# Surgical Stress and Accelerated Tumor Growth

HENK B. KAL[1], HENK STRUIKMANS[2] and ANGELIQUE D. BARTEN-VAN RIJBROEK[1]

[1]*Department of Radiation Oncology, University Medical Center, Utrecht;*
[2]*Department of Radiotherapy, Leiden University Medical Center, Leiden, The Netherlands*

**Abstract.** *Background: Delay in the initiation of radiotherapy after surgery is associated with an increase in local regional recurrence. A possible mechanism might be that remaining tumor cells proliferate significantly faster as a result of induced angiogenic cytokines. The growth rate of tumors arising from the inoculation of L44 tumor cells in the wound bed after surgical removal of L44 tumors was determined. Materials and Methods: L44 tumors growing in the flank of female BN rats were surgically removed. In the wound bed, 5x10$^6$ L44 cells, harvested from the in vitro cell line, were injected. L44 cells were also injected in the contralateral flank and in control rats with and without surgical intervention. Tumor volumes as a function of time after injection of cells were recorded. From the attained volume at day 7, the cell doubling time was calculated, assuming 10$^9$ cells per cm$^3$. Results: Tumors arising in the wound bed had the fastest growth rate as compared to the tumors in the contralateral flank or tumors in control rats with or without surgical intervention. Conclusion: The results clearly indicate accelerated tumor growth after surgical stress. This indicates that delay in the initiation of radiotherapy after surgery with tumor cells remaining, results in a larger tumor burden and hence a higher probability of local recurrence.*

Local recurrence of cancer after surgery is a great concern in cancer management. Several reports revealed the importance of the time interval between surgery and radiotherapy in patients with breast cancers and head and neck tumors. A possible mechanism might be that remaining tumor cells proliferate significantly faster as a result of induced angiogenic cytokines (1-4).

In experimental animals, increased tumor growth after laparotomy *versus* laparoscopy or pneumoperitoneum was observed (5-7). Surgical stress promoted lung tumor metastasis (8). Connective tissue growth factor expression together with transforming growth factor-beta and platelet-derived growth factor are up-regulated in wound healing (9, 10). These observations led to the assumption that growth factors, induced postoperatively, cause remaining tumor cells to proliferate faster.

In this study, a rat tumor model was used to determine the growth rate of tumors arising from the inoculation of L44 tumor cells in the wound bed after surgical removal of L44 tumors and of tumors arising in the contralateral flank of the same rats after L44 cell inoculation. In addition, the growth rate was measured of tumors arising after cell inoculation in control animals with or without surgical intervention.

*Correspondence to:* H.B. Kal, Ph.D., University Medical Center, Department of Radiation Oncology, Q00.118, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. Tel: +31 88 7558800, Fax: +31 88 7555850, e-mail: H.B.Kal@umcutrecht.nl

*Key Words:* Surgical stress, growth rate, experimental lung tumor.

## Materials and Methods

*Tumors.* The L44 tumor is a radiation-induced undifferentiated carcinoma, originally diagnosed as an adenosquamous lung carcinoma, and grows in female BN(Orl)Ico rats, (Charles River, Maastricht, the Netherlands) with a tumor volume-doubling time of about 4 days (11, 12). Tumors were serially propagated in the flank of syngeneic animals. Tumor volumes (V) were measured twice per week with callipers and were based on two orthogonal cross-sectional diameter measurements using $V=0.5a^2b$, with a being the smallest diameter. The weight of the animals at an age of ~12 weeks at the start of the treatments was about 170 g. The number of rats used per treatment group was 2-3. The animals were housed in Macrolon type IV cages, 4 per cage, with free access to tap drinking water and chow. Cage enrichment was applied in the form of plastic tunnels. The room temperature was 20-24˚C and the air humidity 40-60%. The day/night cycle was 12/12 with lights on at 7AM.

L44 tumor cells were also grown *in vitro* in Minimal Essential Medium (MEM) with 10% fetal calf serum (FCS) supplemented with L-glutamine and gentamicin. The cell doubling time was about 24 h.

*Surgical stress.* Tumors were allowed to grow to a volume of ~2 cm$^3$ and were then surgically removed. The skin was closed with surgical clips. 5x10$^6$ L44 tumor cells in 0.5 ml medium were inoculated into the wound bed and the contralateral flank. In the flank of surgical control animals, a 3-cm incision in the skin was made and the skin was detached from the underlying tissues. After closing of the skin with surgical clips, 5x10$^6$ cells were inoculated into the wound bed and the contralateral flank. In addition, L44 cells were inoculated into control animals without surgical intervention. Pain relief was obtained with injections of Temgesic on days 1 and 2.

