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Experimental basis for the use of pre-operative irradiation to prevent growth of tumor cells disseminated at surgery

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Present day cancer therapy continues to fail in a great many instances because of the development of local recurrent cancer in the operative site. This is usually followed, or associated with, the growth of tumor emboli in distant organs. Repeat studies of invasive head and neck cancers reveal a 40% incidence of local recurrence [1]; and a distant metastasis rate as high as 50% [2]. In a series of 119 radical pelvic surgery cases for carcinoma of the cervix, local recurrence was demonstrated in 35 [3]. A smaller group that came to autopsy had an 80% incidence of metastatic spread above the pelvis.

Numerous hypotheses have been suggested to «explain» this high local recurrence rate. If the primary tumor is incompletely excised, regrowth will take place. The increasing use of more radical surgery in an effort to correct this possible error has not materially changed the overall survival rate. Other factors must also be involved, for local recurrences develop in instances where more than adequate surgical margins can be demonstrated. The multicentric character of some local wound recurrences has suggested that the cancer was «seeded» at the time of surgery, possibly from tumor emboli present in blood or lymph bathing the wound, or deposited in the wound from the surface of ulcerated surface tumors.

The presence of tumor emboli in operative wounds has been well established. A continuing study of washings taken from operative wounds after removal of all cancer and before skin closure revealed a 26% incidence of tumor cell contamination [4, 5]. Other studies have demonstrated cancer cell wound contamination in 10–17% instance, depending upon type of wounds studied. In a recent series of 98 pelvic surgery cases, 23 had positive wound washings and an additional 12 had suspicious washings. Efforts to correlate positive wound washings with prognosis of

the patients gave negative results [1]. However, when the positive wound washings were restudied on the basis of the number of cancer cells washed from the wound, degree of hyperchromasia, nucleolar size, state of cell preservation and staining characteristics of the cancer cell, a positive correlation was observed [6]. In this study local recurrence developed in 89% instances where the cytologist predicted such an occurrence.

Another factor in the relatively poor correlation between positive wound washings and the development of local recurrences is the inability of wound washings to demonstrate all free cancer cells present in a wound. It is recognized that wound washings can only portray the findings at one specific time, i.e., after completion of the excisional surgery and before closure of the wound. The amount of fluid used to wash the wound, type of spray and collection of the washings are also factors which may influence the efficiency of the method. The development of the Millipore filter technique of the study of body fluids for tumor cells has made it possible to study the cytology of large volumes of fluid. When this technique was applied to wound drainage collected via suction catheter placed under skin flaps, cancer cells could be demonstrated in wound drainage as late as 72 hours after surgery. In 7 of 63 patients studied, wound drainage contained tumor cells and wound washings were positive in 12. However, only 3 patients had tumor cells in both wound washings and drainage. Thus the local recurrence rate can be calculated on the basis of 16 contaminated wounds rather than 12. The prognostic value of these combined «positive» results remains to be proven. A preliminary follow-up of the 63 patients revealed local recurrence in 6 of the 16 contaminated wounds, while a similar number of local recurrences was noted in 23 with completely negative washings and drainage.

The other associated cause of failure of cancer therapy is the development of distant metastases. It seems reasonable to assume that these metastatic growths must have been initiated by deposits of circulating cancer cells into distant organs. Certainly not all of these deposits implant, and/or grow. Nor are methods available to demonstrate the true incidence of circulating tumor cells. In 38 of the patients described above in the wound washing study, circulating tumor cells were demonstrated in 7. Efforts to correlate prognosis with the finding of circulating tumor cells is as difficult as with wound washing studies.

These and other studies demonstrate that present day therapy of cancer is frequently associated with operative wound cancer cell contamination, and in many instances with implantation of viable cancer

cells in distant organs. Preliminary studies with local chemotherapy of operative wounds suggest that local drug therapy is of value in preventing local recurrence when the degree of wound contamination is so small that wound washings are negative for tumor cells [5]. When wound contamination can be demonstrated, local chemotherapy did not alter the local recurrence rate.

Local wound therapy has an opportunity to be of value only in those instances in which distant tumor embolization has been limited to the tolerance of the host, or those in whom distant metastases develop from tumor recurrent in the wound. It is suggested that any effective adjunct to surgical treatment must either alter host resistance to cancer so that emboli can be destroyed by natural host-immune factors, or the intact cancer cells must be altered so that those which break off and form tumor emboli will not be able to implant and/or grow.

