Phosphorus-32-Chromic Phosphate for Ovarian Cancer: I. Fractionated Low-Dose Intraperitoneal Treatments in Conjunction with Platinum Analog Chemotherapy

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For many years, ^{32}P-chromic phosphate (^{32}P-CP) intraperitoneal instillations and platinum analogue chemotherapy have been used to treat disseminated ovarian cancer. To investigate possible enhancement of ^{32}P-CP irradiation due to the concomitant administration of chemotherapy, in vitro studies were undertaken. Based on those laboratory investigations, a clinical regimen of combined ^{32}P-CP and platinum analogue chemotherapy was developed. **Methods:** In vitro enhancement of ^{32}P-CP cytotoxicity by cisplatin was studied in cultured human ovarian adenocarcinoma (CHOA) cell lines and in a fibroblast cell strain. In addition, ovarian cancer cells obtained from the malignant abdominal ascites and pleural effusions of 10 individual patients were also studied ex vivo. As part of routine clinical care, 30 patients with disseminated ovarian adenocarcinoma underwent up to eight monthly cycles of platinum analogue chemotherapy with concomitant intraperitoneal instillation of 5 mCi of ^{32}P-CP at each monthly chemotherapy cycle. **Results:** There was an enhanced and possibly supra-additive effect of cisplatin on the cytotoxicity from ^{32}P-CP irradiation. For the 30 patients, the survival rate at 3 yr was 63%. **Conclusion:** Phosphorus-32 CP low-dose intraperitoneal treatments in conjunction with platinum analogue chemotherapy is a promising approach for the treatment of disseminated intraperitoneal ovarian cancer.

Key Words: ovarian cancer; phosphorus-32 chromic phosphate; cisplatin; carboplatin

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In the United States there are approximately 22,000 new cases of ovarian cancer each year (*1*). Unfortunately, only 25%–30% of these patients have tumor confined to the ovary and pelvis when their disease is first detected. Common routes of tumor spread include extension to other pelvic structures, lymphatic spread to regional lymph nodes with further dissemination to retroperitoneal and mediastinal lymph nodes and implantation on peritoneal surfaces throughout the pelvis and abdomen (*2*). Despite cytoreductive surgery and newer chemotherapeutic agents, overall 5-yr survival for patients with ovarian cancer is slightly less than 39% (*1*). Common patterns of tumor recurrence include carcinomatosis, lymph node metastases and extension above the diaphragm to produce mediastinal lymph node metastases and malignant pleural effusions. Ovarian cancer metastases to bone, liver, the lung parenchyma and the brain rarely occur before patients reach the near terminal stage. Clearly, there is a need for improved treatment for this tumor which characteristically disseminates to the peritoneal surfaces of the abdominal cavity.

Instillations of ^{32}P chromic phosphate (^{32}P-CP) have been used to treat intraperitoneal and pleural space malignancies including ovarian cancer for many years (*3–22*). Most frequently this treatment has been given as a 10–20-mCi intraperitoneal injection which may be repeated as necessary to control malignant ascites and tumor spread. The goal is to disseminate ^{32}P-CP throughout the abdominal cavity in a distribution similar to the spread of exfoliated or ruptured ovarian carcinoma cells. The distribution of intraperitoneal ^{32}P-CP becomes largely fixed by 24 hr postinjection. Metastatic deposits directly adjacent to sites of ^{32}P-CP localization receive therapeutic radiation from the beta-minus particle emitted during ^{32}P decay (half-life 14.3 days, mean beta-minus energy 0.695 MeV). The average soft-tissue penetration of beta-minus from ^{32}P is 1–4 mm. Clearly, the center of bulky intraperitoneal metastases will not receive significant radiation therapy, and in addition the complications associated with ^{32}P-CP therapy will be limited to the superficial layers of exposed structures such as the bowel wall.

A previously published review indicated that there are mostly minor, short-lived complications from ^{32}P-CP therapy (e.g., nausea, abdominal pain, vomiting, diarrhea and low-grade fever) in 21.9% of treated patients (*23*). The

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frequency of associated major complications, usually taking the form of bowel obstruction or perforation, was 1.5%. Following ^{32}P-CP therapy, histologic changes within the bowel wall take the form of "a thickening and hyalinization of the serosal peritoneum and outer muscularis without discernable changes in the submucosa or its vasculature" (*10*). Using fractionated intraperitoneal ^{32}P-CP therapy, patients have been reported to receive up to a total of 42 mCi of ^{32}P-CP in six divided weekly doses without developing serious complications (*6*).

In recent years, the platinum analogues cisplatin and carboplatin have been demonstrated to have a broad range of cytotoxic effects against many epithelial tumors including ovarian cancer (*24*). While these agents exert many effects, the principle mechanism appears to be the formation of lethal platinum-induced cross links between DNA bases. The principle toxic effects are renal insufficiency, peripheral neuropathy, ototoxicity and nausea with carboplatin possibly being better tolerated than cisplatin when used to treat ovarian cancer (*25*). Given that the absorbed radiation dose from ^{32}P-CP is largely confined to the peritoneal cavity and superficial layers of the bowel wall, there is no reason to assume that the clinically relevant toxicities from platinum analogue chemotherapy and ^{32}P-CP would in any way depend on whether the treatments were given independently or in combination.

We previously reported in abstract form that cisplatin enhanced the effect of ^{32}P-CP irradiation on cultured human ovarian carcinoma (CHOA) (*26–28*). A minimum dose of ^{32}P-CP was needed to induce this enhancement, and the effect was significantly greater when cisplatin was added to CHOA shortly before rather than shortly after the addition of ^{32}P-CP. Based in part on these laboratory results, we have defined a protocol for the clinical use of ^{32}P-CP and cisplatin or carboplatin, all of which are FDA-approved substances being employed in approved ways, to provide for continuous low-dose rate intraperitoneal beta-minus irradiation treatments in conjunction with routine monthly cycles of platinum analogue chemotherapy. As part of therapy planning, ovarian cancer cells from malignant ascitic fluid or pleural effusions might be incubated with various combinations of cisplatin and ^{32}P-CP so as to document for individual patient use the potential efficacy of the planned therapy.

