in vivo *20*: 373-376 (2006)

Cutaneous and Intra-abdominal Abscess Formation in Rats Following Radio Frequency Ablation of Liver Tumors in Combination with Celecoxib Treatment

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Abstract. *Background: The present study evaluated the safety of treatment of colorectal liver metastases with radio frequency ablation (RFA) in combination with high doses of the selective cyclooxygenase-2 inhibitor celecoxib. Materials and Methods: The study was performed in the CC531 rat model for colorectal cancer. The rats were inoculated with CC531 tumor cells subcapsularly in the liver. They were then randomized for treatment with celecoxib, RFA, or their combination. Celecoxib treatment was started at tumor induction. Three weeks later the liver tumors were treated with RFA and the effects on the health of the rats were monitored. Results: Treatment that included RFA resulted in significantly ($p=0.003$) more deaths than sham-operated rats. Including celecoxib in the treatment resulted in significantly increased cutaneous wound abscess formation after surgery ($p<0.0001$). In addition, the combination of celecoxib treatment with RFA resulted in intra-abdominal abscess formation ($p<0.0001$). Conclusion: The results indicated that the combination of high-dose celecoxib and RFA for treating liver metastases should be used with caution when applied as an anticancer treatment modality since additional side-effects are induced.*

Epidemiological studies have indicated that the long-term use of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) reduces the risk of colorectal cancer by 40-50 percent (1). Treatment with cyclooxygenase-2 (COX-2)-specific NSAIDs, such as celecoxib, has been shown to reduce polyp size and number in familial adenomatous polyposis patients with a predisposition to colorectal adenoma and cancer (2). The use of selective inhibitors in combination with additional treatment modalities is being investigated in clinical trials in colorectal cancer patients. Patients with metastases of their colorectal cancer confined to the liver can benefit from radio frequency ablation (RFA) if the metastases are not surgically resectable (3). Peri-operative treatment with selective COX-2 inhibitors was associated with impaired wound healing and anastomotic failure in animal models (4), but their effects in combination with RFA are unknown.

The aim of this *in vivo* study was to evaluate the effects and safety of celecoxib, a COX-2-specific NSAID, treatment in combination with RFA in an animal model. To this end, an *in vivo* colorectal cancer liver metastasis rat model and the syngenic CC531 cell line (5) were used.

Materials and Methods

The principles of laboratory animal care according to Dutch law were followed. The Animal Welfare Committee of the Leiden University Medical Center, The Netherlands, approved the study.

Forty Wag/Rij rats (Charles River, Zeist, The Netherlands) were inoculated with CC531 tumor cells subcapsularly at two sites in the liver, as previously described (6), and ten rats each were randomly assigned either to celecoxib treatment, starting on the day of tumor inoculation, or to no celecoxib treatment. The celecoxib was mixed into standard chow at a concentration of 1500 mg per kg chow. In addition, ten rats per group were randomly assigned to treatment with RFA or a sham operation. RFA was performed on one of the two tumors in each rat on day 21 after tumor induction. This resulted in four treatment groups: 1, RFA + celecoxib; 2, sham + celecoxib; 3, RFA; 4, sham. RFA was applied using a RITA 1500x RF generator (RITA Medical Systems, Mountainview, CA, USA). A 2-cm expandable needle was inserted in the right tumor in the upper liver lobe and expanded to 1.5 cm, resulting in a lesion of 1.5 cm in diameter. The power output was set at 90 W and when a temperature of 90˚C had been reached, a 2-minute ablation was performed. The tumor in the left liver lobe remained untreated as a negative control. All the rats were operated on under semi-sterile conditions. Sham operated animals were subjected to the same surgical procedures without performing RFA. The weight and general health of the rats

Abbreviations: RFA, radio frequency ablation; COX-2, Cyclooxygenase-2.

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Key Words: Celecoxib, colorectal cancer, radio frequency ablation, rat model, cyclooxygenase-2.

Table I. *Postoperative events registered in rats randomized for liver tumor radio frequency ablation (RFA) or a sham operation, 3 weeks after induction of liver tumors, as described in Materials and Methods. The rats had previously been randomized to treatment with celecoxib 1500 mg/kg, which started immediately after tumor induction, or to no treatment. None of the rats had died before the RFA or sham treatment. Cutaneous and intra-abdominal abscesses were also present in the rats that died following the RFA treatment.*

Event of side-effects	Rats per treatment group (no. of rats showing event/ total no. of rats)				Analysis of event occurence	
	1. RFA + celecoxib	2. sham + celecoxib	3. RFA	4. sham	*p*-value	Likelihood ratio
post-RFA/sham deaths	5/10	0/10	3/10	0/10	0.003 (RFA *vs.* sham) 0.01 (RFA + celecoxib *vs.* RFA)	13.1 13.95
rats with cutaneous abscesses	10/10	10/10	0/10	0/10	<0.0001 (celecoxib *vs.* no celecoxib)	55.5
rats with intra-abdominal abscesses	10/10	0/10	0/10	0/10	<0.0001 (RFA + celecoxib *vs.* any other treatment)	17.3

were monitored three times a week until sacrifice, under general anesthesia on day 40. The data were entered into a statistical database (SPSS® version 12·0; SPSS, Chicago, IL, USA). Descriptive statistics were used for the operative outcomes. The Fisher's exact test was used to test categorical variables. A p-value of <0.05 was considered statistically significant.