The ability of ionizing radiation to destroy some cancers has been well documented over the past 20–30 years. The use of radiation as an adjunct to surgical excision has not been established. It seems reasonable to suggest that since radiation can control some cancers, it may also be able to alter cells so that they would not implant and/or grow when disassociated from the parent cancer. This paper reports a series of experimental studies of the use of irradiation to test this hypothesis.

Materials and methods

The observations being reported at this time represent a collection of experimental studies of Dr. *Peter Olch*, Mr. *Richard Eck*, Dr. *Robert Hoye* and the author, performed in the laboratories and clinics of the National Cancer Institute over the past 6 years [8, 9, 10, 11].

Five different transplantable tumor and strain specific mice were used, i.e., 1. Melanoma S91 Cloudman in DBA and CDBA mice; 2. Sarcoma DBA, 49 in DBA mice; 3. Sarcoma T241 Lewis in C57bL mice; 4. Carcinoma DBA, 59 in DBA 2 Jn mice; 5. Lymphoma CD, 5 in BALB/c mice. The tumor cell suspension used for transplant was prepared by passing fresh tumor through a Snell cytosieve. During each experiment a control group was studied concurrently with each treated group. All injections were given through a 27 gauge needle into the tail vein for intravenous studies and into thigh muscles for intramuscular growth. The axillary wounds were made to simulate a radical mastectomy wound and the tumor suspension was deposited into the open wound. The donor tumor was prepared by an intrathigh injection of tumor inoculum. When the tumor in the thigh reached the size of 10–12 mm., radiation was given

to the tumor with a 200 KV x-ray machine so that 341 r per minute could be given. The animal was protected in a lead box with only the tumor-bearing thigh exposed. The amount of x-ray used was varied in the different studies to measure the alteration in the growth curve of "primary tumor". These results have been reported in detail and will not be given here [11]. The radiated tumor was then removed from the donor animal at varying time-intervals after radiation and injected into the strain specific recipient mice, either intravenously, intramuscularly or into an open axillary wound. Tumor growths in control animals and those from irradiated inoculum was measured by: 1. counting the number and size of the lung metastases at a standard time post-inoculation, in the intravenous group; 2. noting time when tumor first became palpable for the axillary and intramuscular groups; and 3. the time to death of the latter groups.

Results

Effect of time interval between irradiation and transplantation

In early studies the interval between the in-vivo tumor radiation and the intravenous inoculation of the irradiated tumor suspension into recipient mice varied from three hours to 20 days. The control animals (those with unirradiated inoculum) had an average of 600 lung tumors per mouse when they were sacrificed three weeks post-inoculation. Tumor inoculum that had been irradiated three hours before transplant with 3000 r produced an average of 14.5 tumors per mouse. There was a gradual decrease in the number of lung tumors in the 24–48–72 hour groups. (The 72 hours groups had 1–7 tumors per mouse). The groups that were transplanted later than 72 hours after radiation had a progressive increase in the number of lung tumors. By 14–20–days the treated inoculum had almost completely lost its radiation effect and was able to produce the same number of tumors as the control groups. The average size of the lung tumors was found to parallel the above findings. The size of lung tumors reached a minimum by the 8th post-radiation day and was back to control range by the 14–17th day.

From these observations it can be seen that the time interval between irradiation and transplant is an important factor in determining the effectiveness of radiation in limiting implantability and growth of tumor emboli. If surgery would be delayed more than 14–20 days post-radiation, the circulating cells would be able to implant and grow normally unless the tumor was completely radiosensitive and all cells were killed.