MATERIALS AND METHODS

In vitro enhancement of ^{32}P-CP cytotoxicity by cisplatin was tested in triplicate in CHOA cell lines (362-1 or FriM) in addition to a fibroblast cell strain. Cell line 362-1 is rapidly growing while FriM is slowly growing. These two cell lines were chosen to reflect the heterogeneity of growth characteristics found in human ovarian adenocarcinoma. Each cell system was incubated for either 7 or 14 days under the following conditions:

1. plain culture media.
2. varying doses of ^{32}P-CP (0.3–6.0 μCi/ml).
3. varying doses of cisplatin (0.02–20.00 μg/ml).
4. LD50 concentrations of cisplatin (as determined from experiment 3) and varying doses of ^{32}P-CP (0.5–1.5 μCi/ml).

Furthermore, once the enhanced cell-killing effect of cisplatin on ^{32}P-CP irradiation was noted, the dependence of this effect upon the time when therapeutic agents were introduced into tissue culture (time sequence of cisplatin and ^{32}P-CP administration) was investigated in three different experiments: LD50 concentration of cisplatin was added to CHOA cell line 362-1 at 6 hr before, at the same time and 48 hr after a 1-μCi/ml dose of ^{32}P-CP.

The FriM CHOA cell line, a papillary serous cystadenocarcinoma, was established in this laboratory. Cells were grown in 50% Waymouth media MB 752-1, diluted with 30% Gey's balanced salt solution and supplemented with 20% fetal bovine serum (GIBCO, Grand Island, NY). The fibroblast cell strain (ElCo) was also established in this laboratory and grown in Waymouth media MB 752-1, supplemented with 10% fetal bovine serum. The 362-1 ovarian cancer cell line was obtained from Dr. Peter Schrier (University Hospital Leiden, Leiden, The Netherlands). This cell line was grown in Waymouth media MB 752-1, supplemented with 10% bovine serum, with the addition of 1 m*M* of L-asparagine (GIBCO). The continuous cultivation of the cells and all the experiments were performed in 25-cm^2 T-flasks (Corning, Corning, NY) and incubated at 37°C. For maintaining optimum cell growth conditions, cells were passaged weekly using 0.5% trypsin, with 5.3 m*M* of EDTA respectively. The media was changed two times per week.

Cell growth was assessed at specified intervals using the hemocytometer technique. Cell viability was determined by trypan blue dye exclusion. Cells in the supernatant were also included. Results are reported as percent of surviving cells after the specified treatment.

Thermoluminescence dosimetry (TLD) discs were used to verify the delivery of the expected radiation doses to the cells exposed to the different μCi/ml concentrations of ^{32}P-CP for the specified incubation times. TLD discs were taped to the bottom of the culture flasks so as to be directly opposite the ovarian tumor cell surfaces throughout the incubations.

Thirty patients with metastatic ovarian carcinoma were treated with fractionated low dose intraperitoneal ^{32}P-CP instillations (plus pleural space instillations for six patients) in conjunction with up to eight monthly cycles of cisplatin (100 mg/m^2) or the pharmacologically equivalent dose of carboplatin (360 mg/m^2). No patients with multiple malignancies were included in this report. Patients gave informed consent for these FDA-approved therapies. Table 1 describes the International Federation of Gynecology and Obstetrics (FIGO) staging of ovarian cancer with Table 2 showing the stage and grade for the 30 patients. Nine of the 30 patients had previously undergone multi-drug chemotherapy. Based on recent surgical exploration or CT imaging, 16 of the Stage III or Stage IV patients were known to have residual intraperitoneal disease measuring greater than 2 cm in greatest diameter.

For treatments, patients were hospitalized for 2 days each month, receiving chemotherapy in the late evening of the first day and ^{32}P-CP intraperitoneal instillations the next morning. Patients received premedication consisting of Ativan (lorazepam), Zofran (ondansetron), Benadryl (diphenhydramine) and prednisone. Zofran was administered at 0 hr, 4 hr and 8 hr for emesis control. Repeated monthly peritoneal cavity access was achieved either by a subcutaneously placed peritoneal access catheter (placed at the time of the prior ovarian cancer surgery) or by puncture of the

TABLE 1
FIGO Staging of Ovarian Cancer

Stage	Characteristics
I	Growth limited to one ovary.
IA	Growth limited to one ovary; no ascites.
IA_1	No tumor on the external surface; capsule intact.
IA_2	Tumor present on the external surface, and/or capsule ruptured.
IB	Growth limited to both ovaries; no ascites.
IB_1	No tumor on the external surface; capsules intact.
IB_2	Tumor present on the external surface and/or capsule(s) ruptured.
IC	Tumor either Stage IA or IB, but with ascites present or with positive peritoneal washings.
II	Growth involving one or both ovaries with pelvic extension.
IIA	Extension and/or metastases to the uterus and/or tubes.
IIB	Extension to other pelvic tissues.
IIC	Tumor either Stage IIA or Stage IIB, but with ascites present or with positive peritoneal washings.
III	Growth involving one or both ovaries with intraperitoneal metastases outside the pelvis, or positive retroperitoneal nodes, or both. Tumor limited to the true pelvis with histologically proven malignant extension to small bowel or omentum.
IV	Growth involving one or both ovaries with distant metastases. If pleural effusion is present there must be positive cytology to allot a patient to Stage IV. Parenchymal liver metastases equals Stage IV.

peritoneal cavity by introduction of a 20-gauge angiocath needle. All peritoneal access procedures were performed by an experienced gynecologic surgeon (R.A.P.).