Results

All the rats were operated on twice. During the first operation, tumors were inoculated subcapsularly in two separate liver lobes. Rats that had been randomized for celecoxib treatment received the drug in their chow at a dose of 1500 mg per kg chow. This dose level was the highest used in a study by Reddy *et al.* and had provided optimal inhibition of tumor growth (7). Treatment with RFA or sham operation was performed 3 weeks after tumor induction. The weights of the rats were followed during the entire study and did not significantly differ among the treatment groups ($p=0.76$). The clinical outcome of both operations are summarized in Table I. No deaths occurred after tumor inoculation. Eight rats (20%) died after the second operation, of which five (12.5%) had received celecoxib. Treatment with RFA resulted in significantly more deaths (8 rats, 20%) as compared to the sham operation, regardless of celecoxib treatment (0 rats) ($p=0.003$, likelihood ratio: 13.1). There were significantly more deaths in the RFA-treated rats in the celecoxib groups than in the groups that did not receive celecoxib ($p=0.01$, likelihood ratio: 13.95). Rats in the celecoxib groups developed intra-abdominal abscesses after RFA treatment ($p<0.0001$, likelihood ratio: 17.3) (Figure 1, Table I). Significantly more rats in the celecoxib groups compared to the groups that did not receive celecoxib developed cutaneous abscesses after surgery ($p<0.0001$, likelihood ratio: 55.5) (Figure 2, Table I).

Discussion

The use of COX-2 inhibitors in the treatment of inflammation and pain has increased in recent years, since these compounds are not expected to show many of the complications associated with regular NSAIDs (8). Furthermore, they showed a potential benefit in the prevention and treatment of various malignancies (9). Clinical studies have employed the use of high-dose COX-2 inhibitors in combination with other treatment modalities, since COX-2 inhibition could potentially enhance the effectiveness of such treatment, minimize side-effects and decrease the development of postoperative micrometastases (9). However, recent studies have indicated that COX-2 inhibitors affect physiological processes at several levels, causing an increase in cardiovascular complications at high doses and affecting wound and anastomosis healing (4, 10). In this study, treatment with celecoxib was associated with an increase in deaths after RFA treatment. Most notable was the formation of cutaneous abscesses after surgery in all the rats receiving celecoxib treatment and additional intra-abdominal abscess formation in the rats that had received celecoxib treatment in combination with RFA. RFA treatment has been associated with a complication rate of approximately 9%, including hepatic abscess formation (11). The addition of celecoxib here increased the incidence of complications following RFA treatment. Selective COX-2 inhibitors have anti-inflammatory and anti-angiogenic properties (9). Impairment of the neovasculature of the postoperative skin wounds and RFA lesions, in combination with the suppression of an adequate immune response against opportunistic infections, could have been responsible for the side-effects seen in our study (4, 9).

In summary, the use of high-dose celecoxib in combination with surgery, and especially with RFA of

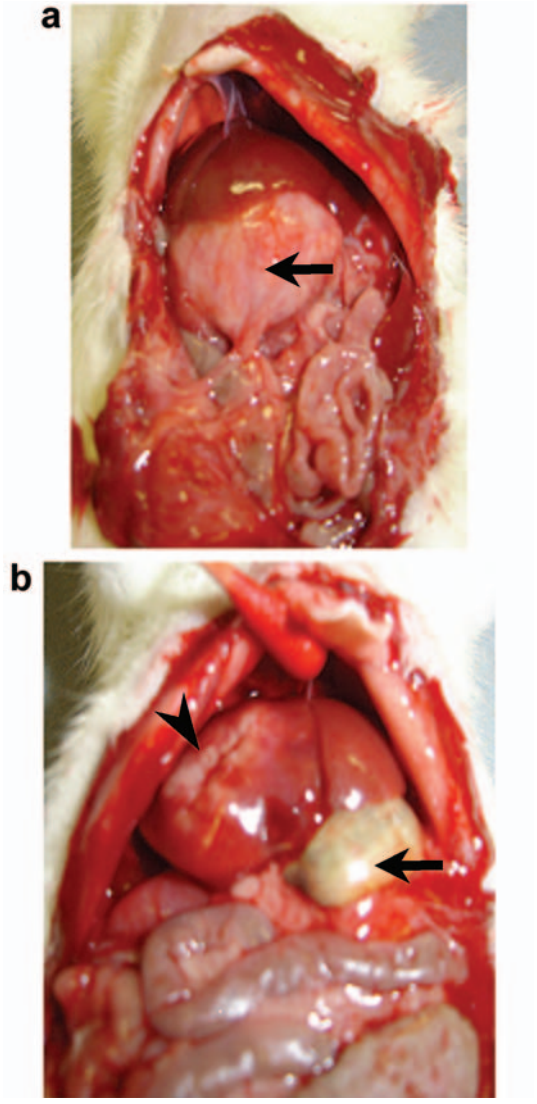

Figure 1. *a) Intra-abdominal abscess covered with omentum after RFA treatment of one of two liver tumors in combination with celecoxib treatment (arrow). b) Rudimentary liver lobe after RFA treatment in a rat in the control group (group 2) (arrow); untreated tumor is visible in upper liver lobe (arrowhead).*

tumors, should be treated with great caution due to an increased risk of additional side-effects.

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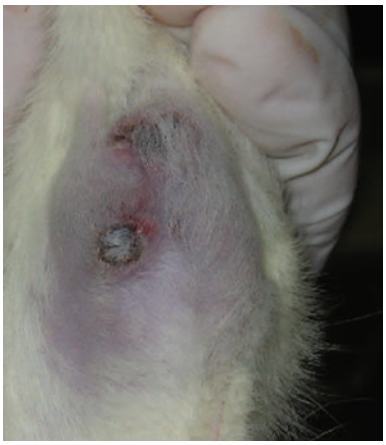

Figure 2. *Presence of postoperative cutaneous abscesses in a rat that received celecoxib treatment.*

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Received January 30, 2006
Accepted March 22, 2006