Table 1
Effect of radiation on "Primary" and artificial metastases

Tumor & Host	Effect	Control	170r	715r	2000r
S91 in DBA	on primary	0	0	+°	++*
	on lung tumors	314	256	66	3
S91 in CDBA	on primary	0	0	+	++
	on lung tumors	440	263	133	2
SA49 in DBA	on primary	0	0	0	0
	on lung tumors	113	107	60	22
T241 in C 57	on primary	0	0	0	+
	on lung tumors	43	30	0	0

° = + interruption of growth for 1–7 days
* = ++ interruption of growth for 7–14 days

Effect of changing amounts of irradiation in growth of primary tumor and its artificial metastases

In this group of experiments the time interval between in-vivo radiation of the donor tumor and transplantation was 24 hours. The effect on the "primary tumor" has been reported in detail elsewhere [10, 11]. The only transplantable tumor that showed more than a 4–6 days' delay in its growth curve was the lymphoma CD5. When this tumor received over 1020 r a "cure" was obtained and further growth was not seen. The effect of different amounts of irradiation on development of artificial metastases is shown in Table 1.

In the above instances following interruption of growth, each of the tumors had a rate of growth that paralleled the control group. It can also be seen that even when the irradiation did not change growth of the "primary tumor", a significant reduction in the number of artificial lung metastases was observed.

The same type of experiment was repeated, using several tumor host systems to demonstrate the inhibition of growth of implants when the irradiated inoculum was given intravenously, intramuscularly and in an axillary wound. The results are presented in Table 2.

Similar results were obtained for the other two tumor host systems. With the lymphoma CD5, the reduction in growth of the disseminated cells after 245 r and 715 r was only as effective as it was against the other tumors. The 2000 r tumor dose was not used, since it would be curative by itself. In the animals where no takes occurred after injection of the irradiated inoculum an injection of normal tumor cells was followed

Table 2

Effect of radiation on growth of tumor cells disseminated locally and systemically

Tumor & Host	Irradiation to Cells in-vivo	Route of Inoculation			
		Intravenous	Intramuscular and axillary		
		Lung Met.	Takes	Onset*	Deaths**
S91 in DBA	Control	343±22	46/46	19 days	46 days
	715r	44±10	42/47	33 days	62 days
	2000r	3±0.5	1/55	45 days	80 days
DBA 49 in DBA	Control	121±5	36/50	17 days	43 days
	715r	60±3	—	—	—
	1020r	—	28/50	20 days	45 days
	2000r	20±4	—	—	—
	2500r	—	1/50	26 days	49 days
T241 in C57B	Control	46±2.6	31/36	11 days	30 days
	715r	9±2	23/33	13 days	33 days
	2000r	3±1	3/36	19 days	33 days

± = standard error of mean
* = days from inoculation to time tumor became palpable
** = days from inoculation to time of death

Table 3

% Reduction of growth of cells disseminated 24 hrs. After 2000r in-vivo

Primary Tumor	% Reduction Systemic Spread	% Reduction Local Spread
S91	99%	98%
DBA49	85%	98%
T241	94%	90%
DBA59		95%

by tumor growth in 100% of the animals. This would demonstrate that the lack of growth in the irradiated groups was not due to a homograft reaction.

The effectiveness of radiation in preventing growth of tumor emboli can be expressed as the percent reduction of growth of disseminated cells (Table 3).

Discussion

In these studies, attempts have been made to test the effectiveness of x-ray in preventing growth of cancer cells that were later disseminated by injection, intravenous, intramuscular, or into an open wound. In the studies as outlined, the optimal time for transplant of the irradiated

tumor was 24–72 hours in order to obtain maximum reduction of growth of disseminated emboli. This is in agreement with work of *Lorenz* [12] and *Sugiura* [13] who described a decreasing percentage of "takes" the first 24–72 hours the primary tumor remains intact after treatment. This is followed by a gradual return to control levels, so that by 14 days the number of artificial lung tumors approached control levels. If these data could be interpolated to human cancer surgery, the pre-operative x-ray should be followed in 24–48 hours by excisional surgery rather than waiting 4–6 weeks as is commonly practiced today.

The other factor in determining the effectiveness of x-ray in controlling implantation and growth of disseminated emboli was the amount of x-ray given to the intact tumor before being prepared as an inoculation. In the animal tumors used in this study, considerable reduction in the number of disseminated implants was observed when only 715 r was used. However, to approach the 98–100% level of effectiveness, 2000 r was usually necessary. The amount of x-ray necessary to produce the desired reduction in growth of implants varied with each tumor system used. In all instances reduction in growth of disseminated implants was observed with an amount of x-ray that did not produce a "cure" of the primary tumor.

