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Review Article

Intratumoral cancer chemotherapy and immunotherapy: opportunities for nonsystemic preoperative drug delivery

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Abstract

The recent literature documents the growing interest in local intratumoral chemotherapy as well as systemic preoperative chemotherapy with evidence for improved outcomes using these therapeutic modalities. Nevertheless, with few exceptions, the conventional wisdom and standard of care for clinical and surgical oncology remains surgery followed by radiation and/or systemic chemotherapy, as deemed appropriate based on clinical findings. This, in spite of the fact that the toxicity of conventional systemic chemotherapy and immunotherapy affords limited effectiveness and frequently compromises the quality of life for patients. Indeed, with systemic chemotherapy, the oncologist (and the patient) often walks a fine line between attempting tumour remission with prolonged survival and damaging the patient's vital functions to the point of death. In this context, it has probably been obvious for more than 100 years, due in part to the pioneering work of Ehrlich (1878), that targeted or localized drug delivery should be a major goal of chemotherapy. However, there is still only limited clinical use of nonsystemic intratumoral chemotherapy for even those high mortality cancers which are characterized by well defined primary lesions i.e. breast, colorectal, lung, prostate, and skin. There has been a proliferation of intratumoral chemotherapy and immunotherapy research during the past two to three years. It is therefore the objective of this review to focus much more attention upon intratumoral therapeutic concepts which could limit adverse systemic events and which might combine clinically feasible methods for localized preoperative chemotherapy and/or immunotherapy with surgery. Since our review of intratumoral chemo-immunotherapy almost 20 years ago (McLaughlin & Goldberg 1983), there have been few comprehensive reviews of this field; only one of broad scope (Brincker 1993), three devoted specifically to gliomas (Tomita 1991; Walter et al. 1995; Haroun & Brem 2000), one on hepatomas (Venook 2000), one concerning veterinary applications (Theon 1998), and one older review of dermatological applications (Goette 1981). However, none have shed light on practical opportunities for combining intratumoral therapy with subsequent surgical resection. Given the state-of-the-art in clinical and surgical oncology, and the advances that have been made in intratumoral drug delivery, minimally invasive tumour access i.e. fine needle biopsy, new drugs and drug delivery systems, and preoperative chemotherapy, it is timely to present a review of studies which may suggest future opportunities for safer, more effective, and clinically practical non-systemic therapy.

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General perspective for traditional systemic therapies

The toxicity of conventional systemic cancer chemotherapy and immunotherapy has severely limited the safety and effectiveness of such therapy. The quality of life

for patients may also be seriously compromised. Although chemotherapy has been helpful to prolong survival, to increase tissue-conserving surgery, and to increase remission rates for several cancer types, the high mortality breast, lung, and colorectal carcinomas, which account for the majority of cancer deaths, have remained problematic during the past 25 years from the standpoint of achieving significant advances in outcomes. This is true despite major changes in surgical and radiation protocols including combinatorial drug and drug–radiation regimens and new approaches to the staging of treatments. This relatively modest improvement is reflected in National Cancer Institute data as presented in the Surveillance, Epidemiology, and End Results (SEER) 1973–1997 Cancer Statistics Review (NCI-NIH 2000).

From these data, it appears that there has been little change in the ratio of incidence (diagnosis) to mortality rate in the short-term period 1997–2000 for breast cancer (180 200 diagnosed/41 945 deaths in 1997 vs 182 800/40 800 in 2000), for lung cancer (178 100/153 200 in 1997 vs 164 100/156 900 in 2000), and for colorectal cancer (131 200/56 695 in 1997 vs 130 200/56 300 in 2000). Some changes do seem to be attributable to various evolving "conventional" treatments over the long-term when one compares 1950 with 1997 data. However, there have been so many therapeutic, diagnostic, and demographic changes during this extended time period that it is extremely difficult to clearly attribute perceived improvements in outcomes to specific "standard of care" regimens. For example, based on the SEER report, the ratio of the annual% change for diagnosis to the annual % change of the mortality rate during the period 1950–1997 is: breast (+1.3%/−0.1%), lung (+2.3%/+2.9%), and colorectal (−0.1%/−0.9%). Using estimates for the past 20 years, we see here increasing diagnosis for breast (+29%) and lung (+58%) but only a 2% decline in breast cancer mortality and a disturbing 77% increase in lung cancer mortality during this time. Even with the remarkable advances in health care during the past 50 years there appear to be only relatively modest and interpretively complicated changes for outcomes in the treatment of high mortality cancers; except for lung cancer where the diagnosis and especially the mortality have been clearly on the rise.

Conventional or traditional cancer therapies

Among the high mortality cancers which are most resistant to treatment and which are potentially most amenable to intratumoral therapy (lung, colorectal, breast), breast cancer represents a most interesting and instructive example of the modest improvements achieved by an enormous body of research. An historical perspective by Fisher (1996) plus recent NIH-NCI reviews and guidelines, including the conclusions of a 2000 NIH Breast Cancer Consensus Conference, afford a good generic cancer treatment case study for the complex evolution of clinical care for breast cancer. This example will be considered in some detail here to illustrate the highly conservative and often empirical manner in which cancer treatments have evolved.

Breast cancer

The historical development of clinical therapies for breast cancer is a valuable example of the evolution of new ideas contrasted against the highly conservative approach of clinicians to changes in the standard of care. The slow acceptance of the value of preoperative chemotherapy and tissue-conserving lumpectomy surgery, when appropriate, is exemplary of this (Fisher 1996). Reports from the 2000 NIH Consensus Development Conference on Adjuvant Therapy for Breast Cancer (NIH Consensus Conference 2000) and the 1998 International Consensus Panel on the Treatment of Primary Breast Cancer (Goldhirsch et al 1998) are recent primary references for considering the potential for future local or intratumoral therapies.

The 1998 International Panel highlighted the need for more clinical emphasis on preoperative systemic chemotherapy, tissue conserving surgery, and local radiation. Greater use of these strategies in combination with more conservative sentinel lymph node biopsy (rather than complete axillary dissection) was also regarded as promising depending on long-term outcomes of current clinical studies. In brief, the conclusions of this panel may be summarized as follows:

1. Extent of surgery to the breast and to the axilla Breast-conserving surgery (lumpectomy) with planned local postoperative radiation is the treatment of choice for unifocal, invasive breast cancer that can be excised with clear margins (1 cm of normal tissue surrounding the tumour).

2. Preoperative (neoadjuvant) systemic chemotherapy This has been shown to be as safe and as effective as postoperative chemotherapy in terms of disease-free survival. Furthermore, as pointed out by Fisher (1996) and Fisher et al (1997), because neoadjuvant chemotherapy usually results in preoperative tumour regression, there can be a major benefit to patients since they are more likely to be eligible for breast conservation surgery.

3. Local radiation following surgery Comprehensive 50 Gy radiation is clearly indicated after breast conserving surgery with a possible 4-fold decrease in the risk of loco-regional recurrence. Thus, radiation is the only local therapy recommended today for clinical use.

4. Drugs for chemotherapy There was no consideration and no discussion concerning local chemotherapy in 1998 (nor in the 2000 NIH Consensus Conference). For systemic use, tamoxifen and mixed cytotoxic drug regimens were considered to be of primary clinical interest. Tamoxifen is a drug that continues to be an important component of adjuvant treatment for patients with tumours that express steroid hormone receptors; but the optimal way to administer the drug—before, concomitant with, or sequential to chemotherapy—remains uncertain.

5. Mixed cytotoxic drug regimens From semi-empirical studies, an extraordinarily complicated variety of multiple drug systems, dosing, and timing of drug delivery have been used, the most common being "classical" CMF (cyclophosphamide, methotrexate, and fluorouracil (5-fluorouracil)). To illustrate the complexity of these "experiential" modalities, note that the classical CMF protocol involves "six courses of cyclophosphamide, methotrexate, and fluorouracil repeated every four weeks". Furthermore, more toxic anthracycline-based regimens such as four courses of doxorubicin and cyclophosphamide every three weeks have afforded comparable results to CMF. Yet another trial using epirubicin (4-epidoxorubicin) given with cyclophosphamide and fluorouracil (plus antibiotic) produced more favourable results than CMF.

Given the myriad complex systemic chemotherapy regimens that have been explored clinically to date, a 1997 study group concluded that anthracycline combinations, even though more toxic, might represent the most effective conventional regimen. Although it has been generally felt that tamoxifen alone is beneficial, this study also concluded that only with anthracycline and classical CMF therapies was combination with tamoxifen likely to enhance disease-free survival (International Breast Cancer Study Group 1997). The foregoing summary of empirical combinatorial drug delivery schedules, which have each required 5–10 years of large cohort clinical testing to obtain statistically meaningful results, is illustrative of the difficulty in identifying truly improved systemic chemotherapy.

Conclusions from the 2000 NIH Consensus Development Panel on Adjuvant Therapy for Breast Cancer (NIH Consensus Conference 2000) were similar but less detailed than the 1998 International Panel report. Although discussed in presentations to the 2000 panel, the potential future benefits of preoperative systemic chemotherapy (Fisher et al 1997) were virtually ignored in the conclusions. Most important from the perspective of this review, absolutely no consideration was given to future needs and opportunities for less toxic and more tissue-conserving loco-regional or intratumoral therapies.

