

Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer

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Background: In advanced metastatic colorectal adenocarcinoma, the addition of a neo-adjuvant systemic treatment to surgery might translate into a survival advantage, although this is yet to be confirmed by ongoing randomized trials. The objective of this study was to assess the effects of preoperative systemic chemotherapy on the morphology of non-tumoral liver.

Patients and methods: A large series of surgically resected liver metastases ($n = 153$) was selected. Light microscopy, electron microscopy, and immunohistochemistry using antibodies against endothelial cells (CD31) and hepatic stellate cells (α -SM actin, CRBP-1) were performed to identify sinusoidal wall integrity.

Results: We found that 44 (51%) of the 87 post-chemotherapeutic liver resection specimens had sinusoidal dilatation and hemorrhage, related to rupture of the sinusoidal barrier. In contrast, the 66 livers treated by surgery alone remained normal. In 21 out of the 44 post-chemotherapy patients (48%), perisinusoidal and veno-occlusive fibrosis also developed. Sinusoidal injury persisted several months after end of chemotherapy, and fibrosis may progress. Development of lesions was strongly correlated to the use of oxaliplatin; 34 out of 43 patients (78%) treated with this drug showed striking sinusoidal alterations.

Conclusions: Systemic neo-adjuvant chemotherapy in metastatic colorectal cancer frequently causes morphological lesions involving hepatic microvasculature. Sinusoidal obstruction, complicated by perisinusoidal fibrosis and veno-occlusive lesion of the non-tumoral liver revealed by this study, should be included in the list of the adverse side-effects of colorectal systemic chemotherapy, in particular related to the use of oxaliplatin.

Key words: drug liver injury, 5-fluorouracil, neo-adjuvant chemotherapy, sinusoidal obstruction syndrome, veno-occlusive disease

Introduction

Liver metastasis is the most common complication from colorectal cancer and a major contribution to mortality due to this disease. Approximately 60% of patients with colorectal cancer develop metastases, and in ~30% the liver is the only site of metastatic involvement [1]. Hepatic resection currently remains the treatment that offers the best chance of long-term survival to such patients [2, 3].

Increasing numbers of liver metastases are now considered surgically resectable. Various factors explain this trend: progress of surgical techniques, better knowledge of the functional anatomy of the liver, detection of metastases at an earlier stage, introduction of new therapeutic approaches (e.g. cryosurgery, radio-frequency) and use of chemotherapy before resection. This latter approach, aimed at the reduction of the size of metastases, allows

the resection of an additional 16% of metastases previously deemed unresectable [4].

Despite the large number of drugs available, only a few agents have been found to be active in advanced colorectal cancer. The standard treatment for advanced colorectal cancer has been 5-fluorouracil (5-FU)-based chemotherapy combined or not with leucovorin. Recently, new therapeutic approaches have been introduced. These include agents with mechanisms of action unrelated to thymidylate synthetase, such as irinotecan, a topoisomerase I inhibitor [5], and oxaliplatin, the only platinum derivative with significant activity in colorectal cancer [6]. Combined with 5-FU and leucovorin, both irinotecan and oxaliplatin have led to a significant improvement in terms of response rate, disease-free and even overall survival in metastatic disease, and have become standard of care in this setting. Considerable enthusiasm for the use of these new systemic combinations in a neo-adjuvant setting before curative surgical removal of liver metastases has also generated a large set of very encouraging data, but only in the context of non-controlled trials, and confirmation in proper randomized trials is still awaited [7–11].

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Table 1. Types of intravenous chemotherapy undergone before surgical resection

Type of chemotherapy	Number of cases	No hepatic lesion (%)	Hepatic lesions (%)
Total without chemotherapy	66	66 (100)	0 (0)
Total with chemotherapy	87	43 (49)	44 (51)
5-FU alone	27	21 (78)	6 (22)
5-FU and irinotecan	17	13 (77)	4 (23)
5-FU and oxaliplatin	27	7 (26)	20 (74)
5-FU, oxaliplatin and irinotecan	16	2 (12)	14 (88)

5-FU, 5-fluorouracil.