After accessing the peritoneal cavity, a minimum of 100 ml of normal saline was injected followed by 1 mCi of ^{99m}Tc-sulfur colloid. For patients without ascites, larger volumes of normal saline (optimally 1 liter) were used. The patient's abdomen was gently palpated to induce mixing of the injected imaging dose of ^{99m}Tc-sulfur colloid and images were obtained to confirm free distribution throughout the peritoneal cavity. If a diffuse intraperitoneal distribution was not obtained with the initial ^{99m}Tc-sulfur colloid injection, additional injections in other quadrants were performed to find the optimal site for injection of the therapeutic dose of 5 mCi of ^{32}P-CP. If, however, localizing adhesions prevented broad distribution, the 5-mCi ^{32}P-CP dose was divided before injection into each quadrant. For patients with no evidence of loculation, intraperitoneal injection of the ^{32}P-CP was followed by injection of no less than 150 ml and no more than 1,000 ml of normal saline. This dose of normal saline was titrated based on presence of ascites and was appropriately divided for injection into multiple (up to four) quadrants in patients with loculations.

TABLE 2
Ovarian Cancer Stage and Grade for 30 Patients

Stage	Number of patients
II	5
III	17
IV	8
Grade	
1	7
2	4
3	19

After withdrawal of the angiocath, the patient's abdomen was vigorously palpated and the patient turned from side to side. The patient was then rotated at hourly intervals for 24 hr from Trendelenburg to Fowler's positions and to right and left lateral decubitus positions. An electronically operated rotating bed was used for this purpose. Diffused peritoneal distribution of ^{32}P-CP was confirmed the next day by ^{32}P Bremsstrahlung scanning as previously described (*12*).

As part of the planning for the above described therapy, incubations of ovarian carcinoma cells obtained from the individual patient's own malignant abdominal ascites or pleural effusions were carried out for the first 10 patients in this study. The harvested cancer cells from ascitic or pleural fluids were examined and counted by a hemocytometer cell count both immediately after removal from the patient and 14 days following incubation with LD50 concentrations of cisplatin and ^{32}P-CP (using LD50 concentrations for the 362-1 CHOA cell line).

Response of the 30 patients to treatment was measured by CA-125 ovarian tumor antigen when positive, CT scan evidence of tumor status, physical exam and survival time. Toxicity was measured by hematologic evaluation of complete blood counts including WBC and platelet nadirs, as well as clinical chemistry responses of liver and kidney functions and clinical evidence of nausea, vomiting, signs of GI complications including diarrhea or signs of bowel obstruction by abdominal x-rays when indicated.

RESULTS

For the 362-1 CHOA cell line, a ^{32}P-CP LD-50 of 1 μCi/ml and cisplatin LD-50 of 0.4 μg/ml was observed (Figs. 1 and 2). Furthermore, the addition of cisplatin at LD50 concentration to FriM CHOA cell line enhanced the ^{32}P-CP irradiation effect at the 0.5-μCi/ml, 1.0-μCi/ml and 1.5-μCi/ml concentrations ($p < 0.01$) (Fig. 3). This enhanced 362-1 CHOA cell killing was greater when cisplatin was added 6 hr before rather than at the same time, or 48 hr after ^{32}P-CP ($p < 0.01$) (Fig. 4). In addition, there was a 79% or greater survival fraction of the normal fibroblast cell strain when exposed under these experimental conditions to LD-50 doses of ^{32}P-CP and cisplatin either alone or in combination (Fig. 5). Furthermore, simultaneously studied CHOA FriM showed substantially greater toxicity when exposed to these same conditions ($p < 0.0005$) (Fig. 5).

TLD discs verified the delivery of the expected radiation doses to the CHOA cell lines that had been exposed to ^{32}P-CP. For these TLD determinations, there was a linear relationship (R-squared = 0.978) between the μCi/ml of

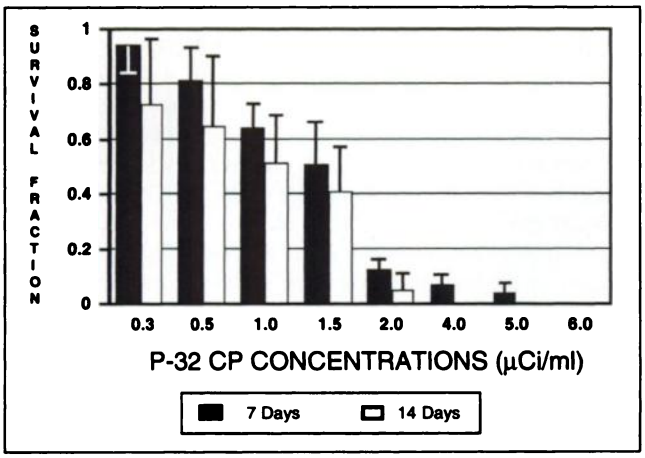

FIGURE 1. Survival fraction at 7 and 14 days of CHOA cells (362-1) in varying concentrations of ^{32}P-CP. LD50 of ^{32}P-CP at 14 days is 1.0 μCi/ml.

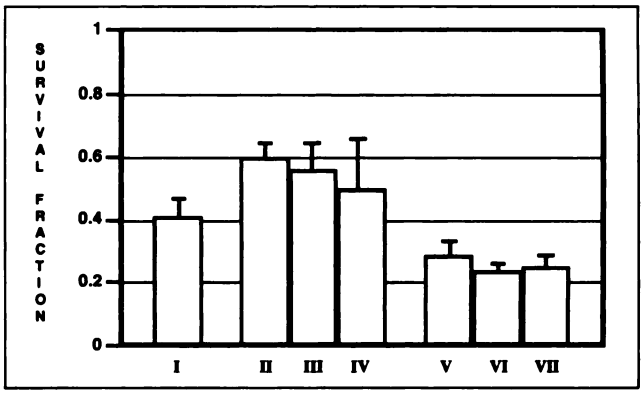

FIGURE 3. Survival fraction at 14 days of CHOA cells (FriM) in both LD-50 cisplatin and various concentrations of ^{32}P-CP. I = LD50 cisplatin; II = 0.5 μCi/ml ^{32}P-CP; III = 1.0 μCi/ml ^{32}P-CP; IV = 1.5 μCi/ml ^{32}P-CP; V = LD50 cisplatin + 0.5 μCi/ml ^{32}P-CP; VI = LD50 cisplatin + 1.0 μCi/ml ^{32}P-CP; and VII = LD50 cisplatin + 1.5 μCi/ml ^{32}P-CP.