It was the view of the 2000 Consensus Panel that "systemic adjuvant therapies are designed to eradicate microscopic deposits of cancer cells that may have spread or metastasized from the primary breast cancer …". Hence, surgery plus local radiation and systemic chemotherapy remain the recommended standard of care. However, perhaps for the first time, more concern was expressed in the conclusions regarding the overall value of toxic systemic chemotherapy, from the perspective of comparing improvements in patient survival with possible compromises in quality of life. In this context, the physician and the patient are confronted with the need to evaluate the potential for acute and chronic adverse health effects and associated psychological distress vs the potential benefits of tissue-conserving surgery and prolonged survival. The enormous literature concerning systemic (adjuvant) breast cancer chemotherapy is impossible to consider further here. For those interested, more than 2200 literature citations for just the 1995–2000 time frame are compiled in an excellent NIH bibliography (NIH-NLM 2000).

Systemic preoperative (neoadjuvant) chemotherapy

The foregoing discussion of breast cancer has already noted the significance of preoperative systemic administration of various cytotoxic drugs based on advantages which may accrue from reduction of tumour burden at surgery (thereby increasing the number of patients indicated for tissue-conserving surgery), and reduction of micrometastasis to enhance the prospect of longer disease-free survival after surgery. It is hardly possible here to do more than sample further some of the more instructive additional recent reviews and reports in this field from among the more than 300 papers published on this topic during the period January 2000 to March 2001 (and approximately 1000 papers cited since 1993 in a PubMed search).

There are a number of good reviews and papers that spell out the likely advantages and disadvantages of

neoadjuvant therapy for breast cancer. Stebbing & Gaya (2001) suggested that response rates were generally high ranging from 70 to 90% with tumour regression facilitating breast conservation surgery. They used the term "induction chemotherapy" for preoperative systemic treatment. Clinical issues illustrated with case by case examples have been discussed in a Journal of Clinical Oncology editorial (McMasters & Hunt 1999) and a paper in that journal by Kuerer et al (1999a). Those authors reported results of two prospective preoperative chemotherapy clinical trials using daunorubicin, surgery, and radiation. A significant conclusion was that "neoadjuvant chemotherapy has the capacity to completely clear the breast and axillary lymph nodes of invasive tumour before surgery". Indeed, this group from the MD Anderson Cancer Center go so far as to state that neoadjuvant chemotherapy is a "standard of care" for patients with locally advanced breast cancer and offers the potential for use of sentinel lymph node biopsy as an alternative to much more invasive axillary resection (Kuerer et al 1999b). Another good discussion of surgical issues concerning breast cancer was presented by Margolese (1998).

Recent clinical review papers on neoadjuvant treatments for other cancers should also be cited here. Studies concerning non-small cell lung cancer were reported by Larson et al (1999) and by Felip & Rosell (2000). Both reviews suggest significantly improved survival using preoperative chemotherapy. Improved survival was also noted in cervical carcinoma clinical studies in which cisplatin, fluorouracil, ifosfamide, and mesna were administered preoperatively according to a complex multiple dose schedule (Etcheverry et al 2000). Similarly, clinical studies of preoperative chemotherapy with advanced cervical cancer using cisplatin or cisplatin plus fluorouracil concurrently with radiation (Morris et al 1999; Rose et al 1999) yielded prolonged survival. Results of yet another clinical trial, pelvic radiotherapy with cisplatin followed by hysterectomy, indicated significantly reduced risk of cervical cancer recurrence (Keys et al 1999). Thus, we see increasing evidence for the benefits and growing acceptance of systemic preoperative chemotherapy, a development of fundamental significance to future consideration of localized intratumoral chemotherapy as a preoperative adjuvant modality.

Intratumoral and loco-regional therapies

The foregoing necessarily brief consideration of traditional toxic systemic chemotherapy hopefully affords a useful background against which to realistically evaluate the needs and opportunities for improving the safety of drug treatments. For the purpose of this discussion, we wish to make clear that the term chemo-immunotherapy, when used, is intended to refer to treatments which result in immunological effects which may be induced by the local administration of chemotherapy alone as well as the combination of local chemotherapy and immunotherapy. The focus of this review is consequently on the need to place much more emphasis on various localized intratumoral approaches that have been virtually ignored to date by the majority of clinicians. Our goal is to attempt to encourage more research aimed at reduction of systemic toxicity, eradication of metastasis, and achievement of greatly improved disease-free survival.

Rationale for local and intratumoral therapy

Safer and more aggressive (higher dose) administration of toxic chemotherapy or immunotherapy directly to a tumour site, i.e. intratumoral therapy, is an obvious and attractive alternative to systemic treatment. However, during the past three decades, the conventional wisdom in clinical and surgical oncology has tended to discourage this approach. The rationale for this reluctance has presumably been based upon three notions: why inject drugs into primary lesions when they may usually be readily resected?; won't metastasis be stimulated along an injection needle track?; and won't local chemotherapy have little benefit in dealing with metastasis in view of low systemic drug levels? However, research in the field of local and regional therapy has made great strides in addressing these questions with findings that suggest systemic immune response for intratumoral therapies, enhanced benefit when local therapy is coupled with subsequent surgery, and widespread clinical experience with fine needle aspiration biopsy with little evidence of complications attributable to needle track metastasis.

Growing recognition of the need to alter the standard practice of systemic therapy is reflected in the proliferation of animal and human clinical research in the literature. These studies have been devoted to the evaluation of concepts for drug delivery at therapeutic levels to a tumour site by regional vascular infusion using vessels that supply a tumour site, injection of drug directly into the tumour, infusion of drug into a tumour through a catheter (sometimes with an implantable pump), or intracavitary placement of a drug or drug containing composition with or without resection of a tumour mass. Because conventional systemic chemotherapy involves prolonged and aggressive use of toxic

multidrug protocols, dose-dependent drug toxicity often makes it impossible to achieve necessary therapeutic drug concentrations at primary and secondary sites of malignancy. Although we will place primary emphasis upon direct intratumoral injection in this review, a recent book devoted to regional chemotherapy (Kerr & McArdle 2000) and a number of pertinent representative regional chemotherapy studies with different cancers are also worthy of note here.

Evolution of loco-regional chemotherapy

Bladder cancer One clinical approach has been reported for what may be termed "regional chemoprophylaxis" following Nd-Yag laser treatment of bladder neoplasms (Szemes & Kovacs 1994). Laser treatment has been a less invasive tissue-conserving replacement for transurethral resection in many cases during the past decade. Catheter instillation of 20 mg mitomycin C in 40 mL saline for 2 h post-laser into the bladder of patients on a regular weekly schedule for six weeks followed by less frequent instillations was reported to result in a statistically significant improvement in outcomes for treatment of primary tumours in 46 patients with primary disease with no relapse for 72% of patients at 44-month examination. It should be noted that postoperative regional immunotherapy of bladder cancer (e.g. with preparations of bacillus Calmette & Guerin, BCG) by intravesicular irrigation has also become a current standard of care.

Lung cancer An interesting recent clinical study of regional chemotherapy involved 68 patients having malignant pleural effusions associated with non-small cell lung cancer. Cisplatin with etoposide (both at 80 mg m^{-2}) was evaluated clinically by 72-h intrapleural catheter infusions (Tohda et al 1999). An increase in survival was reported and valuable results for drug localization and pharmacokinetics were obtained. For both drugs, local drug concentrations in the pleural cavity were reported to be more than two orders of magnitude higher than systemic plasma levels and the local active drug half-life was shown to be much greater for etoposide (65.5 h) than for cisplatin (10.5 h). The highly significant differences in drug stability and localization for different drugs when given regionally were well illustrated in this study.

Liver cancer Primary hepatocellular carcinoma (HCC) is one example of a high mortality tumour of worldwide significance where surgery is often not possible. Although it is generally accepted that surgical resection, hepatectomy, gives the best chance of a cure, less than 20% of patients can undergo hepatectomy. However, even hepatectomy is highly problematic since a major proportion of patients, approximately 70%, have tumour recurrence after surgical resection. Therefore, improved non-surgical treatments have been required. Three recent reviews on non-surgical treatments have appeared (Farmer et al 1994; Liu & Fan 1997; Langer 1998). Although a large number of drugs and drug combinations have been administered systemically, there has been little success for HCC chemotherapy (Ravoet et al 1993; Farmer et al 1994). The poor results for systemic chemotherapy have prompted research on loco-regional regimens such as hepatic artery infusions in an attempt to deliver high local concentrations of drugs to the liver.