Tumor volumes were determined up to ~8 to 12 days after the inoculation of L44 tumor cells.

*Cell doubling time.* The cell doubling time, Td(h), was calculated as $Td(h)=24xTxln2/ln(10^6xV_7/N_0)$ where T was the time between inoculation of cells and measuring the tumor volume, $V_7(mm^3)$ at day seven after inoculation, T=7d; $N_0$ was the number of cells inoculated at T=0 ($N_0=5x10^6$). It was assumed that 1 $mm^3$ of tumor volume contained $10^6$ cells.

*Approval.* The Animal Experiments Ethical Committee of the University Medical Center approved the experiments.

## Results

After inoculation of $5x10^6$ cells into the wound bed of the surgically removed tumor, rapid proliferation was observed. One week after inoculation, a mean tumor volume of 8,710 $mm^3$ was found. The calculated cell doubling time Td in that period was 16.49 h. In the contralateral flank of operated animals, cell proliferation was slower than at the operated site: a mean tumor volume of 999 $mm^3$ at day 7 was found. The Td of tumor cells in the period of seven days was 22.16 h.

After inoculation of cells in the wound bed of sham-operated animals, a mean tumor volume of 1,129 $mm^3$ was found at day 7 after inoculation. The Td in sham-operated animals in that period was 21.96 h. In the contralateral site in the same animals, tumor growth was slower than at the sham-operated site: the attained tumor volume at day 7 was only 474 $mm^3$ with a Td of 26.26 h.

In control animals, the mean tumor volume at day seven after inoculation was 305 $mm^3$ with a Td of 29.82 h.

Table I summarizes the tumor volumes attained at day seven after inoculation of $5x10^6$ cells, and the cell doubling times, as well as the number of rats. Examples of growth curves of tumors, with a growth pattern close to that of the mean tumor volumes, are shown in Figure 1. It is clearly shown that the largest tumor volumes were seen for the wound sites. Tumors arising in the non-wounded control animals had the longest lag time. In Figures 2 and 3, the volumes attained at day 7 and the cell doubling times with the standard deviations, respectively, are shown.

Table I. *Mean tumor volumes attained at day 7 after inoculation of $5x10^6$ cells into the tumor bed (surgery site) and the contralateral site in the same animal, at the operated site (control surgery) and contralateral site in control animals, and the calculated mean cell doubling time Td.*

| Tumor at | n | Volume ($mm^3$) at day 7 | Td (h) |
|---|---|---|---|
| Surgery site | 9 | 8710±5637 | 16.49±2.58 |
| Contralateral | 8 | 999±248 | 22.16±1.21 |
| Control surgery | 6 | 1129±411 | 21.96±2.22 |
| Contralateral | 7 | 474±114 | 26.26±2.49 |
| Control | 14 | 305±174 | 29.82±4.09 |

Values are means of n tumors±standard deviation.

## Discussion

In wound repair, cells that ordinarily divide infrequently are induced to proliferate rapidly, extracellular matrix and connective tissues are invaded and remodelled, epithelial cells and stromal cells migrate, and new blood vessels are recruited. A wound response would appear to provide a highly favourable milieu for cancer progression (13, 14).

In mice, increased tumor establishment and growth after laparotomy *versus* laparoscopy or pneumoperitoneum was found (5). Shiromizu *et al.*, using a murine model, determined that laparotomy accelerated tumor metastasis to the lung and that laparoscopy did not increase the frequency or growth of metastasis (6). The laparoscopic approach may suppress hematogenous metastasis to the lung because of reduced surgical stress and reduced cytokine response. In addition, Southall *et al.* showed that both colon-26 adenocarcinoma and B-16 melanoma tumors in a murine model grew larger after laparotomy than after pneumoperitoneum (7). Balb/c mice, after injection with colon-26-L5 carcinoma cells, were subjected to several degrees of surgical manipulation. Increased surgical stress augmented cancer metastasis *via* surgical stress-induced expression of proteinases in the target organ of metastasis (8). Inkinen *et al.* demonstrated that connective tissue growth factor (CTGF) expression together with transforming growth factor-beta (TGF-beta) and platelet-derived growth factor (PDGF) are up-regulated in wound healing (9). Increased PDGF released after laparotomy stimulated systemic growth of colon-26 carcinoma in Balb/c mice (10).