^{32}P-CP placed in the tissue culture flasks and the TLD readings.

Results with malignant ascitic and pleural fluid specimens obtained from the first 10 patients also demonstrate an enhanced, and possibly supra-additive, effect between ^{32}P-CP and cisplatin. At the previously determined LD-50 doses of cisplatin and ^{32}P-CP (based on 362-1 CHOA results described above), 17 incubation experiments using ovarian cancer cells obtained from 10 patients showed that the cells survived at a rate of 78% ± 22% (mean 1 ± s.d.) in the presence of cisplatin alone, 92% ± 26% in the presence of ^{32}P-CP alone, and only 34% ± 18% in the presence of both cisplatin and ^{32}P-CP (Fig. 6). These results indicate that when the combination of cisplatin with continuous low-dose rate beta-minus irradiation from ^{32}P-CP was used, the cell killing was in the range of eight times greater than when ^{32}P-CP was used alone. Cell killing was also in the range of approximately two to three times greater than when cisplatin was used alone.

These results suggest a supra-additive effect between continuous low dose rate beta-minus radiation and cisplatin. Particularly, assuming independent action of cisplatin and beta-minus irradiation, the expected surviving fraction for this combination should be the product of the surviving fractions for ^{32}P-CP alone (0.92) and cisplatin alone (0.78). This would yield an expected surviving fraction for the combination of ^{32}P-CP and cisplatin of 0.72. The observed surviving fraction of 0.34 suggests a supra-additive effect (*29–31*). However, in absence of proof that dose-response curves are linear or can be made linear with a mathematical transformation, claims for a supra-additive effect with combined chemotherapy and irradiation are difficult to establish. In fact, as has been pointed out by Steel, "It would not be unreasonable in this situation to maintain that since the agents are not additive with themselves, one has no basis for expecting them to be additive in combination" (*29*). All 10 patients initially studied by incubation of their own malignant ascitic or pleural fluid tumors showed evidence of this cisplatin-induced enhancement of ^{32}P-CP cytotoxicity.

Once ovarian cancer cells from the first 10 patients had

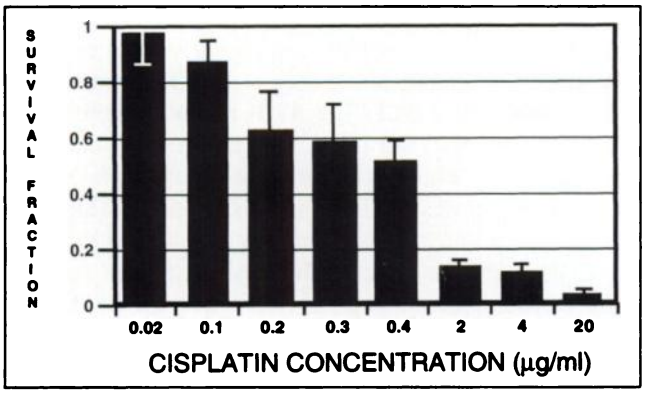

FIGURE 2. Survival fraction at 7 days of CHOA cells (362-1) in varying concentrations of cisplatin. LD50 of cisplatin at 7 days is 0.4 μg/ml.

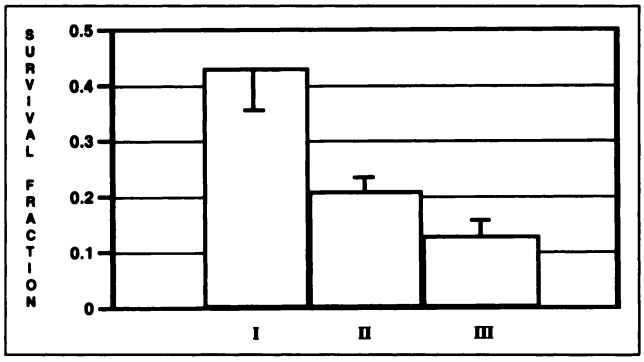

FIGURE 4. Dependence of the survival fraction at 14 days of CHOA cells (362-1) on time sequence of cisplatin and ^{32}P-CP administration. I = cisplatin 48 hr after ^{32}P-CP; II = simultaneous administration; and III = cisplatin 6 hr before ^{32}P-CP (II and III are significantly different, $p < 0.01$).

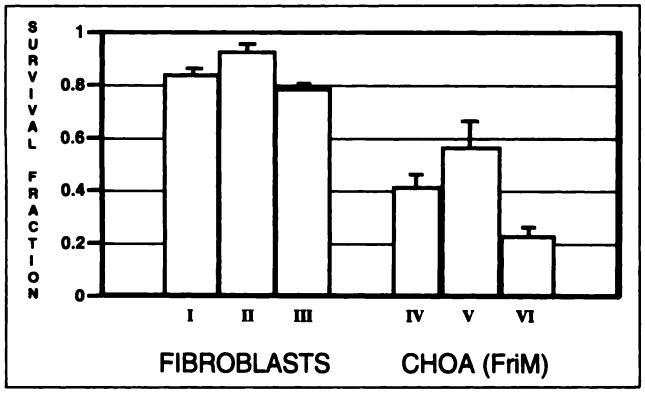

FIGURE 5. Survival fraction at 14 days of both a normal fibroblast cell strain and CHOA cells (FriM) in LD50 concentrations of cisplatin and/or ^{32}P-CP. I vs IV in LD50 cisplatin ($p < 0.0005$); II vs V in LD50 ^{32}P-CP ($p < 0.0005$); and III vs VI in LD50 cisplatin and LD50 ^{32}P-CP ($p < 0.01$).

shown the expected enhanced response to combined cisplatin and ^{32}P-CP therapy, an additional 20 patients were treated at a standard dose of 100 mg/m^2 of cisplatin or 360 mg/m^2 of carboplatin and 5 mCi of ^{32}P-CP at each monthly chemotherapy cycle. Stage IV ovarian cancer patients received up to eight treatments. However, in the presence of loculations, fewer treatments might be given with the patients continuing on chemotherapy alone. With a particularly favorable initial clinical response, some patients did not complete the full course of treatments. The goal for these patients was six ^{32}P-CP treatments with two additional treatments if disease persisted and the presence of abdominal loculations did not severely limit the distribution of the therapeutic radionuclide.