The rationale for regional treatment of HCC via the hepatic artery is based on the fact that the blood supply to hepatomas is delivered by the hepatic artery (Breedis & Young 1954), whereas the blood supply to normal liver tissue is from both portal and arterial sources. As a result, regional infusion of the hepatic artery with percutaneous catheters was attempted clinically as early as 1964 but infection and mechanical complications with these catheters led to the implantation of drug infusion pumps in the late 1980s. Several studies have examined their clinical use, reporting favourable tumour responses ranging from 29 to 88% (Buchwald et al 1980; Balch et al 1983; Cohen et al 1983; Shepard et al 1985). A randomized clinical trial comparing tumour response for systemic vs regional (intra-arterial) delivery of chemotherapy also suggested improvement for regional therapy. Unfortunately, there was still significant hepatic toxicity associated with this regional treatment (Chang et al 1987).

Early attempts at treatment of HCC involving ligation or embolization of the hepatic arterial branch supplying the tumour were associated with serious abdominal complications and limited long-term clinical success. However, one current clinical technique, transarterial chemoembolization (TACE), uses partial embolization of the hepatic artery with gelatin sponge particles for delivery of drugs suspended in an oil emulsion. Doxorubicin, mitomycin, epirubicin or cisplatin are emulsified with lipidol, a contrast medium with high hepatic affinity. Lipidol is an oil derived from poppy seeds that has a highly selective affinity for HCC when injected into the hepatic artery. It acts as both an embolic material and a carrier that can slowly release the drug, significantly increasing the amount of drug delivered locally to the tumour. Thus, combining partial embolization of the

hepatic artery with delivery of a drug/oil emulsion has significantly prolonged survival of patients with HCC (Yamashita et al 1991; Bismuth et al 1992; Bronkowski et al 1994). Several reports have described chemoembolization with various drug-loaded microspheres delivered through the hepatic arterial branch and trapped in the tumour capillary bed (Fujimoto et al 1985; Ichihara et al 1989; Audisio et al 1990; Beppu et al 1991).

Currently, intratumoral percutaneous ethanol injection (PEI) is considered one of the most effective treatments for small (usually less than 3 cm) well defined liver tumours. This procedure is performed under local anaesthesia, using a fine needle placed in the tumour with ultrasonograph or computer tomography (CT) scan guidance. Slow injection of absolute (99.5%) ethanol directly into the tumour causes tissue necrosis within and around the tumour site. Since initial research in 1983, this procedure has become an effective and low-cost treatment for patients having as many as three small lesions (Livraghi et al 1995a, b). Positive results for percutaneous intratumoral injection have been reported for mitoxantrone/oil emulsions (Farres et al 1998) and other drugs. Finally, a recent review of regional therapy for hepatocellular carcinoma is useful in clarifying important differences in evaluating problems for regional treatments of HCC as compared with hepatic colorectal metastases because of important differences in biology and patient morbidities (Venook 2000). Thus, borne of necessity in the case of refractory or inoperable liver cancers, we have examples of the evolution of clinical treatments from systemic and/or surgical methods to more likely successful local and intratumoral clinical procedures.

For hepatic metastasis from colorectal cancer, the benefit of postoperative regional therapy was indicated in two recent studies. In one (Kemeny et al 1999), 156 patients were randomly assigned following liver surgery to hepatic artery infusion of floxuridine plus dexamethasone with fluorouracil given intravenously. This treatment was compared with only intravenous fluorouracil following liver resection. After two years, 90% of the regional plus intravenous group were disease-free compared with 60% for the systemic intravenous therapy group. Yet another approach to regional chemotherapy of hepatic metastasis, hepatic artery chemoembolization, was evaluated in the second study (Tuite et al 1999). All 59 patients in this study had failed systemic fluoropyrimidine therapy. Chemoembolization with cisplatin, doxorubicin, and mitomycin with iodized oil and polyvinyl alcohol particles was performed monthly for 2–7 months (mean 2.4 months). This type of regional therapy was stated to afford survival twice that of systemic chemotherapy and worthy of a further phase III clinical trial.

Bone cancer The history of osteosarcoma treatment is another highly instructive example of changes in approach toward preoperative and local therapies for consideration here. Before the 1970s, osteosarcoma was considered to be resistant to most chemotherapy and patients had only a 10–20% probability of long-term survival (i.e. more than 1–2 years). However since that time, three drugs (methotrexate, adriamycin, and cisplatinum) were shown to prolong survival when combined with surgery. Recent reviews of chemotherapy in the treatment of osteosarcomas have been published (Picci et al 1994; Jaffe et al 1995). Surgical amputation followed by aggressive systemic chemotherapy to treat micrometastases present at the time of surgery was reported to achieve disease-free survival of 50–80%.

Improved survival for postoperative chemotherapy in the treatment of metastases led to interest in the possibility of treating the primary tumour preoperatively with chemotherapy to avoid amputation. This neoadjuvant approach was hoped to afford limb salvage or limb preservation. This is a significant development since osteosarcoma thus became one of the first cancers to be treated clinically by a non-conventional preoperative approach. Introduced in the early 1980s, preoperative chemotherapy was reported to regress tumours in the majority of patients and enhance the prospect for limb salvage. One further scientifically important advantage that resulted from the use of preoperative chemotherapy in osteosarcoma was the opportunity, for the first time, to evaluate the response of the primary human tumour phenotype to the drugs used for chemotherapy. Due to the similarity in phenotypes, the micrometastases and the primary tumour appear to have the same chemosensitivity. Better decisions for postoperative treatments, when necessary, were thereby possible. Response to preoperative chemotherapy has also been found to be a useful prognostic factor in predicting ultimate survival i.e. the better the primary tumour response, the better the prospect for prolonged survival.

Consideration of loco-regional therapy for osteosarcomas resulted in randomized studies which compared preoperative systemic with intra-arterial regional chemotherapy. Greater tumour necrosis using local intra-arterial drug delivery (Bacci et al 1992) was reported. A significantly larger proportion of patients receiving preoperative intra-arterial cisplatin, 78%, exhibited tumour necrosis compared with 46% for systemic treatment. In another effort to localize treatment,

CT-guided ethanol injections directly into bone cancer metastases was reported to effect 26% tumour regression, reduce pain, and improving the quality of life for patients. Thus, these and other related regional treatment studies have indicated a definite advantage for local vs conventional systemic therapy.

Intratumoral chemotherapy

Previous reviews of intratumoral chemotherapy

There have been few comprehensive reviews devoted to intratumoral studies in recent years. A review of "targeted" local cancer chemotherapy by Gupta (1990) shed little light on intratumoral therapy but affords some useful information on drug delivery systems which may be applicable to intratumoral therapy using water-in-oil emulsions, liposomes, or microspheres prepared from starch, ethyl cellulose, and albumin. Two other reviews (Tomita 1991; Walter et al 1995) are narrowly focused on malignant gliomas of the brain. A proliferation of intratumoral research on gliomas has occurred because of inherent surgical limitations (due to tumour accessibility and the peculiar highly irregular tumour morphology) and problematic systemic drug delivery across the blood–brain barrier. These two reviews are primarily instructive for their discussion of a wide variety of drugs and polymer–drug compositions that have been evaluated clinically, some of which may be of broader interest for intratumoral therapy of more common cancers.

Another more recent review devoted to brain tumours (Haroun & Brem 2000) affords a very helpful up to date discussion of several approaches to intratumoral and intracavitary (local post-surgery) chemotherapy applicable to other malignancies. One approach, for which there was FDA approval in 1996, involves the treatment of gliomas with carmustine-impregnated polymer wafers following 80% tumour resection. The polymers studied in clinical trials included poly(carboxy-phenoxypropane sebacate) and an ethylene-vinyl acetate copolymer (Gliadel, Guilford Pharmaceuticals, Baltimore, MD). It was reported that high local drug concentrations were maintained for 30 days following polymer–drug implantation with little systemic toxicity and with significant prolonged patient survival. Other compositions discussed for intratumoral glioma treatment included poly(DL-lactide-co-glycolide) microspheres loaded with fluorouracil which biodegrade within 1–4 weeks. Due to fluorouracil being regarded as a radiosensitizer, human clinical studies were conducted with 59.4 Gy of local radiation given after surgery with intracavitary injection of fluorouracil microspheres. A doubling of the median survival time was reported for patients treated in this way (Menei et al 1999).

The review by Haroun & Brem (2000) discussed local immunotherapy for glioma treatment; a concept that is broadly pertinent to many other types of cancers. Emphasis here is on the local delivery of interleukins-2 and -12, cytokines that stimulate recruitment of cytotoxic T-lymphocytes and lymphokine-activated killer cells. Local delivery of these interleukins in controlled-release microspheres (i.e. alginate/chitosan) reportedly enhanced survival in animal studies (Liu et al 1997; Shah & D'Souza 1999). The most significant aspect of the review from the standpoint of the intriguing potential for intratumoral chemo- and chemo-immuno-therapies was reference to chemo-immunotherapy studies, which afforded good evidence for increased antigen expression by tumours following exposure to cytotoxic drugs. For example, using the combination of cyclophosphamide with IL-12, it was suggested that more intracellular tumour peptide antigens were released by local tumour cell killing thus enhancing tumour-specific antigenicity for greater tumour-specific T-cell/killer cell activity (Pardoll 1993; Tsung et al 1998).