Despite the increasingly extensive use of neo-adjuvant chemotherapy in advanced colorectal cancer, its functional and histopathological effects on the liver remain inadequately described. The aim of this study was to attempt to fill this gap by analyzing the morphological effects of preoperative systemic chemotherapy on the non-tumoral hepatic parenchyma, in a large series of livers resected for metastatic involvement by colon carcinoma. The study was conducted in two centers that have used preoperative chemotherapy since 1995.

Materials and methods

Patients

We reviewed the clinical data for all patients who had undergone surgical liver resection for hepatic metastases from colorectal carcinoma between 1994 and 2002 at the University Hospital, Geneva, Switzerland ($n = 82$) and at the Hôpital Cochin, Paris, France ($n = 92$). Our study was performed in accordance with the ethical standards of the Declaration of Helsinki of the World Medical Association. After evaluating clinical data, we examined the pathological material available for each case, and determined that of the 174 resections, 153 had adequate clinical information and sufficient material for pathological studies. The 21 excluded cases had incomplete clinical data and/or $<2 \text{ cm}^2$ of non-tumoral liver tissue on the slide analyzable for morphological evaluation.

The group included 93 men and 60 women. The mean age was 62 years (range 36–80). The liver resections consisted of 80 right and 31 left hepatectomies, and 42 segment-oriented resections. Vascular exclusion (Pringles' maneuver or vascular efferent exclusion) was performed during surgery in 55 cases. Preoperative portal embolization was performed in six cases. Eighteen patients underwent two or more liver resections, because of recurrence of hepatic metastases in 16 cases, and two time resections in two cases.

Surgical resection was the only treatment undergone for the metastases in 66 cases. Systemic chemotherapy was undergone before hepatic surgery in 87 cases. Several different protocols were used: 16 patients received oxaliplatin in combination with irinotecan and 5-FU; 27 received oxaliplatin in combination with 5-FU; 17 received irinotecan in combination with 5-FU; and 27 received 5-FU alone (Table 1). The administration was exclusively intravenous.

Tissues

All archival slides (originally prepared from formalin-fixed, paraffin-embedded tissue) were reviewed, and representative slides of non-tumoral tissue located at distance of the tumor were selected for quantitation of the lesions and immuno-

histochemical studies. The morphological analyses were based on hematoxylin and eosin, Masson trichrome and reticulin stainings. Slides were examined blindly by pathologists working on the study (L.Rubbia-Brandt, B.Terris, V.Audard and P.Sartoretti), who were unaware of the subject's clinical data; specifically, no information was available on the administration of preoperative chemotherapy.

The following histological features were evaluated: sinusoidal dilatation, centrilobular vein fibrosis, perisinusoidal fibrosis, nodular hepatic regeneration, hepatocellular necrosis and steatosis. Sinusoidal dilatation was graded semi-quantitatively as follows: 0, absent; 1, mild (centrilobular involvement limited to one-third of the lobular surface); 2, moderate (centrilobular involvement extending in two-thirds of the lobular surface); 3, severe (complete lobular involvement). Steatosis was estimated as the percentage of involved hepatocytes, and was categorized as follows: 0, absent; 1, mild (steatosis in $<30\%$ of the hepatocytes); 2, moderate (steatosis in 30–60% of the hepatocytes); 3, severe (steatosis in $>60\%$ of the hepatocytes).

The χ^2 -test was used to correlate the different groups of patients according to the presence or not of preoperative treatment, and the type of chemotherapy versus the presence of sinusoidal dilatation. A result was considered statistically significant if $P < 0.05$.

Immunostaining

Five cases of hepatectomies showing representative sinusoidal lesions and five control cases of hepatectomies for colorectal metastasis without lesions were selected for immunohistochemical studies, in order to better characterize the morphology of the sinusoidal lesions.