In patients with breast, and head and neck cancer, the importance of the time interval between surgery and radiotherapy was evaluated. Slotman *et al.* reported that a delay of radiotherapy after breast conserving surgery of more than 7 weeks could adversely affect the local tumor control rate (1). Trotti *et al.* concluded that their data on high-risk head and neck tumors support the notion that microscopic tumor cell aggregates escaping surgical excision repopulate rather quickly before treatment completion (2). For advanced head and neck cancer to gain a full benefit from treatment acceleration, the surgery-radiotherapy gap and the overall treatment time should not exceed 6 and 10 weeks, respectively (3). Connolly *et al.* compared growth rates of primary cancer and prostatic fossa recurrence after radical prostatectomy. They concluded that tumors that recur locally after prostatectomy appear to have a higher proliferative rate compared to the parent tumors (4).

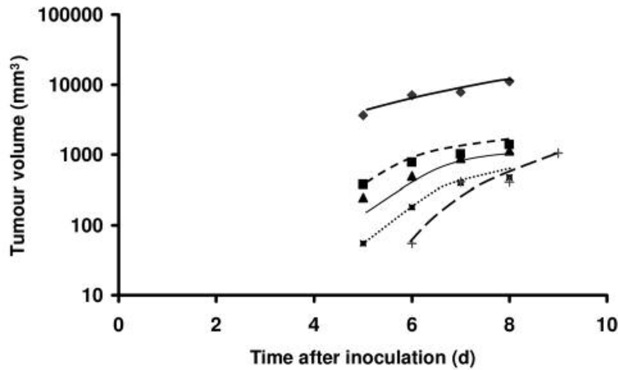


Figure 1. *Mean tumor volumes as a function of time after cell inoculation at day 0. Cells inoculated into the tumor bed (surgery site), ◆, and the contralateral site in the same animal, ■; cells inoculated into the operated site (control surgery), ▲, and the contralateral site, x; cells inoculated into control animals, +.*

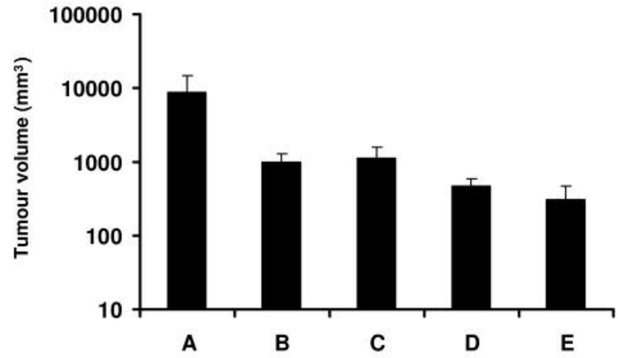


Figure 2. *Mean tumor volumes attained at day 7 (± standard deviation) after cell inoculation. Cells inoculated into the tumor bed (surgery site), A, and the contralateral site in the same animal, B; cells inoculated into the operated site (control surgery), C, and the contralateral site, D; cells inoculated into control animals, E.*

In the present experiments, we were able to show that tumor cells inoculated in the wound bed of animals from which the tumor was excised had a faster growth rate than cells inoculated in the lateral site, 16.5 h *versus* 22.2 h. The wounding conditions made surgically promoted tumor growth. The same phenomenon was observed in control animals in which an incision was made and in which cells were inoculated *versus* the contralateral site, 22.0 h *versus* 26.3 h. In control animals, the growth rate was the slowest, almost double of that of the cells inoculated in the tumor bed, 29.8 h *versus* 16.5 h. The tumor mass in control animals appeared later than in the wounded rats.

The faster growth rate observed for tumor cells in the wound bed is probably due to the abundance of cytokines and growth factors at the site of the excised tumor. In addition, the wound induces an inflammatory reaction, recruiting leukocytes. These cells and growth factors have a growth-promoting effect on tumor cells (13, 14). Circulating cytokines and growth factors also enable inoculated cells in the contralateral flank to grow faster, although slower than cells in the wound bed. In the sham-operated rats, the incision in the skin and detaching the skin from the underlying tissues induced growth factors for healing the wound, also enabling the cells in the wound bed to grow faster than cells in control animals. The induced growth factors may also have stimulated cell growth in the contralateral site of the same animals. In control animals without wounding conditions, growth factors must be induced by the tumor cells themselves which takes time, and therefore the calculated doubling time is relatively high.

In conclusion, both experimental and clinical data indicate that surgical stress promotes growth of inoculated or remaining tumor cells. It indicates that a delay in the initiation of radiotherapy after surgery may result in a larger tumor burden and hence a higher probability of local recurrence.