Perhaps also appropriate to cite here is an early review of topical chemotherapy for the treatment of various skin cancers (Goette 1981). It is pertinent since topical drug delivery may be regarded as a special type of localized tumour chemotherapy. Topical application of fluorouracil in ointments containing up to 5% drug has become a clinically effective and economical treatment for many benign and cancerous skin lesions. Fluorouracil has also been of clinical value in special topical formulations at concentrations up to 20%.

The review of intratumoral chemo-immunotherapy by McLaughlin & Goldberg (1983) affords the most comprehensive presentation and critical evaluation of intratumoral research to that time. A goal of the present review is therefore to provide a comprehensive current perspective. However, several key points concerning mechanical aspects of intratumoral procedures and drug pharmacology that were discussed in some detail in the 1983 review are still important and may be helpful to restate here.

Drug localization and complete perfusion of the lesion is fundamental for complete tumour response. Rapid diffusion of drug away from the injection site into the general circulation must therefore be minimized. In animal studies, injection duration and timing of multiple injections (e.g. every few hours for four to six injections) were shown to be important parameters. For perhaps the most extensively studied animal model, a metastatic

line-10 guinea-pig hepatocellular carcinoma, repeated intratumoral injections of 0.5 mL of drug solutions every few hours into 1-cm tumours was readily feasible and multiple injections were superior to single injections for a given total dose.

Inflammation and necrosis at the injection site is an acute outcome which must not be excessive to the point of severe patient pain and discomfort and undue toxicity to healthy surrounding tissue. The use of polymer–drug compositions that control and prolong highly localized drug activity may therefore be desirable.

Physical accessibility of the primary lesion is necessary. Methodology for deep percutaneous access has been greatly facilitated by developments in the past 10–15 years involving needle guidance for fine needle aspiration biopsy (FNA) with 20–25-gauge needles and core-needle biopsy (CN) with 11–14-gauge needles. Precisely the same methodology that is currently used to percutaneously access deep internal lesions for FNA or CN can be readily adapted for intratumoral therapy. New and superior injection methodologies for guided intratumoral therapy are thereby now available.

Although a thorough discussion of FNA and CN is beyond the scope of this review, hundreds of studies have been published during the past five years concerning the value of these minimally-invasive biopsy techniques and their accuracy, safety and economy for diagnosis of a wide variety of malignancies compared with open surgery. For reference, a few useful recent FNA clinical papers and reviews are appropriate to cite here (Abdul-Karim & Rader 1998; Arisio et al 1998; de Paredes et al 1998; Nguyen & Akin 1998; Guy & Ballo 1999; Iwamoto 1999; Jorda et al 2000; Wakely 2000; Wakely & Kneisel 2000). It should be noted that recent reviews suggest that the development by several device manufacturers of newer CN instrumentation, with advanced mammographic or ultrasound guidance, will likely displace FNA in many cases (Edwards et al 1997; Velanovich et al 1999).

High local drug concentrations with minimal systemic toxicity is a key potential benefit of intratumoral therapy. Due to the normally short half-lives of most free cancer drugs, polymer–drug compositions that can enhance drug stability and prolong local activity with limited diffusion away from the tumour site are important to the intratumoral modality. Drug-loaded microspheres, liposomes, and polymer gels all offer potential benefits for much more successful intratumoral therapy in the future.

Biological response to intratumoral drug delivery must at least be helpful to subsequent surgical resection (if deemed necessary) by reducing the local tumour burden. Stimulation of a tumour-specific systemic immune response to eradicate metastasis is also a vitally important outcome. There is evidence cited in the 1983 review for systemic immune response in animal experiments and some human clinical studies using intratumoral chemotherapy. Consistent with the previously cited work of Tsung et al (1998) and Pardoll (1993), it now appears even more reasonable to conclude that this systemic immune response results from the processing of tumour-specific antigen expressed by tumour cell debris in immune-competent individuals following intratumoral chemotherapy. However, confirmation of this in-vivo vaccine-like phenomenon needs considerable further clinical study. Furthermore, protocols coupling intratumoral with preoperative systemic chemotherapy as well as multiple intratumoral drug regimens and intratumoral immune modulator combinations with chemotherapy remain to be investigated.

Theon (1998) reviewed applications of lesional chemo- and immunotherapy in veterinary medicine with principal attention to equine tumours. The relatively brief review of intratumoral chemotherapy by Brincker (1993) is the only other fairly recent general overview of the subject. Although that review did not consider the potential for systemic immunological response for intratumoral therapy, it did draw attention to a number of additional important points. These included the fact that general textbooks on chemotherapy and cancer treatment do not mention intratumoral chemotherapy at all despite the extensive experimental and clinical literature; and that clinical and experimental studies consistently show significantly higher drug concentrations in target tissues (e.g. 6–10-times higher) with greatly reduced systemic toxicity compared with conventional intravenous chemotherapy thus affording a rationale for more serious clinical attention.

Comments on early (pre-1990) intratumoral research

Earliest clinical reports

Although many early studies have been referenced in previously cited papers and reviews, probably the earliest clinical reports for direct intratumour injection of chemotherapy were more than 40 years ago by Bateman (1955, 1958). The innovative and provocative 1958 study used two phosphoramides which first became available in 1953 (Thio-TEPA: N,N′,N″- triethylene thiophosphoramide, and ODEPA: N-(3-oxapentamethlene)-N′,N″- diethylene phosphoramide). This study involved 486 patients with far-advanced cancers of a few

different types including breast and liver. It is instructive to this day. In general, significant tumour regressions were reported. Patients were initially treated weekly and then at intervals of four to five weeks as regression was observed. For 177 mammary carcinoma patients, the overall positive response was 66%. However, it is difficult to draw firm conclusions concerning the value of intratumoral therapy from this report because of the advanced disease status and the various surgical and adjuvant radiation treatments experienced by patients. Nevertheless, the breast and liver cancer case studies reported and statements concerning hepatic cancer patients such as "no improvement was noted in any patients treated with Thio-TEPA until the drug was injected intrahepatically", make a strong case for intratumoral vs systemic chemotherapy even with these early cytotoxic drugs in this mixed cohort of highly advanced cancer patients. The clinical insight of this investigator is also remarkable and reflected in the comment that "since survival time alone is hardly worth achieving, some measure of the quality of survival is desirable".

Immune response due to intratumoral chemotherapy and chemo-immunotherapy

Probably the most intriguing aspect of the results from early research has been the suggestion that intratumoral chemotherapy may provoke a systemic tumour-specific immune response. This in-vivo vaccine-like effect, if validated in human clinical trials, would represent an exciting therapeutic advance. Although discounted for a variety of reasons associated with the animal models used (or forgotten in recent years), pioneering studies during the late 1970s by NCI-NIH groups at Bethesda (directed by H. Rapp) and at Hamilton, Montana (directed by E. Ribi) provided strong evidence for the immuno-therapeutic benefit of intratumoral chemotherapy and combination intratumoral chemo- and immuno-therapy with BCG dispersions in transplanted guinea-pig hepatomas and spontaneous bovine ocular carcinomas (Bast et al 1976; Borsos et al 1976; Cantrell et al 1979; McLaughlin & Goldberg 1983). These studies generally gave favourable results in eradicating draining lymph node metastasis with actinomycin D, melphalen, Adriamycin (doxorubicin), mitomycin, and 1,3-bis(2-chloroethyl)-1-nitrosourea. Methotrexate, fluorouracil, and mercaptopurine (6-mercaptopurine) were not effective. Two intratumoral studies using mitomycin (McLaughlin et al 1978; Cantrell et al 1979) were of particular interest. In the line-10 hepatocarcinoma in strain 2 guinea-pigs, McLaughlin et al (1978) established a dose response to intratumoral drug in the range 50–500 μg mitomycin; with 80% complete tumour regression at 250–500 μg drug dropping to 50% at 100 μg and 17% at a non-necrotic dose of 50 μg. This study used day-6 post intradermal tumour transplant of 10^6 cells when the hepatoma was 6–12 mm diameter with lymph node metastasis. Even intratumoral injections with more advanced disease and larger tumours (16–20 mm) produced statistically significant complete tumour regressions using 500 μg drug (70% at 11 days and 29% at 14 days). Animals with complete tumour regressions, which had been treated only with intratumoral mitomycin, rejected a second 10^6 tumour cell challenge and were therefore regarded as "cured". However, an immunotherapeutic BCG cell wall emulsion preparation was even more effective when given intratumorally one day after intratumoral drug injections (90% cures with day-11/12 injections). Cantrell et al (1979) investigated intratumoral treatment of a subcutaneous EL-4 lymphoma transplant in male B57BL/10 mice using mitomycin alone or in combination with a soluble tumour-specific antigen extract of EL4(G-) cells. Drug alone at 25 or 100 μg induced 20–26% cures but intratumoral antigen in an oil-in-water emulsion (given two days after intratumoral drug) cured 50–80% of mice. Note that the intratumoral immunotherapy alone had no anti-tumour effect. In other early papers from the group directed by Fisher et al (1979) at the University of Pittsburgh, combined preoperative intratumoral chemo-immunotherapy was also found to be effective using cyclophosphamide chemotherapy with *Corynebacterium parvum* immunotherapy in a spontaneous mammary carcinoma from C3H/HeJ mice. In this case, chemotherapy alone did not appear to be beneficial.