For immunostaining, additional serial 3–5 μm sections were prepared. Sections were mounted on silane-coated glass slides, air-dried overnight, deparaffinized, rehydrated and pretreated with H_2O_2 /methanol to block endogenous peroxidase activity. The following primary antibodies were used: mouse monoclonal α -smooth muscle (α -SM) actin (DakoCytomation, Zug, Switzerland; 1:400 dilution); rabbit polyclonal cellular retinol binding protein-1 (CRBP-1) (gift from Professor G. Gabbiani; 1:200 dilution); and mouse monoclonal anti-human CD31 (DakoCytomation; 1:50 dilution). For α -SM actin and CD31 antibodies, microwave pretreatment was used. For CRBP-1, pretreatment with a pressure cooker was performed. Sections were incubated for 1 h at room temperature with the diluted primary antibodies, which were then revealed by ENVISION (DakoCytomation). Peroxidase activity was revealed with 30% 3,3'-diaminobenzidine as chromogen in phosphate-buffered saline containing 0.015% H_2O_2 . Sections were weakly counterstained with Mayer's hematoxylin and mounted in Eukitt (Kindler GmbH, Freiburg, Germany). Negative controls were prepared by omitting the first antibodies.

Electron microscopy

During the study period, new available liver specimens with macroscopic lesions were fixed in glutaraldehyde and embedded in Epon. Semi-thin sections were stained with Toluidine Blue. Regions of the liver with representative sinusoidal lesion were selected for thin sections. Thin sections were treated with uranyl acetate and lead citrate and examined using a Zeiss electron microscope (EM CR 10; Zeiss).

Results

Histopathological aspects

Among the 153 hepatic resections analyzed, 44 (29%) exhibited histological lesions in the non-tumoral liver. They consisted mainly of injury in centrilobular zones characterized by severe sinusoidal dilatation with erythrocytes congestion, occasionally