For Stage II and III ovarian cancer patients, the treatment protocol was similar except that the goal for these patients was six combined platinum analogue chemotherapy and ^{32}P-CP treatments with no provision for additional ^{32}P-CP treatments. Nine of the 30 patients received six or more ^{32}P-CP treatments.

For 25 of the 30 patients, peritoneoscintigraphy performed at the time of the ^{32}P-CP therapies showed free distribution throughout the peritoneal cavity. Three patients had at least one image showing a radionuclide distribution limited to approximately half of the peritoneal cavity and two patients had at least one image showing a very limited distribution typical of loculation in a small space.

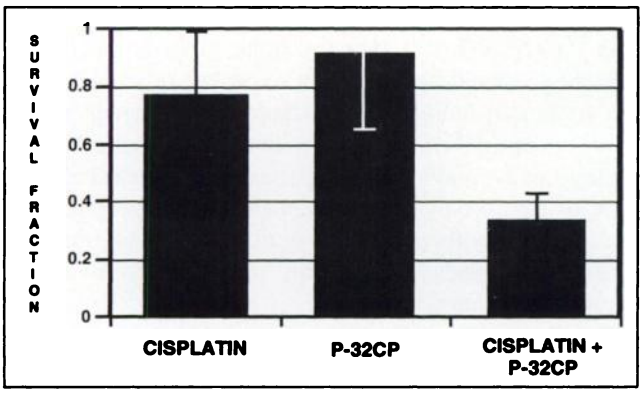

FIGURE 6. Survival fraction of ovarian cancer cells harvested from 10 individual patients. Concentrations of ^{32}P-CP and cisplatin are 1 μCi and 0.4 μg/ml respectively.

TABLE 3
Therapeutic Response for 30 Ovarian Cancer Patients

	Stage				
	II	III	IV	Total	%
Number of patients	5	17	8	30	100
Complete response	4	6	4	14	47
Partial response	0	10	2	12	40
No response	1	1	2	4	13

The results of the total clinical experience with 30 ovarian cancer patients showed a complete response in 14 of the patients (no evidence of disease); partial response in 12 (50% or greater reduction of measurable disease on CT or 50% or greater decrease in CA-125 antigen) and no response in 4 (Table 3). Beta-minus irradiation from ^{32}P-CP, combined with platinum analogue chemotherapy was associated with nausea and vomiting (at least one episode for 16 of 30 patients); WBC nadirs below 3,000/mm^3 (18 patients); and platelets less than 100,000/mm^3 (9 patients). There were no instances of bowel perforation or obstruction attributed to ^{32}P-CP. One bowel obstruction and perforation secondary to cancer recurrence was due to tumor penetration through the colon wall.

There were no significant prolonged temperature elevations and there were no other bowel obstructions which required surgical intervention. Six patients underwent laparotomy or laparoscopy after therapy. Random peritoneal biopsies showed only minor fibrosis with no significant toxic radiation changes. Of the 30 patients treated, 9 died of their disease and two died of other causes (Table 4). Nineteen of the 30 patients remain alive, 11 with no evidence of disease and 8 with disease under continuing salvage chemotherapy. At 3 yr, 63% survival is observed with a mean survival time of 17.1 mo (Table 5). For the 16 patients with residual disease measuring greater than 2 cm in greatest diameter, the median survival at 3 yr of 18.8 mo was not significantly different from the 15.2 mo median survival at 3 yr experienced by the 14 other patients in this study.

TABLE 4
Survival for 30 Ovarian Cancer Patients

	Stage			
	II	III	IV	Total
Number of patients	5	17	8	30
No evidence of disease	4	5	2	11
Alive with disease	0	5	3	8
Died of disease	1	6	2	9
Died other cause	0	1	1	2

TABLE 5
Survival for 30 Ovarian Cancer Patients at Three Years*

Stage	Patients surviving	Percent surviving
II	4 of 5	80%
III	10 of 17	59%
IV	5 of 8	63%
All stages	19 of 30	63%

*Median survival 17.1 mo.

DISCUSSION

The goal of combined ^{32}P-CP and platinum analogue chemotherapy is to achieve an enhancement in ovarian cancer cell kill without increasing the clinically significant toxicity for the patient. Prior in vitro, animal model and human studies, which combined cisplatin with various forms of radiation, observed enhancement in cell kill ranging from none to marked (*30,32–41*). DNA is thought to be the most important target for both radiation and cisplatin, and a theoretical model examining the probability of interactions occurring between cisplatin-induced DNA lesions and radiation-induced DNA lesions has been proposed (*41*). As has been pointed out by Fu (*42*), possible mechanisms and interactions between chemotherapy and radiotherapy which result in enhanced cell kill include:

1. changes in the slope of the dose-response curve;
2. decreased accumulation or inhibition of repair of sublethal damage;
3. decreased recovery from potentially lethal damage;
4. perturbation of cell kinetics with an increased portion of cells in the sensitive cell cycle and proliferative state;
5. decreased tumor bulk and improved blood supply leading to reoxygenation and recruitment and increased radiosensitivity and chemosensitivity; and
6. increased drug delivery and uptake.

It is particularly likely that cisplatin increases the slope of radiation dose-response curves in mammalian cells (*42*). Working with continuous low-dose rate irradiation and concurrent infusion of chemotherapeutic drugs in murine models, researchers have reported a cisplatin-induced enhancement of radiation effects (*30,42*). A variety of other factors which might influence this enhancement include the time sequence for drug and radiation administration and the radiation dose/fractionation schedule (*36*).

This investigation reports that cisplatin enhances the tumor cell killing effect of ^{32}P-CP both for CHOA cell lines and for ovarian carcinoma cells isolated from individual patients undergoing therapy planning. In theory, such enhancement might be additive, subadditive or supra-additive (*29*). As described in the Results section, the magnitude of cell kill for this therapeutic combination for ovarian carcinoma cells from individual patients is greater than would be expected if the effects of cisplatin and ^{32}P-CP were additive and independent: this implies a supra-additive form of enhancement.

