The Tumoricidal Activity of Tumor Necrosis Factor-alpha – The Antitumor Effect of Inducible Nitric Oxide Synthase and The Protective Role of Endothelial Nitric Oxide Synthase

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Introduction. The mechanism of the antitumor effect of tumor necrosis factor-alpha (TNF) is not fully understood. Using a mouse model with TNF-responsive (MethA) and non-responsive (LL2) tumors we have previously demonstrated a correlation between tumor nitric oxide (NO) levels after TNF and response to TNF. The present study uses selective inhibition of nitric oxide synthase (NOS) to further evaluate the role of NO in the antitumor response to TNF.

Methods. Balb/C mice were tumored s.c. with 1 x 10^6 MethA cells and C57BL/6 mice were tumored similarly with LL2 cells. When the tumors reached 10 mm in maximum dimension the mice were treated with 15 μg i.v. TNF (Knoll Pharmaceuticals, Whippany, NJ). Mice were placed into three groups. One group received no NOS inhibitor. Another group received TNF and the non-selective NOS inhibitor L-NAME (Alexis Corporation, San Diego, CA) 10 mg/kg i.p. which inhibits both inducible NOS (iNOS) and endothelial NOS (eNOS). The last group received TNF and the selective iNOS inhibitor 1400W (Alexis Corporation, San Diego, CA) 7-10 mg/kg s.c. Tumor NO was measured continuously from 15 min before TNF to 1.5-4.5 hr after TNF using a 10 micron electrochemical microsensor. Tumors were measured in two dimensions and tumor weight was calculated by the formula, $w=0.5ab^2-0.5cd^2$, where w=weight (mg), a=tumor length (mm), b=tumor width (mm), c=ulcer/eschar length (mm), d=ulcer/eschar width (mm).

Results. When TNF was given without a NOS inhibitor, all of the MethA tumors (n=7) ulcerated with a mean 62% decrease in tumor weight by day 4; however, 8 of 8 LL2 tumors continued to grow with a mean tumor weight of 173% of baseline by day 4. The NO in the MethA tumors without NOS inhibitor rose from a mean baseline of 1.15 ± 0.05 μM to 1.85 ± 0.20 μM at 15-30 min ($p<0.05$) and 1.98 ± 0.16 μM at 60-75 min ($p<0.05$). The mean NO level in LL2 was 1.60 ± 0.31 μM at baseline and did not change significantly after TNF. When TNF + L-NAME was given to the MethA group, 9 of 15 mice died within 12 hr, 4 tumors ulcerated (mean 90% decrease in tumor weight by day 6), and 2 continued to grow (tumor weight doubled by day 6-7). The control group (TNF i.v. + PBS s.c., n=3) all ulcerated. Four mice from this treatment group underwent NO measurement. The NO decreased from a mean baseline of 1.44 ± 0.15 to 0.43 ± 0.13 at 45-60 min and remained below baseline from 0-90 min. Eight LL2 mice were treated with TNF + L-NAME. The addition of L-NAME resulted in significant ulceration of these tumors (mean 56% decrease in tumor weight by day 5). The control group (BSA i.v. + L-NAME s.c., n=2) continued to grow and were 192% of baseline weight by day 5. Four MethA mice were treated with TNF + 1400W. One mouse died within 24 hrs, 1 tumor ulcerated and 2 exhibited no ulceration and were unchanged from baseline; whereas, 5 of 5 controls (TNF + PBS i.p.) ulcerated (mean 49% decrease in tumor weight by day 3).

Conclusions. A pathologic rise in NO levels in MethA tumors after TNF correlates with response to TNF, and absence of a rise in NO levels in LL2 tumors correlates with lack of response to TNF. The prevention of ulceration in the MethA tumors after TNF plus 1400W suggests that iNOS plays a pathologic role in the antitumor effect of TNF, since iNOS inhibition prevented tumor ulceration. Administration of TNF and L-NAME in the MethA tumors resulted in a high mortality rate and persistence of ulceration, despite documented suppression of NO levels. Additionally, TNF and L-NAME caused the previously non-responsive LL2 tumors to ulcerate. These findings suggest that eNOS is protective for the tumor after TNF, since inhibition of eNOS causes ulceration in either tumor even when the pathologic NO levels are suppressed. Thus, it appears that manipulation of tumor iNOS and eNOS activity affects the outcome of the tumor after TNF.

ABSTRACT

Local Excision and Chemoradiation for Low Rectal T1 and T2 Cancers Is an Effective Treatment

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BACKGROUND: Lesions located in the distal third of the rectum are usually treated with abdominoperineal resection (APR) or a low anterior resection with a colo-anal anastomosis. However, in a select group of patients with favorable histology and a low probability of lymphatic spread, sphincter sparing procedures will afford long term disease-free survival and cure without the need for extensive, complicated surgery.