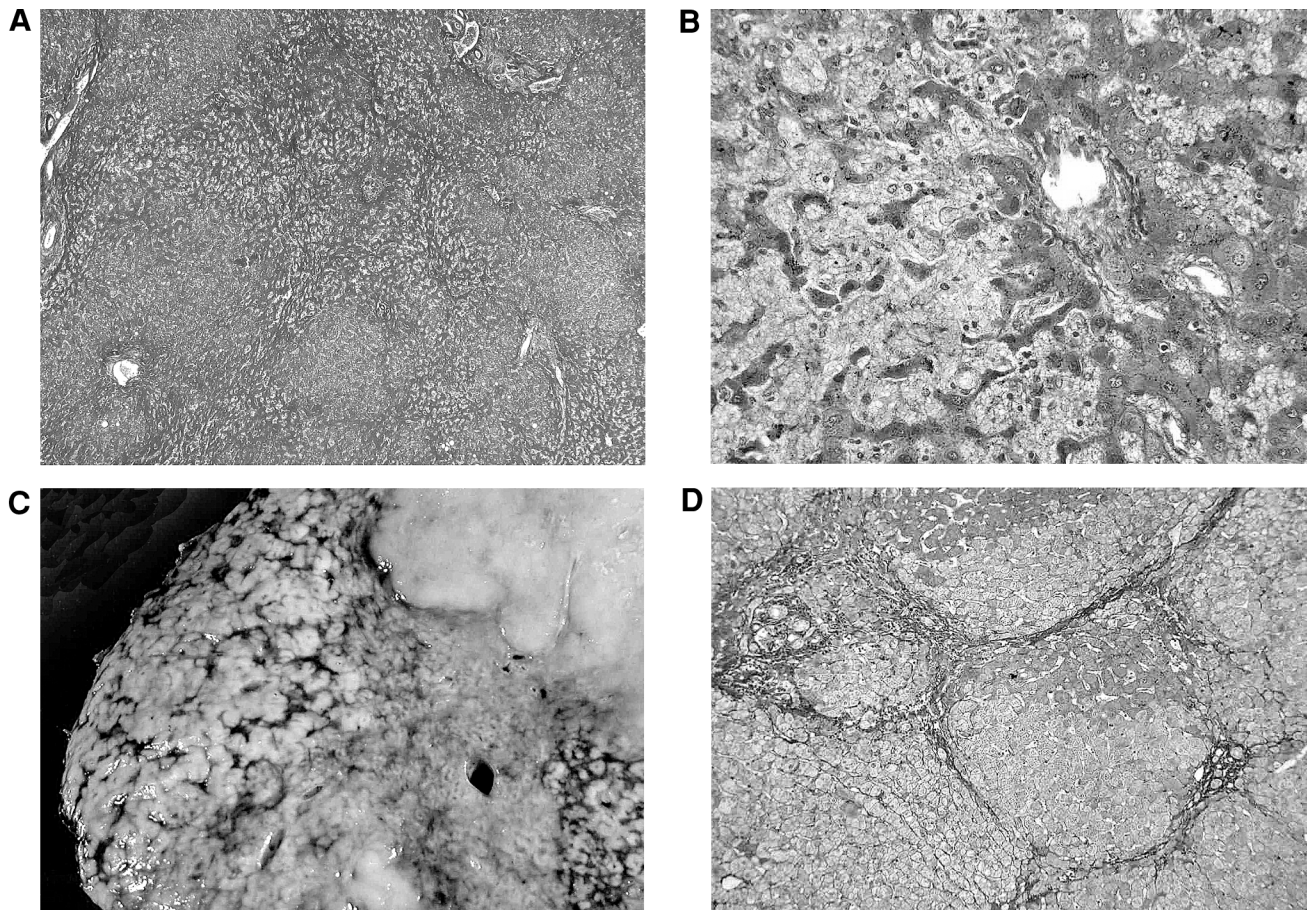


Figure 1. (A) Low-power examination on trichrome Masson stain of the liver revealed areas of sinusoidal congestion involving centrilobular and mediolobular lobular surface. At high-power examination, sinusoidal congestion is severe, outlined by atrophic hepatocyte trabeculae. (B) The small hepatic vein is partially occluded by fibrous tissue; on the cut surface, the liver around and at the distance of the metastasis is diffusely nodular. (C) Nodules are outlined by congested areas. (D) In a third hepatectomy, performed in one patient 49 months after chemotherapy, extensive fibrous septa delimit parenchymal nodules on trichrome Masson.

accompanied by perisinusoidal fibrosis and fibrotic venular occlusion (Figure 1A and B). In most cases, there was an irregular distribution of the lesions, with presence of abnormal lobules intermingled with intact lobules. Sinusoidal congestion was grade 1 in 16 patients, grade 2 in 16 cases and grade 3 in 12 patients. In some centrilobular areas of cases with grade 2 or 3, the severity of sinusoidal distension was accompanied by extravasation of erythrocytes, characterized by erythrocytes lining down directly along hepatocellular plates (Figure 2A). Atrophic hepatocellular plates or few necrotic cells bordered dilated areas. In atrophic zones, the reticulin staining was focally increased, corresponding to perisinusoidal fibrosis (data not shown). However, a component of parenchymal collapse with consequent visualization of the reticulin cannot be completely ruled out. Several centrilobular veins were also clearly affected by fibrosis, resulting in variable degrees of luminal occlusion (Figure 1B). These patterns were observed in 21 out of 44 patients (48%). The periportal lobular zones appeared with widened hepatocellular plates; these regenerative foci involved the affected lobules in most of the cases, but reached an aspect of diffuse nodular regenerative hyperplasia (NRH) in seven cases (data not shown). In the most florid cases, diffuse heterogenous

hemorrhagic foci and parenchymal nodularity were evident even macroscopically (Figure 1C). Steatosis was present in 75 of the 153 patients (49%). It was grade 1 in 44 of 75 (59%) cases, grade 2 in 22 of 75 (29%) cases and grade 3 in nine of 75 (12%) cases.