This investigation also found that the effect of ^{32}P-CP on the 362-1 CHOA cell line was greater when cisplatin was added 6 hr before rather than at the same time or 48 hr after ^{32}P-CP. It is known that cisplatin-DNA adducts may take several hours before reaching a maximum concentration and, furthermore, that the cisplatin-induced DNA lesions can remain present for several days (*41,43–45*). If it is assumed that both cisplatin-DNA adducts and radiation-induced DNA breaks must be present simultaneously for enhancement to occur, it then follows that adding cisplatin to the tissue culture flasks approximately 6 hr before exposing 362-1 CHOA to ^{32}P-CP would increase the enhancement effect.

In clinical practice, we used fractionated 5-mCi intraperitoneal doses of ^{32}P-CP up to a total after eight treatment cycles of 40 mCi. To account for the intraperitoneal volume and uneven distribution, the 1-μCi/ml flask dose (the LD50 dose at which enhancement occurred in vitro) was extrapolated to a total dose of 5 mCi for patients. The 5-mCi dose also is less than the 10–20 mCi single intraperitoneal instillations commonly used elsewhere in current clinical practice. After eight ^{32}P-CP and cisplatin chemotherapy cycles, the maximum total cumulative dose of 40 mCi of ^{32}P-CP is less than the maximum doses reported in the scientific literature (*6*).

The observed toxicities for the 30 patients in this study are similar to what has been reported with platinum analogue chemotherapy alone: nausea and vomiting (16 patients), WBC nadirs below 3000/mm^3 (18 patients) and platelets less than 100,000/mm^3 (9 patients). Among the 30 patients, the one instance of bowel obstruction and perforation was secondary to cancer recurrence with tumor penetration through the colon wall. We also note that all 30 patients had prior surgery, with 9 patients having prior multiple drug chemotherapy. It is difficult to determine with certainty which portion of the observed toxicities was (1) due to prior surgery or chemotherapy; (2) would have occurred with platinum analogue chemotherapy alone; (3) would have occurred with ^{32}P-CP therapy alone; or (4) should be ascribed to the combined use of platinum analogue chemotherapy and ^{32}P-CP. Because prior experience with ^{32}P-CP indicates that the bone marrow receives a clinically insignificant radiation exposure (*3,46–48*), one can argue that the observed hematologic toxicities would have occurred with platinum analogue chemotherapy alone. Finally, while only six patients underwent laparoscopy or laparotomy after combined ^{32}P-CP and platinum analogue chemotherapy, it is worth noting that random peritoneal biopsies showed only minor fibrosis with no significant radiation changes.

Careful attention to instillation of ^{32}P-CP within the peritoneal cavity so as to avoid loculation may have been responsible for the apparent absence of clinically significant ^{32}P-CP toxicities in this series. Others have previously reported that the use of peritoneoscintigraphy, introduc-

tion of fluid volumes sufficient to produce a free and diffuse radionuclide distribution throughout the peritoneal cavity (particularly important in patients without ascites) and vigorous palpation of the abdomen with changes in patient positioning can contribute to a ^{32}P-CP distribution throughout the peritoneal cavity and a lower incidence of complications (*12,49–55*). We also note that all of our peritoneal access procedures were performed by an experienced gynecological surgeon (R.A.P.). For a single 5-mCi ^{32}P-CP therapy, patients would receive as many as four punctures of the peritoneal cavity with a 20-gauge angiocath needle in an attempt to find either an optimal site for injection or to divide the ^{32}P-CP dose between loculated intraperitoneal spaces. This technique for instillation of ^{32}P-CP in this series should be compared with prior clinical practice in which 10–20 mCi of ^{32}P-CP was often injected at one site. Clearly, the risk of injecting a larger dose of ^{32}P-CP into a loculated space, a procedure which has been associated with complications, was minimized by our treatment protocol and procedures.

A majority (70%–75%) of ovarian cancer cases are advanced when first diagnosed. In one study, Stage III and Stage IV patients with residual disease of 2 cm or greater after surgical debulking had only a 15% survival at 4 yr and an 8% survival at 5 yr when treated with a cisplatin-based regimen (*56*). In this study, the authors present a survival curve indicating that for patients with advanced ovarian adenocarcinoma treated in this fashion, survival at 36 mo was between 25% and 30%. Two subsequent studies respectively found 20% and 18% survivals at 5 yr for patients with advanced ovarian carcinoma treated with similar cisplatin-based regimens (*57,58*). The survival rate of 63% at 3 yr in the current study of advanced stage patients receiving ^{32}P-CP and platinum analogue chemotherapy is encouraging.

Based on these initial laboratory investigations and limited clinical findings, we conclude that ^{32}P-CP fractionated low-dose intraperitoneal treatments in conjunction with cisplatin or carboplatin chemotherapy is a promising approach for the treatment of disseminated intraperitoneal ovarian carcinoma. This combination may be more effective than ^{32}P-CP alone as a single agent for consolidation therapy (*21*). Prospective controlled clinical trials of this combined ^{32}P-CP and platinum analogue chemotherapy approach to ovarian cancer are being developed.