Additional early evidence for an immune stimulus from intratumoral chemotherapy comes from several other reports. Bier et al (1980), using ^{57}Co-labelled bleomycin, observed high drug concentration in the injected tumour and draining lymph nodes with "cures" in a guinea-pig hepatoma. Prolonged drug activity, hence a better result, was achieved with bleomycin–oil emulsions compared with free drug.

In further research with this hepatoma using intratumoral cisplatin and vincristine, it was found that a key role was played by T-lymphocytes in the chemotherapy induced immune response e.g. in T-lymphocyte suppressed animals, the curative immune effect of the intratumoral chemotherapy was negated (Bier 1987). Two early human clinical studies should also be mentioned in which possible systemic immune benefits might be ascribed to intratumoral chemotherapy. In one, involving 32 cases of metastatic stage III–IV ovarian cancer, disease was arrested in 15 patients, tumour

regression was seen in 14, and treatment failed in the remaining three cases. The drugs administered intratumorally were thioTEPA, cyclophosphamide, merphalan (sarcolysin), and methotrexate (Vinokurov & Mitrokhina 1983). The other clinical study tested the feasibility of percutaneous ultrasound guided intratumoral chemotherapy through a 22-gauge needle with methotrexate, fluorouracil, and cyclophosphamide in 12 patients with a variety of cancers (i.e. pancreatic, lung, etc). Stable disease or tumour regression was reported in 60% of patients (Livraghi et al 1986).

More recently, intratumoral administration of the cytotoxic drugs etoposide and Z7557 (an active cyclophosphamide derivative) was shown to induce cell-mediated antigen-specific immunity to an antigen (KLH) but required subcutaneous priming to be effective (Limpens et al 1990). In this case, draining lymph node cells were shown to proliferate with enhanced IL-2 production. Another study by Claessen et al (1991) followed up previous research where they showed that low dose local 4-HPCY (an active cyclophosphamide derivative) strongly enhanced T-cell mediated immune response in mouse and guinea-pig tumours. Their results with the metastatic line-10 guinea hepatoma were consistent with previous results (McLaughlin et al 1978) in that early stage intratumoral treatment (day-7 after tumour implant) achieved 75% cured animals which rejected tumour-specific rechallenge. Late stage intratumoral treatment (14 days) was much less successful. However, if day-14 intratumoral treatment was preceded by a low systemic dose of cyclophosphamide (which alone was ineffective), 57% cure was achieved. It was reported by Claessen et al (1991) that immunopotentiation was also achieved using intratumoral cisplatin and etoposide.

Intratumoral chemotherapy during the past decade (1990–2000)

The rapid recent proliferation of intratumoral chemotherapy research and clinical reports is evidenced by the more than 300 papers that have appeared in this field during just the past two years. These 300 papers actually represent one-half of all studies that have appeared since 1979. We will sample from among the more interesting and most important of the animal and human clinical studies as well as recent related intratumoral immunotherapy with emphasis upon future clinical prospects. In addition, some results from our own laboratory on intratumoral research will be summarized. In general, the improved efficacy and reduced toxicity suggested by the majority of these studies clearly warrants even greater attention to the development of practical clinical modalities for intratumoral chemotherapy and immunotherapy.

Animal intratumoral chemotherapy studies

Gliomas remain a focus of attention for animal and human experimentation. In rat glioma models, intratumoral implanted drug-loaded biodegradable rods and disks were indicated to maintain high local drug concentrations and were much superior to systemic or intratumoral free drug in regressing tumours. Methotrexate, alkyllysophospholipids, and alkylphosphocholines in polylactide compositions, and halogenated pyrimidines in carboxyphenoxysebacate polymers were used (Zeller et al 1990; Williams et al 1998). Targeted protein toxin conjugates have also shown significant efficacy for the treatment of gliomas by intratumoral administration in a nude mouse model (Laske et al 1994).

Papers on intratumoral therapy of hepatomas in rabbit models revealed further positive results using cisplatin in a viscous collagen gel for sustained local drug release (Korey et al 1993) and with free mitoxantrone (Ramirez et al 1996). In this research, excellent drug localization was demonstrated. For example, high cisplatin concentrations were uniformly distributed and maintained in tumours for at least 24 h ($> 400\ \mu g\ g^{-1}$ at 0.5 h; $50\ \mu g\ g^{-1}$ at 24 h) with minimal levels in adjacent tissues ($< 2\ \mu g\ g^{-1}$) and in the plasma ($< 0.5\ \mu g\ g^{-1}$). With mitoxantrone at 1.5 mg kg^{-1}, systemic intravenous administration exhibited toxic myelosuppression with no tumour suppression whereas the same intratumoral dose produced no major side effects, blue-stained areas of tumour necrosis, and resulted in a 3.5-fold reduction in tumour growth rate.

In a BD IX rat colon tumour model, Benoit et al (1999) reported prolonged survival with intratumoral mitomycin and cisplatin. Inhibition of growth for tumours that were not yet established as well as regression of established tumours was reported even though there was evidence of non-uniform drug distribution in injected tumours. However, these investigators felt that this approach still failed to consistently cure these animal tumours. Using nude mice xenografts of a human pancreatic adenocarcinoma (BxPC-3), Smith et al (1994) examined intratumoral delivery of fluorouracil (50 mg kg^{-1}), cisplatin (8 mg kg^{-1}), or doxorubicin (8 mg kg^{-1}) in a collagen gel containing epinephrine (intended to prolong drug delivery by local vasoconstriction). Doxorubicin was most potent but all intratumoral systems reduced tumour burden by $> 70\%$. In more recent research with fluorouracil/epinephrine gel (Smith et al 1999), it was again shown

that much higher concentrations were achieved in tumours for intratumoral gel injections (18.4 mM) compared with fluorouracil solutions given intravenously (2.02 mM) or intraperitoneally (0.07 mM). Ravichandran et al (1998) also showed the benefit of intratumoral delivery of lithium linolenate for the treatment of pancreatic carcinoma in a nude mouse model.

In one interesting study, various spontaneous malignancies in baboons (i.e. myxoma, squamous cell carcinoma, lymphosarcoma, adenocarcinoma) were treated by intratumoral therapy with toremifene (Wurz et al 1998). Here again, drug concentrations remained localized and partial responses were observed for most tumours. In yet another intratumoral evaluation, a slow release polymer paste formulation with paclitaxel was tested in a human prostate tumour model in castrated athymic mice (Jackson et al 2000). The drug–polymer paste consisted of 10% paclitaxel in a biodegradable triblock copolymer of a random copolymer of DL-lactide with caprolactone (PLC) and poly(ethyleneglycol) (PEG) blended with a methoxypoly(ethyleneglycol) (MPEG) i.e. 40:60 PLC-PEG-PLC:MPEG. Following intratumoral injection, phase separation of MPEG at 37°C produced a semisolid intratumoral drug delivery depot. Depending on treatment timing, single intratumoral injections of 100 μL of the drug–polymer paste through a 22-gauge needle reportedly reduced tumours of 43–233 mm^3 to nonpalpable size with some minor ulceration at the injection site as the only side effect.

A series of papers by Theon et al (1993, 1996, 1997) reported on the veterinary use of intratumoral chemotherapy in horses, dogs, and cats. In equine sarcoids and squamous cell tumours (Theon et al 1993), cisplatin in a slow-release sesame oil emulsion, given at two-week intervals for four courses (0.97 mg cm^{-3} for tumours ranging from 10 to 20 cm^3), gave a complete response in 18 of 19 sarcoids and 5 of 7 squamous cell carcinomas. For periocular squamous cell carcinomas in horses (Theon et al 1997), intratumoral bleomycin and cisplatin were also effective with animals 78% disease-free at one year. Nasal plane squamous cell carcinoma in cats was treated safely and effectively by intratumoral injection of cisplatin in a sesame oil formulation (Theon et al 1996). The oil emulsion reduced drug leakage from the injection site and limited systemic drug toxicity. Four weekly doses of 1.5 mg cm^{-3} of tumour tissue resulted in 70% complete tumour clearance.

Radiation combined with intratumoral chemotherapy A synergistic benefit may accrue to the use of some interesting combinatorial adjuvant regimens. In one study involving 12 dogs with various spontaneous malignancies, a bovine collagen gel/cisplatin formulation (0.25 mg drug kg^{-1} total dose) was given intratumorally at four treatment sessions immediately before radiation therapy (48 Gy total dose). The collagen gel formulation significantly limited systemic toxicity and a complete response was observed for 10 of 12 dogs (Theon et al 1994). A similar murine tumour study with intratumoral implantation of poly-bis(*p*-carboxyphenoxy)propane sebacate polymer rods containing 17% cisplatin plus radiation indicated that intratumoral drug delivery was much more efficient than systemic cisplatin and that prolonged higher levels of drug were maintained in the tumour tissue (Yapp et al 1997). Local chemotherapy coupled with radiation therefore appear to be synergistic. Similar results were obtained for intratumoral placement of slow release polymer–cisplatin rods in an RIF-1 mouse tumour study (Yapp et al 1998). These results were further confirmed in a SCCVII squamous cell murine carcinoma model with cisplatin given intratumorally in a polymer gel (Ning et al 1999). Here again the intratumoral protocol was regarded as safer and more effective than systemic drug delivery and the combination of local chemotherapy with radiation further enhanced antitumour efficacy.