Immunohistochemical studies

In control liver without sinusoidal lesions, CD31+ endothelial cells were present in portal tract and vessel walls (hepatic artery, portal vein and centro-lobular vein), and diffuse along lobular sinusoid. α -SM actin-positive cells were observed in vessel walls (data not shown). Sparse hepatic stellate cells (HSC) positive for α -SM actin were focally observed in a small proportion of the lobules. In the lobule, CRBP-1 was expressed at a high level in HSC (data not shown).

In livers with sinusoidal lesions, CD31 positivity was absent from areas of severe sinusoidal dilatation. In contrast, CRBP-1 showed the same distribution as observed in normal liver (data not shown). These results suggest loss of sinusoidal endothelial cells but a persistence of perisinusoidal HSC along dilated sinusoid. However, the expression of α -SM actin in HSC was drastically modified compared with normal liver. A strong reactivity was

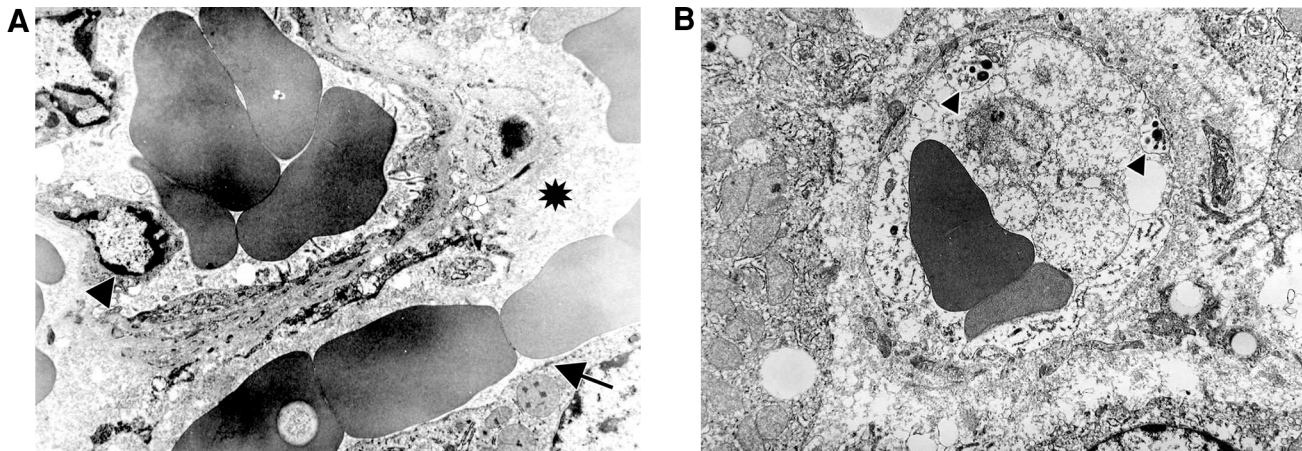


Figure 2. (A) A sinusoid containing four red blood cells. The perisinusoidal space of Disse (star) is extensively dilated and contains several erythrocytes in close contact with hepatocytes (arrow). Endothelial cells are rounded up and protrude into the lumen of the sinusoid (arrow head). Magnification, $\times 7200$. (B) A sinusoid containing two red blood cells. The rest of the sinusoid lumen is filled with cytoplasmic blebs, two of which contain organelles (arrow heads). The perisinusoidal space is enlarged and contains collagen fibers. Magnification, $\times 7200$.

present in almost all HSC within the lobules, and was present both along dilated sinusoids and intact sinusoids (data not shown). This indicates an activated state of HSC.

