal verbeteren.

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