Electric field effects combined with intratumoral chemotherapy Favourable synergistic effects have been reported for electrochemotherapy. This technique involves the application of a localized electric field to a tumour site shortly before, during, or soon after the administration (either intravenous or intratumoral) of a chemotherapeutic drug, which has relatively poor cellular permeability (such as bleomycin or cisplatin). The electric field is intended to enhance the permeability of cellular membranes to the drug thereby promoting greater diffusion into tumour cells, a process that has been termed electroporation. In addition to achieving higher intracellular concentrations of cytotoxic drugs within the tumours, electrochemotherapy is believed to affect the microvasculature of the tumour and reduce the tumour blood supply (Cemazar et al 2001). There have been several reviews concerning the theory of electroporation (Mir & Orlowski 1999; Neumann et al 1999) and on pre-clinical and clinical trials to date (Dev & Hofmann 1994; Heller et al 1999; Hofmann et al 1999b; Mir 2001). The results of a few selected trials are noted here. An in depth discussion of electrochemotherapy is beyond the scope of this review. The interested reader is referred to the cited reviews and additional literature for more detailed discussion of the topic.

In one electrochemotherapy study in mice using intratumoral cisplatin delivered at doses up to 8 mg kg^{-1}

combined with eight electrical pulses of 100 ms at 1 Hz frequency and approximately 1000 V, cures were reported for 67% of tumours when electrical pulses were given 5 min before or during injection (Cemazar et al 1998). In a similar experiment with intratumoral bleomycin in a hamster tongue cancer model, high voltage treatment following drug injection was reported to induce rapid tumour necrosis (within 48 h) and progressive tumour regression. After three weeks, three of six animals showed no palpable tumour and experienced no major side effects (Omura et al 2000). In a rat model of a rhabdomyosarcoma, a single electrochemotherapy treatment using intratumoral bleomycin in small tumours (250 mm^3) resulted in 100% of the treated tumours being nonpalpable at day 28, and 42% of the animals remained tumour free after 100 days. Much larger tumours (3000 mm^3) required multiple treatments, but 100% complete tumour regression was reported (Hyacinthe et al 1999).

Human intratumoral chemotherapy studies

Drug–gel compositions In addition to the earlier mentioned human evaluations, a growing number of clinical intratumoral drug delivery studies have appeared during the past decade. A few representative examples will be referenced here. One promising approach has been to use polymer gels as drug carriers to prolong intratumoral drug residence time. Basal cell carcinoma was treated by intratumoral administration of a sustained release fluorouracil–polymer composition (Matrix Pharmaceutical MPI 5003) in a 20 patient double blind trial. Histologically confirmed cures were reported for 8/10 patients (Orenberg et al 1992). More recently, intralesional injection of a cisplatin/epinephrine gel composition (Matrix IntraDose) was evaluated in a phase II hepatocellular carcinoma clinical trial (Johnson 1999). The treatment was reported to be safe and yielded encouraging preliminary data for 29 patients with six achieving a complete response and 10 in remission at 10 months post-treatment.

A phase III trial was conducted with 102 patients having advanced breast, melanoma, and oesophageal tumours (most of whom had previous systemic chemotherapy) using intratumoral injection of a cisplatin/epinephrine gel. This local drug–gel treatment was associated with a lower incidence of toxic effects compared with systemic chemotherapy. A reduction in tumour burden of more than 50% was reported for 45% of patients with a strong correlation of reduced tumour size and quality of life. Repeated injections appeared necessary for larger tumours of more than $\sim$ 0.5–3 cm (Hardbord et al 1999). In a related phase I trial of intratumoral injection of a bovine collagen gel containing cisplatin/epinephrine in 82 patients for treatment of a variety of highly refractory solid tumours (i.e. head and neck), there were 39% complete responses and 11% partial responses. Injections were through 20–26-gauge needles. Although the total dose was much less than normal for systemic therapy (5–20 mg intratumoral vs 160–220 mg intravenous), the tumour concentration of cisplatin was 10–100-times greater than for systemic therapy (Agerwal 1998). Finally, in a study of eight patients with inoperable colorectal liver metastasis, CT-guided intratumoral injections of cisplatin/epinephrine gel compositions (repeated 3–5 times) resulted in improvement and lesional necrosis at six months; 38% for metastases and 83% for the hepatocellular carcinomas (Engelmann et al 2000).

Percutaneous intratumoral injection of mitoxantrone with CT-guidance has been reported for treatment of primary and secondary liver tumours in nine patients for whom previous therapies had resulted in failure or serious complications (Farres et al 1998). Drug with contrast medium was used with 1–3-monthly treatments. Tumour necrosis was observed, but there was recurrence in five of nine patients within nine months after treatment. It was concluded that further study of this therapeutic modality was warranted. Prolonged release formulations and multiple injection regimens may be of particular value for such difficult cases. In another study using CT-guided mitoxantrone injection, repeated minimally invasive intratumoral treatment was performed on 110 patients with bone and soft tissue metastases and 35 patients with vertebral metastases from primary hepatic tumours. Pain reduction of 75% was achieved in most patients but tumour regression was found for only approximately 25% of patients. A decisive factor for this therapy of far-advanced disease was a major improvement in patient quality of life (Gronemeyer & Seibel 1993).

Intratumoral injection of ^{32}P-chromic phosphate is being evaluated in patients with refractory metastatic gastrointestinal malignancies with remarkable results for tumour regression. Of 17 patients, a positive response was seen for 70% of patients with complete remission in seven patients (41%). However five of 17 patients showed no response (Firusian & Dempke 1999). An interesting and unusual application of intratumoral chemotherapy involved treatment of inoperable tracheal/airways tumours which severely obstructed breathing (Celikoglu et al 1997). The endobronchial tumours were treated via flexible fibre optic guided bronchoscope with multiple 1–3 mL injections using a complex multi-drug regimen: 50 mg fluorouracil, 1 mg

mitomycin, 5 mg methotrexate, 10 mg bleomycin, 2 mg mitoxantrone. The reported results were impressive with relief of airway obstruction for 81 of 93 patients. These investigators regarded this intratumoral treatment as a well tolerated life-saving palliative without systemic side effects.

Finally, local electrochemotherapy has been reported to show promising results in several human clinical trials. In a phase I/II trial for treatment of head/neck tumours with bleomycin, application of an electrical pulse to the injected tumour site resulted in complete tumour regression for five of eight patients and partial responses ($>50\%$ shrinkage) for three of eight patients. The treatment was apparently well tolerated but duration of the complete response was only 2–10 months (Hofmann et al 1999a). In another trial, 143 cutaneous or subcutaneous tumour nodules in 34 patients were treated with intratumoral bleomycin followed by 6–8 pulses of 99 ms using a 1.3 kV cm^{-1} electric field. Twelve weeks after treatment, 142 of the 143 nodules (99%) had at least a 50% reduction in tumour volume and 91% of the nodules were undetectable (complete response) (Heller et al 1998). The results of this study were more favourable than a similar study reported by the same group in which bleomycin was given intravenously before the electroporation treatment. That study resulted in a tumour response of 72%, but only 33% had a complete response (Heller et al 1996). The combined results of independent electrochemotherapy trials at five cancer centres were published by Mir et al (1998). In these clinical studies, 291 cutaneous or subcutaneous tumours in 50 patients were treated with either intravenous or intratumoral bleomycin resulting in 85% tumor regression of at least 50% with 56% complete regression 30 days after treatment.

Intratumoral immunotherapy

Complementing recent research and clinical trials for intratumoral chemotherapy, there has been a significant growth in research concerning intratumoral immunotherapy (more than 100 PubMed literature citations during the past four years). We will briefly re-emphasize here some of the more important pertinent earlier works and provide a number of examples from recent immune modulator research and especially studies which have utilized cytokines and gene therapy approaches.

Bacterial and other immunomodulators

One of the most interesting and provocative early intratumoral immunotherapy studies used thermally killed *Corynebacterium parvum* in a mammary adenocarcinoma model (CAD_2) in DBA/2 mice (Likhite & Halpern 1974). At the time of treatment, 14 days after subcutaneous inoculation with 10^7 tumour cells, the disease was metastatic and tumours were 9-mm diameter. *C. parvum* (400 μg in 0.2 mL Hank's solution) was given intratumorally as six injections into different parts of the tumour site. Although a variety of controls and test groups received immunotherapy at other than tumour sites (i.p., contralateral s.c., and 1 cm away from the tumour), only the animals receiving intratumoral treatment survived. For the intratumoral group, all animals survived (12 of 12), and they could be regarded as "cured" because they also rejected challenges with 10^7, 5×10^7, and 10^8 CAD_2 cells. Furthermore, these cures were tumour-specific since all animals died when challenged with a different mammary adenocarcinoma cell line (T1699). Another previously mentioned report (McLaughlin et al 1978) demonstrated the efficacy of intratumoral immunotherapy using a BCG cell wall skeleton preparation containing trehalose dimycolate in an oil-in-PBS emulsion. Although 50% cures were achieved in the line-10 guinea-pig hepatoma model, this intratumoral immunotherapy was more effective when used one day following intratumoral drug injection with mitomycin. The authors suggested that the systemic immune response (with eradication of metastases) observed for intratumoral chemotherapy alone and the more effective combination with an immune modulator was due to processing of cell debris with greater expression of tumour cell antigens to the host immune system.