REFERENCES

1. Boring CC, Squires TS, Tong T. Cancer statistics, 1993. *CA Cancer J Clin* 1993;43:7–26.
2. Burghardt E, Girardi F, Lahousen M, Tamussino K, Stettner H. Patterns of pelvic and paraaortic lymph node involvement in ovarian cancer. *Gynecol Oncol* 1991;40:103–106.
3. Root SW, Tyor MP, Andrews GA, Kniseley RM. Distribution of colloidal radioactive chromic phosphate after intracavitary administration. *Radiology* 1954;63:251–257.
4. Van Den Brenk HAS, Clarke KH, Holman WP, Winkler C. Studies of the effect of colloidal radioactive chromic phosphate (Cr-$^{32}PO_4$) in clinical and experimental malignant effusions. *Br J Cancer* 1959;13:181–194.
5. Card RY, Cole DR, Henschke US. Summary of ten years of the use of radioactive colloids in intracavitary therapy. *J Nucl Med* 1960;1:195–198.
6. Hester LL, White L. Radioactive colloidal chromic phosphate in the treatment of ovarian malignancies. *Am J Obstet Gynecol* 1969;103:911–918.
7. Hilaris BS, Clark DGC. The value of postoperative intraperitoneal injection of radiocolloids in early cancer of the ovary. *AJR* 1971;1112:749–754.
8. Clark DGC, Hilaris B, Roussis C, Brunschwig A. The role of radiation therapy (including isotopes) in the treatment of cancer of the ovary (results of 614 patients treated at Memorial Hospital, New York, NY). *Prog Clin Cancer* 1973;5:227–235.
9. Alderman SJ, Dillon TFF, Kremmerman MS, Phillips BP, Chung AF. Postoperative use of radioactive phosphorous in stage I ovarian carcinoma. *Obstet Gynecol* 1977;49:659–662.
10. Julian CG, Inalsingh A, Burnett LS. Radioactive phosphorus and external radiation as an adjuvant to surgery for ovarian carcinoma. *Obstet Gynecol* 1978;52:155–160.
11. Pezner RD, Stefens KR, Tong D, Allen CV. Limited epithelial carcinoma of the ovary treated with curative intent by the intraperitoneal instillation of radiocolloids. *Cancer* 1978;42:2563–2571.
12. Kaplan WD, Zimmerman RE, Bloomer WD, Knapp RC, Adelstein SJ. Therapeutic intraperitoneal ^{32}P. A clinical assessment of the dynamics of distribution. *Radiology* 1981;138:683–688.
13. Piver MS, Barlow JJ, Lele SB, Bakshi S, Parthasarathy KL, Bender M. Intraperitoneal chromic phosphate in peritoneoscopically confirmed stage I ovarian adenocarcinoma. *Am J Obstet Gynecol* 1982;144:836–840.
14. Piver MS, Lele SB, Bakshi S, et al. Five and ten year estimated survival and disease-free rates after intraperitoneal chromic phosphate; stage I ovarian adenocarcinoma. *Am J Clin Oncol* 1988;11:515–519.
15. Varia M, Rosenman J, Venkatraman S, et al. Intraperitoneal chromic phosphate therapy after second-look laparotomy for ovarian cancer. *Cancer* 1988;61:919–927.
16. Potter ME, Partridge EE, Shingleton HM, et al. Intraperitoneal chromic phosphate in ovarian cancer: risks and benefits. *Gynecol Oncol* 1989;32: 314–318.
17. Spencer TR, Marks R, Fenn JO, et al. Intraperitoneal P-32 after negative second-look laparotomy in ovarian carcinoma. *Cancer* 1989;63:2434–2437.
18. Young RC, Walton LA, Ellenberg S, et al. Adjuvant therapy in Stage I and Stage II epithelial ovarian cancer: results of two prospective randomized trials. *N Eng J Med* 1990;322:1021–1027.
19. Walton LA, Yadusky A, Rubinstein L. Intraperitoneal radioactive phosphate in early ovarian carcinoma: an analysis of complications. *Int J Radiat Oncol Biol Phys* 1991;20:939–944.
20. Soper JT, Berchuck A, Clarke-Pearson DL. Adjuvant intraperitoneal chromic phosphate therapy for women with apparent early ovarian carcinoma who have not undergone comprehensive surgical staging. *Cancer* 1991;68: 725–729.
21. Peters WA, Smith MR, Cain JM, Lee RB, Yon Jr, JL. Intraperitoneal P-32 is not effective consolidation therapy after a negative second-look laparotomy for epithelial carcinoma of the ovary. *Gynecol Oncol* 1992;47:146–149.
22. Rogers L, Varia M, Halle J, et al. Phosphorus-32 following negative second-look laparotomy for epithelial ovarian cancer. *Gynecol Oncol* 1993;50:141–146.
23. Van Nostrand D, Silberstein EB. Therapeutic uses of ^{32}P. In: Freeman LM, Weissmann HS, eds. *Nucl Med Ann* New York: Raven Press; 1985:285–344.
24. Advanced Ovarian Cancer Trialists Group. Chemotherapy in advanced ovarian cancer: an overview of randomized clinical trials. *BMJ* 1991;303: 884–893.
25. Swenerton K, Jeffrey J, Smart G, et al. Cisplatin-cyclophosphamide versus carboplatin-cyclophosphamide in advanced ovarian cancer: a randomized phase III study of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 1992;10:718–726.
26. Pattillo RA, Abdel-Dayem HM, Ruckert AC, Ozker K, Collier BD. Phosphorus-32 chromic phosphate irradiation and cis-diamminedichloroplatinum in cultured human ovarian adenocarcinoma. *Radiology* 1990;177:185.
27. Pattillo RA, Abdel-Dayem HM, Ozker K, Ruckert AC, Wilson CR, Collier BD. Supraadditive effect of P-32 chromic phosphate and cisdiaminedichloroplatinum in cultured human ovarian carcinoma. *Radiology* 1991:181–184.
28. Pattillo RA, Collier BD, Ozker K, et al. Supraadditive effect of P-32 chromic phosphate and cis-diamminedichloroplatinum. *J Nucl Med* 1993;34:56P.
29. Steel GG, Peckham MJ. Exploitable mechanisms in combined radiotherapy-chemotherapy: the concept of additivity. *Int J Radiat Oncol Biol Phys* 1979;5:85–91.
30. Fu KK, Rayner PA, Lam KN. Modification of the effects of continuous low dose rate irradiation by concurrent chemotherapy infusion. *Radiat Oncol Biol Phys* 1984;10:1473–1478.