METHODS: A 10-year retrospective review was performed, including pathologic examination of specimens by a single pathologist, in an attempt to identify factors associated with a decreased disease-free survival.

RESULTS: Thirty-five patients (median age 71y, range 48-88) with low rectal carcinomas were treated with full-thickness disc excision (+/- chemoradiation) with curative intent. Median follow-up was 46 months (range, 8-120). There were 15 T1, 16 T2, and 4 T3 lesions. Tumors with poor histologic factors or greater than T1 received adjuvant radiation (+/- 5-FU). Four patients developed a local failure at a median of 21.5 months (range, 9-30) and were salvaged with APR. The five year cancer-specific survival was 91%. Negative margins approached statistical significance ($p<0.07$) in influencing local control.

CONCLUSIONS: When combined with chemoradiation for lesions deeper than submucosa or with adverse histologic factors, local resection of rectal cancer is an effective treatment in selected patients.

CATHETER DIRECTED THROMBOLYSIS FOR ILIOFEMORAL DVT IMPROVES HEALTH RELATED QUALITY OF LIFE

OBJECTIVES: Treatment designed to eliminate thrombus in patients with iliofemoral deep venous thrombosis (DVT) is theoretically attractive, however it's definitive benefits compared to anticoagulation have not been demonstrated. Although not previously analyzed, an effective measure of treatment success is likely to be the assessment of health related quality of life (HRQOL). The objectives of this study are to evaluate whether catheter-directed thrombolysis for iliofemoral DVT is associated with improved HRQOL compared to standard anticoagulation, and whether HRQOL outcome in the thrombolysis group is related to lytic success.

METHODS: An 80 item self administered HRQOL questionnaire was developed which contained the Health Utilities Index, Short Form-12 and disease-targeted scales including health distress, stigma, health interference, physical functioning and symptoms (e.g. leg swelling, pain, ulcers). Psychometric testing confirmed that the HRQOL questionnaire is reliable and valid. Questionnaires were administered to 98 patients with iliofemoral DVT treated at least 6 months earlier. 68 were identified through a prospective DVT registry and treated with catheter-directed thrombolysis, and 30 identified by medical record review were treated with anticoagulation alone.

RESULTS: The majority of patients were female (61%), Caucasian (95%), married (65%) with a mean time interval since initial DVT of 16 months. The lytic group was younger (53 ± 17 years) than the heparin group (61 ± 17 years) $P<0.05$. Following treatment, lytic patients reported better overall physical functioning ($P=0.046$), less stigma ($P=0.033$), less health distress ($P=0.022$) and fewer post-thrombotic symptoms ($P=0.006$) compared to anticoagulation alone. Within the lytic group, phlebographically successful lysis correlated with improved HRQOL ($P=0.038$). Lytic failures and heparin treatment outcomes were similar.

CONCLUSIONS: Patients with iliofemoral DVT treated with catheter-directed thrombolysis report better functioning and well-being compared to patients treated with anticoagulation alone. Successful lysis is directly linked to improved HRQOL, with lytic failures having similar outcomes to heparin treated patients. Future randomized trials comparing treatments for DVT should include an HRQOL measure as part of their outcome analysis.

ABSTRACT

GENETIC RISK FACTORS REDUCED NODE POSITIVE
COLON RESECTIONS BY 50%

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Background: 10-20 years ago 67-70% of colon resections for carcinoma had positive nodes but in the last five years there has been significant improvement.

Method: 670 Colon resections in three hospitals were reviewed for years 1994-95-96 and the incidence of positive nodes was recorded. The incidence of synchronous and metachronous adenomas in a personal series of 432 patients was recorded. The incidence of colorectal Ca in first degree relatives in the same group of patients was recorded.

Results: There was a 50% reduction in node positive colon resections in the three hospitals. Synchronous and metachronous adenomas were found to be 40% and 70%. Colorectal Ca was found in a first degree relative in 30% of patients with Colorectal Ca, Villous Adenoma and Tubular adenoma.

Conclusion: Genetic risk factors have identified patients at increased risk who need colonoscopy for early detection which is the key to improved survival. A family history of cancer is essential for early detection in colorectal cancer.

TNF- α Mediates Nicotine Induced Growth Retardation In Human Umbilical Vein Endothelial Cell Growth In Culture

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Purpose: Intimal injury leading to the formation of atherosclerosis is a multifactorial process. The relationship between nicotine use and vascular disease is well established but poorly understood. Our laboratory previously established that nicotine is toxic to human umbilical vein endothelial cells (HUVEC) at low concentrations (10^{-10} mM). We postulated that this toxicity is cytokine mediated and proposed blocking this phenomenon with monoclonal antibodies. We chose TNF-α to determine its role in vascular endothelial injury.