Following the results of these experiments a clinical study was outlined to test this method of pre-operative x-ray in a group of head and neck cancer patients at the clinical center of the National Institutes of Health. The study was designed as a double blind one in which all of the patients were set up in the x-ray department but only the radiologist knew which ones actually received pre-operative x-ray therapy. The x-ray therapy was designed to cover the primary tumor and the area of regional metastases. Since the onset of the study the amount of x-ray delivered at one sitting has been reduced from 2000 r to 1500 r, and at the present time, only 1000 r is being given. The change in radiation dosage has been necessitated by an increase in post-operative morbidity. One of the patients that received 2000 r to a floor of the mouth cancer has had delayed wound healing and some of the soft tissues of the upper-neck have required plastic repair with a tube graft. The degree of post-operative morbidity has not been so severe as to warrant breaking the code to definitely determine which cases actually received x-ray. The radiologist assures the surgeon that some of the post-operative complications the surgeon would ascribe to radiation actually occurred in the control patients (those receiving no irradiation). It is also recognized that since these human tumors were all epidermoid carcinomas and "radiosensitive" there was no way of determining experimentally the amount

of x-ray necessary to prevent growth of disseminated tumor emboli. To date, 32 patients have been included in the study. Approximately 10–12 of these are controls. The remaining 20 have an opportunity to establish the possible efficacy of pre-operative x-ray therapy. Local recurrence, and distant metastases have been observed in this group of patients. In fact, onefourth to one-third are already dead of their cancer. It is difficult to determine whether the mortality rate is any different from that experienced by this group of surgeons in previous studies. The actual value of the study remain for additional case material and time for the data to mature. One factor the study has shown is that pre-operative radiation followed by immediate surgery is feasible. It remains for additional controlled studies to determine if it has any value.

Summary

Clinical laboratory studies demonstrate tumor emboli in wound washings, wound drainage and in circulating blood of patients undergoing definitive cancer surgery. All of these tumor cells do not implant and grow. Follow-up studies of cancer patients suggest that there must be a quantitative factor in growth of tumor emboli, and that a resistance to implantation and growth may be present within the host. In certain instances, the host resistance to spread of tumor is overcome and dissemination occurs.

The study to be reported attempts to alter the intact tumor cell prior to surgical removal so that any cells disseminated in the wound, or at distant sites, would be unable to implant and grow. The laboratory model employed used five different genetically compatible mouse tumor host systems. The tumor in the intact donor mouse was irradiated 24 hours before surgery. The tumor was then removed from the donor animal, cytosieved and injected into recipient mice intravenously, intramuscularly or into an axillary wound. Growth of tumor at these sites was then measured and found to be decreased by more than 90% over control animals. The amounts of X-ray used was not large enough to stop growth of a "primary" tumor or cause regression of a growing implant. It is hypothesized that these cells have been damaged by irradiation in vivo, and that after dissemination from the parent tumor they are unable to implant or grow.

Zusammenfassung

Durch klinische Laboratoriumsstudien können bei Wundwaschungen, in Wunddrainagen und im zirkulierenden Blut von Patienten, die sich

einer definitiven Krebsoperation unterziehen, Tumoremboli nachgewiesen werden. Nicht alle dieser Tumorzellen implantieren sich und wachsen. Fortlaufende Studien an Krebspatienten lassen vermuten, daß im Tumorembolus ein quantitativer Wachstumsfaktor vorhanden sein muß und daß im Wirt eine Resistenz gegenüber Implantation und Tumorwachstum wirksam sein kann. In gewissen Fällen wird die Resistenz des Wirtes gegen Tumorstreuung überwältigt und die Dissemination erfolgt.

In der geschilderten Arbeit wurde versucht, die intakte Tumorzelle vor der chirurgischen Entfernung zu verändern, so daß alle in die Wunde oder an entfernte Stellen disseminierten Zellen sich weder implantieren noch wachsen können. Beim verwendeten Laboratoriumsmodell benützten wir 5 verschiedene, genetisch verträgliche Mäusetumorwirtsysteme. Der Tumor in der intakten Spendermaus wurde 24 Stunden vor der Operation bestrahlt. Der Tumor wurde nachher chirurgisch entfernt, zellgesiebt und Empfangsmäusen intravenös, intramuskulär oder in eine Achselwunde injiziert. Das Tumorwachstum wurde dann an diesen Stellen gemessen und um 90% geringer befunden als bei den Kontrolltieren. Die Dosis der verwendeten Röntgenstrahlen war nicht hoch genug, um das Wachstum eines Primärtumors aufzuhalten oder die Rückbildung eines wachsenden Implantates zu verursachen. Es wird angenommen, daß diese Zellen durch Bestrahlung in vivo geschädigt werden und daß sie nach der Dissemination aus dem Ursprungstumor unfähig sind, sich zu implantieren oder zu wachsen.