31. Steel GG. The search for therapeutic gain in the combination of radiotherapy and chemotherapy. *Radiother Oncol* 1988;11:31–53.
32. Burholt DR, Schenken LL, Kovacs CJ, Hagemann RF. Response of the murine gastrointestinal epithelium to cis-dichlorodiammineplatinum II: radiation combinations. *Int J Radiat Oncol Biol Phys* 1979;5:1377–1381.
33. Luk KH, Ross GY, Phillips TL, Goldstein LS. The interaction of radiation and cis-diamminedichloroplatinum (II) in intestinal crypt cells. *Int J Radiat Oncol Biol Phys* 1979;5:1417–1420.
34. Douple EB, Richmond RC. Enhancement of the potentiation of radiotherapy by platinum drugs in the mouse tumor. *Int J Radiat Oncol Biol Phys* 1982;8:501–503.
35. Haselow RE, Adams GS, Oken MM, Goudsmit A, Lerner HJ, Marsh JC. Cis-platinum with radiation therapy for locally advanced unresectable head and neck cancer [Abstract]. *Proc Am Soc Clin Oncol* 1983;2:160.
36. Begg AC, Bohlken S, Bartelink H. The effect of cisplatin on the repair of radiation damage in RIFI mouse tumors in vivo. *Radiother Oncol* 1989;15: 79–81.
37. Fu KK, Lam KN, Rayner PA. Effects of continuous low dose rate irradiation and concurrent infusion of mitomycin, cisplatin and 5-fluorouracil on three murine tumors. *Endocurietherapy/Hyperthermia Oncol* 1986;2:157–162.
38. Tannock IF, Brownman G. Lack of evidence for a role of chemotherapy in the routine management of locally advanced head and neck cancer. *J Clin Oncol* 1986;4:1121–1126.
39. Basham C, Mills J, Douple EB, Roberts JJ. The independent action or radiation and cisplatin on the survival and recovery of human normal and tumour cells in vitro or in vivo. *Int J Radiat Biol* 1989;55:807–820.
40. Begg AC, Bohlken S, Barteling H. The effect of cisplatin on the repair of radiation damage in RIFI mouse tumors in vivo. *Radiother Oncol* 1989;15: 79–81.
41. Begg AC. Cisplatin and radiation: interaction probabilities and therapeutic possibilities. *Int J Radiat Oncol Biol Phys* 1990;19:1183–1189.
42. Fu KK. Biological basis for the interaction of chemotherapeutic agents and radiation therapy. *Cancer* 1985;55:2123–2130.
43. Zwelling LA, Kohn KW, Ross WE, Ewig RAG, Anderson T. Kinetics of formation and disappearance of a DNA-cross linking effect in mouse leukemia L1210 cells treated with cis- and trans-diamminedichloroplatinum (II). *Cancer Res* 1978;38:1762–1768.
44. Plooy ACM, van Dijk M, Lohman PH. Induction and repair of DNA cross links in Chinese hamster ovary cells treated with various platinum coordination compounds in relation to platinum binding to DNA, cytotoxicity, mutagenicity and antitumor activity. *Cancer Res* 1984;44:2043–2051.
45. Roberts JJ, Friedlos F. Differential toxicity of cis- and trans-diamminedichloroplatinum (II) toward mammalian cells: lack of influence of any difference in the rates of loss of their DNA-bound adducts. *Cancer Res* 1987;47:31–36.
46. Cooper JA, Zorn EN. Distribution of colloidal radioactive chromic phosphate after intracavitary administration in the rat. *J Lab Clin Med* 1953;42: 867–871.
47. Leichner PK, Rosenhein NB, Leibel SA, Order SE. Distribution and tissue dose of intraperitoneally administered radioactive chromic phosphate (^{32}P) in New Zealand white rabbits. *Radiology* 1980;134:729–734.
48. Leichner PK, Cash SA, Backx C, Durand RE. Effects of injection volume on the tissue dose, dose rate, and therapeutic potential of intraperitoneal P-32. *Radiology* 1981;141:193–199.
49. Taylor Jr, A, Baily NA, Halpern SE, et al. Loculation as a contraindication to intracavitary P-32 chromic phosphate therapy. *J Nucl Med* 1975;16:318–319.
50. Vider M, Deland FH, Maruyama Y. Loculation as a contraindication to intracavitary P-32 chromic phosphate therapy. *J Nucl Med* 1975;17:150–151.
51. Rosenshein N, Blake D, McIntyre PA, et al. The effect of volume on the distribution of substances instilled into the peritoneal cavity. *Gynecol Oncol* 1978;6:106–110.
52. Currie JL, Bagne F, Harris C, et al. Radioactive chromic phosphate suspension: studies on distribution, dose absorption, and effective therapeutic radiation in phantoms, dogs, and patients. *Gynecol Oncol* 1981;12:193–218.
53. Sullivan DC, Harris CC, Currie JL, et al. Observations on the intraperitoneal distribution of chromic phosphate (P-32) suspension for intraperitoneal therapy. *Radiology* 1983;146:539–541.
54. Arnstein NB, Wahl RL, Cochran M, et al. Adenocarcinoma of the alimentary tract: peritoneal distribution scintigraphy. *Radiology* 1987;162:439–441.
55. Tulchinsky M, Eggli DF. Intraperitoneal distribution imaging prior to chromic phosphate (P-32) therapy in ovarian cancer patients. *Clin Nucl Med* 1994;19:43–48.
56. Louie KG, Ozols RF, Myers CE, et al. Long-term results of a cisplatin containing combination chemotherapy regimen for the treatment of advanced ovarian carcinoma. *J Clin Oncol* 1986;11:1579–1585.
57. Sutton GP, Stehman FB, Ehrlich CE, Einhorn LH, Roth LM, Blessing JA. Seven-year follow-up of patients receiving cisplatin adriamycin, and cyclophosphamide (PAC) chemotherapy for stage III and IV epithelial ovarian carcinoma [Abstract]. *Proc Am Soc Clin Oncol* 1986;5:120.
58. Hainsworth JD, Grosh WW, Burnett LS, Jones III HW, Wolff SN, Greco FA: Advanced ovarian cancer: long-term results of treatment with intensive cisplatin-based chemotherapy of brief duration. *Ann Intern Med* 1988;108: 165–170.