The effectiveness of local (intravesical) BCG treatment for superficial bladder carcinoma as a prophylaxis has been demonstrated, but appropriate treatment regimens remain empirical and controversial. In a clinical study of 15 cases of in-situ bladder carcinoma treated monthly for one year, patients were disease free at the 18–21 month follow-up. Although still effective, somewhat less success was achieved for 48 cases of superficial bladder cancer treated similarly with BCG where 28% recurrence was observed (Rigatti et al 1990). More recent clinical studies have used an attenuated strain of *Streptococcus pyogenes* (OK-432, Picibanil, Chugai Pharmaceutical, Tokyo) as an intratumoral immune modulator for preoperative intratumoral treatment of gastric cancer. In a clinical trial with 395 patients (Tanaka et al 1994), a significant improvement in five-year survival was demonstrated for intratumoral OK-432 for patients with stage III disease (48% survival with intratumoral OK-432 vs 28% for controls). Quite recently, results were presented for a 10-year clinical study of intratumoral immunotherapy with OK-432 in 370 gastric

cancer patients (Gochi et al 2001). In the intratumoral study group, patients received endoscopically-injected intratumoral OK-432 one to two weeks before surgery followed by postoperative adjuvant therapy with one intravenous dose of mitomycin plus oral tegafur and intradermal OK-432. The intratumoral immunotherapy group with stage III cancer and lymph node metastasis at time of treatment had significantly increased 5- and 10-year survival rates. It was concluded that intratumoral treatment probably acted to eliminate micrometastatic foci in lymph nodes.

Cytokine therapy

There is a rapid proliferation of literature on the intratumoral delivery of interleukins, particularly IL-2, IL-7, IL-12, and IL-stimulators, because of a growing awareness that such local therapy can be more effective and much safer than systemic administration. This literature can be only briefly sampled here. Direct intratumoral injection of IL-2 was evaluated in a murine alveolar carcinoma model with twice-daily injections for three weeks (Dubinett et al 1993). Increased survival for intratumorally treated mice and 24% cures were observed compared with no long-term survivors for controls or intraperitoneal treatment. For the intratumoral group, systemic anti-tumour response was suggested by isolation of tumour-infiltrating lymphocytes (TILs) and splenic lymphocytes with enhanced lytic activity. IL-2 was evaluated using an intratumoral depot formulation that released IL-2 for 24 days in a rat prostate adenocarcinoma (Hautmann et al 1997). Significant inhibition of tumour growth with no toxicity was reported. More promising results were obtained in a study using intratumoral polyethylene glycol (PEG) modified IL-2 (EuroCetus, Amsterdam) compared with recombinant IL-2 in a line-10 guinea-pig hepatoma. PEG-IL-2 was found to be more effective than rIL-2 using a regimen of three injections per week for five weeks. At the optimal PEG-IL-2 dosage, 12 of 12 animals survived. All were believed to have been cured as evidenced by rejection of a second line-10 challenge (Mattijssen et al 1992). No cures were observed using perilymphatic injections or with intratumoral rIL-2.

Prolonged intratumoral delivery of cytokines such as IL-2 appears to be an important therapeutic factor, one that has been reinforced by another report in which IL-2-loaded polylactic acid microspheres were co-injected with tumour cells in a murine model involving a human tumour xenograft (SCID). Sustained IL-2 delivery from the biodegradable microspheres resulted in complete suppression of tumour growth in 80% of mice with mediation by natural killer cells (Egilmez et al 1998). In related research, an rIL-12-loaded polylactic acid microsphere composition was evaluated in tumour bearing BALB/c mice. A single injection of the rIL-12 microspheres prevented metastasis, promoting complete tumour regression, and conferred systemic resistance to a subsequent tumour challenge, and so this system was considered to have acted as an "in-situ vaccination" (Egilmez et al 2000).

In another IL-12 study, transduced bone marrow-derived dendritic cells were injected into murine tumours (MCA205, B16, and D122). Results indicated that the gene-modified dendritic cells effectively expressed IL-12 activity in the tumour and draining lymph nodes thereby regressing injected lesions and conferring systemic tumour-specific immunity (Nishioka et al 1999). Another intratumoral gene therapy strategy for IL-12 used adenovirus-mediated delivery of IL-12/B7.1 genes in a non-transplanted woodchuck hepatocellular carcinoma. Large (2–5 cm) intrahepatic tumours were injected with MRI guidance once with 10^9 U AdIL-12/B7.1 (an adenovirus vector carrying genes for murine IL-12 and B7.1). Within one to two weeks there was substantial tumour regression (assessed histologically) accompanied by massive infiltration of T lymphocytes with increased levels of CD4(+) and CD8(+) T cells and interferon gamma. One tumour followed for seven weeks after treatment was completely eliminated (Putzer et al 2001). It was concluded that intratumoral adenovirus vector-based immunotherapy was effective and promising for treating hepatocellular carcinoma in man.

Intratumoral injection of numerous other interleukin-modified dendritic cells (DCs) and adenoviral (Ad) gene vector compositions have been reported within the last two to three years with consistent reports of tumour regression and tumour-specific immune response. Such intratumoral delivery of gene immunotherapy is clearly becoming a highly significant direction for future research and clinical investigation. A few additional pertinent studies including one small human trial are briefly noted here. DC-AdIL-7 was especially effective (complete tumour regression plus immunity) in two murine lung cancer models (Miller et al 2000). IL-2 is FDA approved for metastatic renal cell carcinoma (but benefits only 10–20% of patients), and so a preclinical human renal carcinoma tumour xenograft safety test was conducted with an IL-2 plasmid DNA/lipid complex given intratumorally. This treatment resulted in complete tumour regression without the adverse systemic effects that are characteristic of intravenous IL-2 therapy (Hoffman & Figlin 2000). Fibroblasts (H-2K) modified to secrete IL-2 were reportedly an effective intratumoral treatment in a murine model of brain

metastasized breast cancer (Deshmukh et al 2001). Intratumoral DCs modified to produce IL-12 induced a complete response with immunity in a transplanted colon adenocarcinoma (Melero et al 1999). Similar results were achieved for gene therapy with compositions expressing IL-2 (Daniels & Galanis 2001). Using a unique polyvinylpyrrolidone-IL-12 gene vector complex, two types of murine tumours were similarly responsive to intratumoral gene therapy (Mendiratta et al 1999). In a human trial with melanoma and breast cancer patients, DCs from monocytes obtained by phlebotomy with granulocyte-macrophage expressing IL-4 were injected in autologous plasma intratumorally at multiple sites. Tumor regression within four days was reported for four of seven melanoma and two of three breast cancer patients (Triozzi et al 2000).

Intratumoral studies from the authors' laboratory

For more than 20 years, our laboratory at the University of Florida has maintained a research programme aimed at achieving less toxic and clinically useful cancer treatments employing localized or intratumoral therapies. A primary strategy has been to use clinically accepted cytotoxic drugs with microsphere–drug modifications designed to promote prolonged local intratumoral activity at high drug concentrations. This research and reports by many others cited in this review tend to support the view that intratumoral chemotherapy may significantly reduce systemic toxicity, regress tumours, and create an in-situ vaccine depot due to tumour-specific antigen expression by killed tumour cells. Our work on microsphere compositions and intratumoral studies in four animal models (as well as some pertinent related research by others) is briefly summarized in the following sections.

Microsphere–drug compositions

It is beyond the scope of this review to provide a detailed discussion of the extensive literature on the synthesis and properties of microsphere-drug compositions. It is sufficient to refer to a few good reviews and some of our work on drug-loaded protein and polysaccharide microspheres that has been principally aimed at intratumoral therapy. This has included a novel synthesis of readily dispersed human and bovine serum albumin microspheres (Longo et al 1982a; Longo & Goldberg 1985), research on the synthesis and properties of hydrophilic albumin, albumin-polyglutamic acid, and dextran microspheres with drug loading by physisorption as well as ionic and covalent binding (Goldberg et al 1984), covalent attachment of mitomycin to aldehyde-dextran microspheres (Iwata et al 1982), albumin–polyglutamic acid–adriamycin microspheres (Longo et al 1982b, 1983) and various compositions designed for affinity binding to targeting receptors (Goldberg et al 1982). Additional studies pertinent to intratumoral therapy include mitoxantrone-loaded albumin microspheres (Quigg et al 1992a; Goldberg et al 1994), casein–mitoxantrone microspheres (Knepp et al 1993), methotrexate–casein microspheres (Jayakrishnan et al 1994), and mitoxantrone–albumin microspheres (Hadba et al 2000).