Résumé

Les recherches de laboratoire clinique ont démontré la présence d'emboli tumoraux dans le liquide de lavage de plaies, dans le liquide de drainage et dans le sang circulant de malades soumis à un traitement chirurgical radical pour cancer. Ces cellules tumorales ne sont pas toutes capables de s'implanter et de croître. Des études en série de malades cancéreux montrent qu'il y a un certain facteur quantitatif pour que les emboli tumoraux puissent se développer, et qu'il doit y avoir dans l'organisme du porteur de cancer une certaine résistance à l'implantation et à la croissance de la tumeur. Dans plusieurs cas, la résistance de l'hôte à la croissance du cancer est submergée et la dissémination tumorale se produit.

La présente étude s'occupe des tentatives faites pour altérer la cellule tumorale intacte avant l'acte chirurgical, de telle manière que les cellules disséminées dans la plaie opératoire ou à distance ne soient plus

capables de s'implanter ni de se développer. La technique de laboratoire employée a été appliquée à cinq possibilités de transmission chez la souris avec des tumeurs génétiquement compatibles. La tumeur de la souris donneuse intacte a été irradiée 24 heures avant l'ablation chirurgicale de la tumeur. Puis, elle a été enlevée, cytofiltrée et injectée dans une souris réceptrice par voie intraveineuse, intramusculaire ou dans une blessure de la région axillaire. Ensuite, on a mesuré l'accroissement tumoral et l'on a trouvé qu'il est dans plus de 90% moins développé que chez les animaux de contrôle. La quantité de rayons X appliquée n'était pas suffisante pour bloquer la croissance de la tumeur primaire, ni pour provoquer une régression des métastases en voie de développement. Cela permet l'hypothèse que ces cellules ont été endommagées in vivo, et qu'après dissémination elles n'étaient plus capables de s'implanter ni de croître.

Riassunto

Ricerche in laboratori clinici, hanno potuto dimostrare la presenza di emboli tumorali nel liquido di lavaggio delle piaghe, nel liquido di drenaggio, e nel sangue circolante dei malati sottoposti a trattamento chirurgico radicale a causa di un cancro.

Queste cellule cancerose non hanno tutte la proprietà di impiantarsi nè di crescere. Studi in serie di malati cancerosi, dimostrano che deve esserci un certo fattore quantitativo, affinchè gli emboli tumorali possano svilupparsi, e che ci deve essere nell'organismo del portatore di un cancro una certa resistenza all'impianto ed alla crescita del tumore. In diversi casi, la resistenza dell'ospite è sommersa, e si produce così la disseminazione del tumore.

Questo studio si occupa dei tentativi fatti per alterare le cellule cancerose intatte prima dell'intervento chirurgico, di modo che le cellule disseminate nella ferita operatoria o a distanza, non siano più capaci di impiantarsi nè di svilupparsi. La tecnica di laboratorio impiegata è stata applicata a 5 possibilità di trasmissione nel topo con dei tumori geneticamente compatibili. Il tumore del topo donatore intatto, è stato irradiato 24 ore prima dell'ablazione chirurgica dello stesso. In seguito esso è stato asportato, citofiltrato ed iniettato in un topo ricevente per via intravenosa, intramuscolare o in una ferita della regione ascellare. In seguito si è misurato lo sviluppo del tumore, constatando che essi sono del 90% meno sviluppati che quelli degli animali di controllo. La quantità di raggi x applicati non era sufficiente a bloccare la crescita del tumore primario, nè a provocare un regresso delle metastasi in via di

sviluppo. Ciò permette di emettere l'ipotesi che queste cellule siano state alterate in vivo, ed a disseminazione avvenuta non siano più state in grado d'impiantarsi e di crescere.

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