Electron microscopy

Extensive ultrastructural abnormalities of sinusoidal endothelial cells were observed. Endothelial cells were rounded up and swollen; their nuclei showed peripheral chromatin condensation, suggesting the apoptotic process (Figure 2A). Areas of sinusoidal dilatation showed diffuse discontinuities along the endothelial sinusoidal lining, allowing red blood cell extravasation in the widened Disse's spaces (Figure 2A and B). A neodeposition of extracellular matrix components in Disse's spaces was evident, mainly by fine collagen bundles (Figure 2A). A striking feature was the presence of numerous cytoplasmic blebs obstructing the sinusoidal lumen, several of them containing organelles (Figure 2B). In contrast, hepatocytes were better preserved, showing mainly a loss of hepatocyte microvilli.

Correlation of histological findings with type of chemotherapy and clinical data

Centrilobular lesions were observed in 44 of the 87 (51%) patients who received chemotherapy. No centrilobular lesions were observed in any of the 66 patients treated by surgery alone, including those in whom preoperative portal embolization or preoperative vascular exclusion had been performed (Table 1). Among the different protocols of chemotherapy, a significant correlation was detected between the presence of liver lesions and the use of oxaliplatin: 34 of the 43 (79%) patients treated with oxaliplatin developed lesions, as opposed to 10 of the 44 (23%) who did not ($P < 0.001$) (Table 1). The amount of oxaliplatin received by our population was quantified as cumulative dose expressed in mg/m^2 ; the range was 280–1600 mg/m^2 . No correlation was found between the cumulative dose of oxaliplatin and the development of sinusoidal lesions ($P > 0.05$).

The mean interval between last administration of chemotherapy and surgical resection was 35 days (range 16–110). Hepatic sinusoidal lesions persisted up to 4 months after last chemotherapy. Steatosis was observed in 42 of the cases 87 (48%) with chemotherapy and in 33 of the 66 cases (50%) without chemotherapy ($P > 0.01$).

Evolution of the liver lesions

To appreciate the evolution of the liver lesions at a time distant from the initial chemotherapy, we analyzed the 18 cases who underwent iterative hepatic surgery. In three patients treated by surgery alone, no centrilobular lesions were observed in the subsequent hepatectomies. Among the 15 remaining patients, nine had initial lesions on the first hepatectomy. Six of them received new cycles of preoperative chemotherapy, which did not allow the evaluation of the natural evolution of the lesions at a time distant from the end of chemotherapy. The three remaining patients showing lesions on the first liver resection had iterative hepatic resections for recurrence of liver metastasis with surgery as the only treatment. The second hepatectomies were performed 5, 11 and 17 months after the first resection, respectively. A third hepatectomy was performed in one patient 32 months after the second one. There was a persistence of sinusoidal dilatation in one case and fibrosis in two cases. In one of these patients, the fibrosis progressed; it no longer remained confined to the centrilobular zones, but it also extended to the rest of the lobule with centro-centro and porto-centro bridging fibrosis, reaching a cirrhotic configuration in the third hepatectomy (Figure 1D). In this patient, α -SM actin-positive HSC were diffusely present along the lobules, while the pattern of CRBP-1 and CD31 expression was similar to that found in normal liver (data not shown). Moreover, this patient was hepatitis B and C virus negative, and had no history of alcohol abuse.

Discussion

At present, surgery is the only curative option for the treatment of liver metastases from primary colorectal carcinoma. Preoperative

chemotherapy is used to achieve conversion from unresectability to resectability of hepatic metastases, thus increasing the number of patients who can benefit from surgical intervention [4, 12]. Expanding the body of data gathered indicates that in metastatic disease, this combined strategy might be beneficial on the overall rate of recurrence [7, 8, 10, 11]. Approximately 40% of patients who undergo this treatment can be expected to survive 5 years or longer [12]; this advantage could have an effect on the outcome of resected patients, and randomized trials testing this hypothesis are currently ongoing. In addition, surgery is also applied in liver metastasis recurrence, and the number of cases of iterative resections, often preceded by new cycles of chemotherapy, is increasing. Therefore, it is important to be aware of, and to prevent, the possible harmful side-effects of the chemotherapeutic regimens administered to these patients.