Methods: Human umbilical vein endothelial cells (HUVEC, ATCC, Rockville, MD) were plated in 12-well plates in endothelial growth medium (EGM-2, Clonetics Inc., San Diego, CA) at $2x10^3$ cells/cm^2. After 48 hours, fresh media was added to control wells. Free base nicotine (Sigma, St. Louis, MO) was diluted in media to 10^{-9} mM which was previously determined to result in statistically significant growth retardation in HUVEC cells. Test media was added to wells with and without 0.9μg/ml anti-TNF-α (Sigma, St. Louis, MO) on T_0 day. Cell counts were performed in triplicate on days T_{2-5} utilizing standard hemocytometry. Data was analyzed using Student's t-test, with a 95% confidence interval.

Results: Cell growth was significantly decreased in wells exposed to nicotine when compared to control on days T_2-T_5 ($p<0.05$). In cells exposed to anti-TNF-α and nicotine there was inhibition of the growth retardation seen in the cells containing nicotine alone. Table I depicts mean cell counts for each group. Differences between the control group and the anti-TNF-α group were not statistically significant.

Growth day	**T2**	**T3**	**T4**	**T5**
HUVEC (10^4 cells) Control	**3.82**	**14.9**	**24.0**	**28.2**
HUVEC (10^4 cells) + Nicotine (10^{-9}mM)	**2.30§**	**13.3§**	**16.5§**	**21.6§**
HUVEC (10^4 cells) + Nicotine (10^{-9}mM) + anti-TNF-α (0.9μg/ml)	**5.78†**	**15.6†**	**23.8†**	**26.9†**

§ p≤0.05 compared to control

† p= NS compared to control

Conclusion: Our data suggests endothelial cell growth retardation as a consequence of nicotine exposure is TNF-α mediated. These results will direct our future efforts in determining response of endothelial cells and their influence of arterial smooth muscle cells in the development of vascular disease.

PANCREATIC CARCINOMA DEEMED UNRESECTABLE AT EXPLORATION MAY BE RESECTED FOR CURE: AN INSTITUTIONAL EXPERIENCE

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Only a minority of patients with a diagnosis of pancreatic adenocarcinoma (PA) has disease amenable to curative resection. Between April 1987 and March 1999, 40 patients with PA deemed unresectable at exploration at other institutions were considered for neoadjuvant treatments and then re-evaluated for possible re-exploration. We retrospectively compared the clinical outcomes, including overall survival (OS) between three groups: 1) Group A: 22 previously unresectable patients who were subsequently successfully resected, 20 after induction therapy, 2) Group B: 31 patients who received preoperative chemoradiotherapy prior to their only operation, and 3) Group C: 33 patients who were primarily resected, 27 of which were then treated with adjuvant therapy.

Of those resectable from Group A, five required portal venorraphy and three had hepatic artery reconstruction. Eighteen of the 40 patients were unresectable due to progression of disease with a mean OS of eight months: 12 were assessed at second laparotomy; six were excluded from second operation based on preoperative imaging studies. Kaplan–Meier curves showed no differences in OS between the three groups: A=34 months (mo), B=21 mo and C=13 mo, respectively (p=0.15). Margin status is comparable in all three groups (p=0.52). As expected, nodal positivity was greatest in Group C (p=0.001). There were no operative mortalities in Group A and the morbidity rate was comparable to that of Groups B and C.

Upon re-evaluation, many tumors (54%) previously deemed "unresectable" were surgically extirpated for cure with a median survival comparable to patients who did not undergo previous exploration.

PANCREATIC RESECTION WITH INTRAOPERATIVE RADIATION THERAPY AND POSTOPERATIVE ADJUVANT CHEMORADIATION

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Pancreatic cancer remains one of the most lethal gastrointestinal malignancies. Five year survival is reported in less than 25% of resected patients. Postoperative chemotherapy and radiation have been shown to increase median survival following resection. Intraoperative radiation therapy (IORT) allows for more precise and effective radiation dosage while potentially reducing toxicity to surrounding tissues. Postoperative morbidity and mortality, patterns of recurrence, and survival were retrospectively reviewed in 29 consecutive patients with ductal adenocarcinoma of the pancreas, who underwent pancreatic resection with IORT and were enrolled in a program of postoperative adjuvant chemoradiation from January of 1989 through July of 1998. Twenty-two patients underwent pancreaticoduodenectomy. Four patients underwent distal pancreatectomy and three were treated by total pancreatectomy. Perioperative morbidity was observed in 12 patients (41%). There were no perioperative mortalities. Median follow-up for the entire group was 45 months. Overall median survival was 23 months. Local recurrence was noted in 17% of patients. Twenty-one (72.4%) of patients completed a postoperative course of 5-Fluourocil and radiation. Median and two year survival was 25 months and 54% respectively. IORT is a safe adjunct to pancreatic resection and when combined with postoperative therapy, improves local control and median survival.