Useful reviews from various research groups on microsphere–drug systems include Davis et al (1984), Davis & Illum (1988), Okada & Toguchi (1995), Kreuter (1996), Ravi Kumar (2000), Emerich (2000). In most microsphere papers and these reviews, microsphere–drug compositions have consistently been shown to be beneficial by exhibiting substantially reduced toxicity compared with free drug, very limited systemic drug exposure, a variety of useful biodegradation and controlled-release profiles, and high intratumoral drug concentrations.

Guinea-pig hepatoma

A number of studies with the transplantable line-10 hepatoma model in strain-2 guinea-pigs, including our own, have been adequately discussed in previous sections of this review, especially concerning immune response due to intratumoral chemotherapy. The applicability of this model (as well as many other widely used animal models) to human disease has sometimes been questioned because it is not a spontaneous tumour and it may also exhibit slight autoimmune characteristics. However, it is noteworthy that intravenous and intratumoral therapies have been extensively evaluated in this highly metastatic larger animal model with results that are consistent with data that have often been obtained with other small animal models (i.e. mouse and rat). The strong evidence afforded by guinea-pig hepatoma studies for local chemotherapy-induced immunity, with eradication of metastasis, remains a compelling stimulus for investigation of intratumoral therapy in high mortality human cancers, especially those that are inoperable.

Mouse ovarian tumour

Local, intraperitoneal (i.p.) delivery of chemotherapy (mitomycin or mitoxantrone) and immunotherapy with MPL (Monophosphoryl Lipid A, a 3-O-deacetylated phospholipid immune modulator, Ribi Immunochem,

Hamilton, Montana) was studied using bovine serum albumin microsphere carriers in collaboration with Dr John Cantrell of Ribi Immunochem. This research (unpublished to date) used a mouse ovarian tumour (MOT) model obtained by intraperitoneal injection of 10^4 ascites tumour cells derived from C3Hb/FeJ mice. In one experimental series, local chemotherapy with 50 μg mitomycin injected intraperitoneally on day-6 after tumour transplant was followed on day-7 with an intraperitoneal injection of MPL-loaded albumin microspheres. The most favourable results were obtained for mice that received this sequence of combination chemo-immunotherapy with 100 μg MPL in 1.4 mg microspheres. At day-66 following treatment, 50% (5/10) of mice in this group were disease-free whereas the median survival time for controls was 20 days. However, local intraperitoneal chemotherapy with mitomycin alone was ineffective.

In a parallel experiment, 10–15 μm-diameter mitoxantrone-loaded albumin microspheres were used to treat mouse ovarian tumour by intraperitoneal injection. The injection of microspheres containing 250 μg drug (more than the LD50 for mitoxantone given intravenously in mice) resulted in 90% disease-free survival at 90 days. A definite dose response was observed with 90-day survival reduced to 40% at 125 μg and 20% at 63 μg of drug. In general, mitoxantrone–microsphere compositions, even without the potential benefit of subsequent immunotherapy, were remarkably effective in this murine mouse ovarian tumour model compared with free drug given intraperitoneally or intravenously and were much less toxic. This is an example of the potential for therapeutic benefit that may be possible for localized (though not intratumoral) drug delivery for treatment of malignancies that do not exhibit discrete primary lesions. One pertinent parallel study on the synthesis and intraperitoneal drug-release properties of mitoxantrone–albumin microspheres in rats afforded data consistent with our mouse ovarian tumour results (Luftensteiner et al 1999). Sustained release of tumouricidal drug levels were found in peritoneal fluid for at least 72 h after intraperitoneal injection with minimal amounts of drug in plasma. Although no animal tumour was tested, it was concluded that such microsphere compositions might overcome dose-limiting toxicity of conventional chemotherapy with mitoxantrone.

Lewis lung carcinoma

Glutaraldehyde cross-linked microspheres of 10–15-μm diameter were prepared for this research from a 5:1 mixture of bovine serum albumin and polyglutamic acid using a modification of the steric stabilization synthesis developed in this laboratory (Longo et al 1982b). Post-synthesis electrostatic and physisorption loading of the basic drug, mitoxantrone, (by microsphere sorption from aqueous drug solutions) was capable of achieving microsphere payloads of mitoxantrone as high as 35%. These BSA–PGA–mitoxantrone microspheres were used to treat a transplantable Lewis lung carcinoma in B6D2F mice. This murine tumour model is an anaplastic, squamous cell carcinoma that is diffi cult to treat by systemic chemotherapy and rapidly metastasizes to the lungs. A variety of different intratumoral treatments were examined at very high dosage (3.0 mg/animal) including varied injection timing, intratumoral plus intraperitoneal chemotherapy, surgical resection without chemotherapy, and intratumoral drug followed by tumour resection. A novel aspect of this study was the use of microsphere suspensions prepared in drug solutions for injection. Typically, preparations contained 0.5-mg drug in solution plus 2.5-mg drug in the microsphere dispersion. Thus, injected tumours were immediately perfused with free drug followed by slower release from microspheres. Preliminary results, indicating reduced toxicity and long-term survival for intratumoral treatment groups, were reported by Quigg et al (1992a). Similar results were obtained with casein–mitoxantrone microspheres (Quigg et al 1992b; Knepp et al 1993). More extensive data now available clearly indicate that intratumoral therapy with BSA–PGA–mitoxantrone microspheres can prolong survival and delay the onset of lung metastasis by a factor of 2–3-times. However, a most significant outcome was the observation that intratumoral microsphere-drug treatment, followed by surgical resection of tumours 10 days after intratumoral injections, resulted in 92% disease-free survival (more than 90 days without lung metastasis compared with 100% deaths at 24 days for untreated control animals). This research affords further evidence for the conclusion that preoperative intratumoral therapy is a promising approach for human clinical evaluation.

Mouse mammary adenocarcinoma

We are currently in the midst of preclinical breast cancer chemotherapy studies in a 16/C murine mammary adenocarcinoma. It is appropriate to mention some preliminary results here (Hadba et al 2001). This model was adapted from Corbett et al (1978) and uses a spontaneous mammary tumour, which can metastasize to the lungs in C3H/HeJ mice. It is reportedly responsive to anthracenediones such as mitoxantrone (Corbett et al 1982). In this model, treatment was started 14–18 days after tumour implantation (when the tumour had reached 1 cm in its longest dimension).

The efficacy of intratumoral injections of mitoxantrone were compared with intravenous injections with or without surgical resection. To date, the most significant results for median day of death after treatment, using a 4 mg kg^{-1} drug dose, were 20.5 days for surgical controls with tumours resected at day 5, 27.5 days for intravenous drug with tumour resection at day 10, and 60 days for intratumoral drug injection followed by day 10 tumour resection. Untreated controls had a median day of death of 7.5 days. As we have seen in other studies, the most effective treatment was intratumoral injection plus surgery for which there were 50% disease-free survivors at 60 days. However, these disease-free survivors failed to reject a challenge with the 16/C tumour line. Tumor-specific immunity was therefore apparently not achieved in these initial experiments with this murine mammary tumour.

In another study, mitoxantrone-loaded albumin microspheres (12% drug) were used to treat the 16/C murine mammary tumour. Although the intravenous LD50 in mice is 6.6 mg kg^{-1}, drug–microsphere compositions were safely administered at doses up to 48 mg kg^{-1} if given by intratumoral injection. The mean day of death for intratumoral drug–microsphere therapy at the 48 mg kg^{-1} dose was 43 days compared with free drug results of 22 days at 8 mg kg^{-1} and 16 days at 4 mg kg^{-1}. Here again intratumoral injection of even free drug prolonged survival significantly (compared with the 7.5 day mean day of death for untreated control animals), but microsphere compositions were most effective since much higher concentrations could be administered safely to the tumour site with little systemic toxicity.

Concluding comments

We have tried to provide the most comprehensive review of localized chemotherapy and related cancer treatments available in the literature to date with particular emphasis on the growing body of research that has appeared during the past decade. Important earlier studies have also been reviewed. Throughout, we have witnessed the very slow evolution of the standard of care for adjuvant chemotherapy in the treatment of different cancers. In the context of this review, it is interesting that the only local therapy, radiation, has become a clinical modality of major importance, often in combination with surgery and systemic chemotherapy. Even the improvements available from the use of preoperative (but still systemic) neoadjuvant chemotherapy as a standard of care for breast, colorectal, lung, and bone cancers have been slow in coming. However, research results presented in this review suggest that we are entering a new era of cancer treatment where surgeons and oncologists in collaborations with various physical and biomaterials scientists will place more emphasis on local therapies. In the short-term, it is reasonable to believe that preoperative intratumoral chemotherapy with drug carriers that prolong local drug activity is ready to achieve more widespread clinical use. It is also predictable that the advantages of immunotherapy with cytokines, immune modulators, and genetically tailored drugs for gene therapy will be best realized when administered by the intratumoral route. Overall, therefore, it is hoped that this review will help stimulate further important research, which will improve the quality of patient survival and will eventually achieve new treatments and cures for cancer.

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