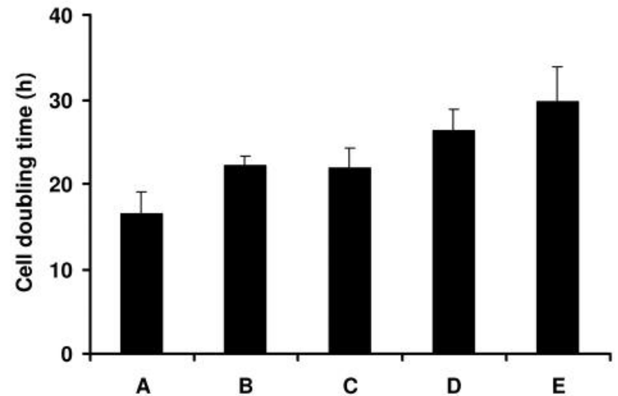


Figure 3. *The calculated mean cell doubling times (± standard deviation) after cell inoculation. Cells inoculated into the tumor bed (surgery site), A, and the contralateral site in the same animal, B; cells inoculated into the operated site (control surgery), C, and contralateral site, D; cells inoculated into control animals, E.*

## References

1 Slotman BJ, Meyer OW, Njo KH and Karim AB: Radiotherapie na mammasparende operatie: na langer uitstel meer lokaal recidief. [Radiotherapy following breast-conserving surgery: more local recurrences after longer delay]. Ned Tijdschr Geneeskd *139*: 2427-2432, 1995.

2 Trotti A, Klotch D, Endicott J, Ridley M and Cantor A: Postoperative accelerated radiotherapy in high-risk squamous cell carcinoma of the head and neck: long-term results of a prospective trial. Head Neck *20*: 119-123, 1998.

3 Awwad HK, Lotayef M, Shouman T, Begg AC, Wilson G, Bentzen SM, Abd El-Moneim H and Eissa S: Accelerated hyperfractionation (AHF) compared to conventional fractionation (CF) in the postoperative radiotherapy of locally advanced head and neck cancer: influence of proliferation. Br J Cancer *86*: 517-523, 2002.
4 Connolly JA, Presti JC Jr, Cher ML, Chew K, Shinohara K and Carroll PR: Accelerated tumor proliferation rates in locally recurrent prostate cancer after radical prostatectomy. J Urol *158*: 515-518, 1997.
5 Allendorf JD, Bessler M, Kayton ML, Oesterling SD, Treat MR, Nowygrod R and Whelan RL: Increased tumor establishment and growth after laparotomy vs laparoscopy in a murine model. Arch Surg *130*: 649-653, 1995.
6 Shiromizu A, Suematsu T, Yamaguchi K, Shiraishi N, Adachi Y and Kitano S: Effect of laparotomy and laparoscopy on the establishment of lung metastasis in a murine model. Surgery *128*: 799-805, 2000.
7 Southall JC, Lee SW, Allendorf JD, Bessler M and Whelan RL: Colon adenocarcinoma and B-16 melanoma grow larger following laparotomy *vs.* pneumoperitoneum in a murine model. Dis Colon Rectum *41*: 564-569, 1998.
8 Tsuchiya Y, Sawada S, Yoshioka I, Ohashi Y, Matsuo M, Harimaya Y, Tsukada K and Saiki I: Increased surgical stress promotes tumor metastasis. Surgery *133*: 547-555, 2003.
9 Inkinen K, Wolff H, Lindroos P and Ahonen J: Connective tissue growth factor and its correlation to other growth factors in experimental granulation tissue. Connect Tissue Res *44*: 19-29, 2003.
10 Lee SW, Gleason NR, Stapleton GS, Zhai C, Huang EH, Bessler M and Whelan RL: Increased platelet-derived growth factor (PDGF) release after laparotomy stimulates systemic tumor growth in mice. Surg Endosc *15*: 981-985, 2001.
11 Kal HB, Meijnders PJ, van Berkel AH and Van Bekkum DW: Response to chemotherapy of non-small cell bronchial rat tumours growing subcutaneously or in the lung. In Vivo *5*: 301-306, 1991.
12 Kal HB, van Berkel AH and Goedoen HH: Growth rate, morphology and drug responses of rat lung tumours growing at different sites. Anticancer Res *14*: 495-499, 1994.
13 Hofer SO, Molema G, Hermens RA, Wanebo HJ, Reichner JS and Hoekstra HJ: The effect of surgical wounding on tumour development. Eur J Surg Oncol *25*: 231-243, 1999.
14 Chang HY, Sneddon JB, Alizadeh AA, Sood R, West RB, Montgomery K, Chi JT, van de Rijn M, Botstein D and Brown PO: Gene expression signature of fibroblast serum response predicts human cancer progression: similarities between tumors and wounds. PLoS Biol *2*: 206-214, 2004.

*Received October 29, 2007*
*Revised December 19, 2007*
*Accepted January 25, 2008*