The results of this study show that neo-adjuvant chemotherapy in patients with metastatic colon cancer may induce important sinusoidal obstruction associated with sinusoidal fibrosis and veno-occlusive lesions, occasionally with nodular regenerative hyperplasia. In our population, this effect was particularly marked in the livers of patients treated with protocols that included oxaliplatin. No such lesions were present in the resected livers from patients who had been treated by surgery alone.

Whereas hepatocellular carcinomas usually arise from a background of fibrosis or cirrhosis, colon cancer metastases often affect normal livers. Frequently in our experience, however, surgeons report gross abnormalities, such as soft, congestive or even pseudocirrhotic livers, which compel them to limit the extension of the resection. Not uncommonly, frozen-section examinations requested to evaluate the non-tumoral liver prior to the performance of the resection indicate sinusoidal lesions.

To our knowledge, these abnormalities have not been systematically investigated. Only the hepatotoxic effects of 5-FU have received much attention: Washington et al. [13] reported in 1993 the development of NRH in five patients treated with 5-FU; bile duct fibrosis and stenosis secondary to hepatic intra-arterial 5-FU treatment have also been reported [14]. Furthermore, experimental studies with the use of intra-arterial 5-FU perfusion in rabbits [15] have shown endothelial toxicity resulting in disruption of the endothelium of small arteries, patchy exposure of the subendothelium and foci of thrombus formation.

The sinusoidal lesions we observed here were morphologically similar to those seen in veno-occlusive disease, a condition that occurs mainly as a complication of high-dose chemotherapy in the setting of stem-cell transplantation, and recently renamed by DeLeve et al. [16–18] as sinusoidal obstruction syndrome (SOS). In our series, the prevalence of SOS in patients who received preoperative chemotherapy was 51%. This figure is substantially higher than the 10–20% reported in patients who underwent bone marrow transplantation for lympho-hematological malignancies [17].

The intensity of the sinusoidal dilatation and congestion was occasionally severe, and included the disruption of the sinusoidal wall integrity. This latter finding was well illustrated by the extravasation of erythrocytes in Disse's space and by immunohistochemical and ultrastructural studies, which showed the com-

plete absence of endothelial lining in the dilated areas. HSC, however, remain present in the perisinusoidal space, as demonstrated by the preserved expression of CRBP-1, a new marker of HSC [19]. Extensive collagenization of perisinusoidal space and centrilobular vein was occasionally identified. This fibrogenesis was highlighted by the presence of α -SM actin-positive HSC, an activation marker of fibrogenic cells, both around sinusoidal dilated areas and in other areas of the lobule. This observation is in agreement with a previous description of veno-occlusive diseases in humans [20].

Based on our observations, we propose the hypothesis that an initial toxic injury to the sinusoidal endothelial cells results in sinusoidal wall disruption; this may be followed by an activation of HSC and the deposition of matrix in the sinusoids. Obstruction was caused by erythrocytes sloughing, but also by blebs, characterized by free fragments of cytoplasmic processes, occasionally containing cellular organelles. Ultrastructural bleb formation has been described in an experimental rat model of hepatic sinusoidal drug toxicity [21].

In addition to sinusoidal lesions, we also observed areas of hepatic regeneration, which reached a pattern of NRH in seven cases. NRH has been reported in one published series of patients who received preoperative 5-FU chemotherapy for metastatic carcinoma of the colon, with a frequency equivalent to that of our study [13]. The pathogenesis of NRH is believed to be related to modifications of intrahepatic blood flow [22, 23]. When symptomatic, patients develop signs of portal hypertension and cholestasis with elevated alkaline phosphatase. Despite the fact that NRH could become clinically evident a long time after chemotherapy, the latter is under investigation in our study population, as well as possible delayed hepatic regeneration after surgical resection.

Different chemotherapy protocols for advanced colorectal carcinoma have been developed over the last decade. To date, oxaliplatin, a novel platinum complex, has proven to be a safe and effective therapy for colorectal cancer in combination with 5-FU [24], and its side-effects have been easy to manage with appropriate awareness from patients and care providers. During clinical trials, the adverse events most often reported were hematological toxicity, gastrointestinal tract toxicity and a neuropathy, none of which has been observed with other therapeutic platinum derivatives [25]. In our series, we found a significant correlation between the use of oxaliplatin and the development of severe sinusoidal injury. Carboplatin, another alkylating agent of the cisplatin family, has been rarely associated with the development of veno-occlusive disease in patients who have undergone stem-cell transplantation [26]. However, to our knowledge this is the first report of a series of patients with significant hepatic adverse effects from oxaliplatin. Several interpretations of these data are possible. First, no targeted liver biopsy studies have been performed in patients with colon cancer who have received chemotherapy that included oxaliplatin. Such studies would be difficult to justify from an ethical viewpoint, and may never be performed. For our study we had access to extensive sampling from the resected livers, which allowed accurate histopathological examination and therefore increased the likelihood of detecting even subtle abnormalities. A second possibility

is that oxaliplatin alone may in fact have little or no liver toxicity, but in combination with other chemotherapeutic agents (e.g. 5-FU) a synergic effect may occur. This is unlikely, because most chemotherapy protocols for colon cancer include a combination of different drugs, and serious liver toxicity has not been reported. Another explanation may be related to the time these lesions require to develop and progress. In our series we were able to study the evolution of the lesions in few patients who underwent a second liver resection for metastasis recurrence. The sinusoidal lesions persisted for several months after chemotherapy had been discontinued. The histopathological findings suggest that fibrosis persists, and, in the few cases we were able to follow for an extended period (>49 months), may continue to progress and reach considerable extension. The risk of evolution may be important, particularly for those patients who have to receive multiple chemotherapy cycles to obtain better tumor responses. Finally, the relationship between tumoral response and type of chemotherapy received is under study. Preliminary results did not identify a significant difference in the size, localization and number of metastasis, evaluated by computed tomography scan prior to neo-adjuvant therapy, between the control group and the group receiving chemotherapy. No significant relationship has been observed between these tumor characteristics and development of sinusoidal lesion in the non-tumoral liver.

Our ability to evaluate the clinical relevance of our findings was limited by the retrospective nature of this study. One would suspect, however, that the constellation of the lesions described here may result in an impairment of liver function in some patients; it is also possible that the vascular lesions may limit the regenerative ability of the liver after large hepatectomies.

In the light of these findings, we are currently carrying out a prospective study to determine the laboratory and clinical effects of oxaliplatin-induced liver toxicity during the course of and after preoperative chemotherapy. We are also exploring ways to prevent these toxic effects by using glutathione, a compound that has recently been reported to be effective in the prevention of chemotherapy-associated neuropathy [27]. The use of glutathione in an attempt to prevent liver injury has both biological plausibility and experimental support: in a rat model, profound glutathione depletion was found to play a role in the occurrence sinusoidal hepatic toxicity [28–30]. Prevention of sinusoidal lesions was also achieved experimentally in rats by maintaining the level of hepatic nitric oxide (NO) through the administration of O(2)-vinyl 1-(pyrrolidin-1-yl) diazen-1-ium-1,2-diolate (V-PYRRO/NO), a liver-selective NO-donating prodrug, metabolized by hepatic enzymes to release NO within the liver [16, 31]. The use of such drugs in humans has not yet been reported in veno-occlusive disease, but it is conceivable that in the future they may acquire a role in the prevention of vascular liver toxicity.

In conclusion, we have shown that half of the patients who underwent neo-adjuvant chemotherapy, and at least three-quarters of patients who received oxaliplatin, had specific liver lesions. Sinusoidal injury complicated by fibrosis and veno-occlusive lesions should be included in the list of the adverse side-effects of systemic chemotherapy for colorectal tumors, particularly in connection with the use of oxaliplatin. The clinical consequences

of these sinusoidal lesions and venular obstruction also need to be evaluated with respect to the potential impairment of liver regeneration after extended resection.

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