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ORIGINAL ARTICLE

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Therapeutic advantage from combining paclitaxel with the hypoxia-selective cytotoxin NLCQ-1 in murine tumor- or human xenograft-bearing mice

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Abstract *Purpose*: The antitumor effect of paclitaxel was investigated against murine tumors and human xenografts in combination with the hypoxia-selective cytotoxin NLCQ-1. *Methods*: The tumor regrowth assay was used as the endpoint and an optimal administration schedule was followed, based on previous studies. In certain cases the hypoxia-selective cytotoxin tirapazamine (TPZ) was included for comparison. NLCQ-1 was given i.p. in saline, whereas paclitaxel was given i.p. (C3H) or i.v. (athymic mice) in an appropriately formulated vehicle. *Results*: In the SCCVII/C3H model, when NLCQ-1 (10 mg/kg) was given 90 min after paclitaxel (8 mg/kg) twice a day 4 h apart on days 0 and 9, tumor regrowth delay was increased by 10.3 days compared to paclitaxel alone, at fivefold the original tumor size. This corresponds to 1.51 log cell kill. In the same study, TPZ resulted in 4.6 days of extra delay compared to paclitaxel alone, which corresponds to 0.91 log cell kill. Paclitaxel alone resulted in 3.9 days of tumor growth delay compared to control, or 0.42 log cell kill, but this delay was not statistically significant ($P < 0.2$). In the FSaIIC/C3H model, when NLCQ-1 (10 mg/kg) was given 90 min after paclitaxel (12 mg/kg) on day 0, tumor regrowth delay was increased by 5.8 days compared to paclitaxel alone, at 20-fold the original tumor size. In athymic nude mice bearing PC-3 prostate xenografts, NLCQ-1 (10 mg/kg) given 90 min before paclitaxel (8 mg/kg) for five consecutive days, increased tumor regrowth delay by 5.6 days compared to paclitaxel alone, at threefold the original tumor size. This corresponds to 0.95 log cell kill whereas the log cell kill for paclitaxel alone was 0.52. No improvement was observed in the tumor regrowth delay at any lower paclitaxel doses given in combination with NLCQ-1. No concurrent enhancement in paclitaxel-induced toxicity was observed in any of the combination treatments or in any of the models tested. NLCQ-1 alone was ineffective at the doses given. *Conclusions*: These results suggest that an enhancement in tumor growth delay can be achieved both in murine tumors and in human xenografts due to a synergistic interaction between NLCQ-1 and paclitaxel.

Keywords NLCQ-1 · Paclitaxel · Combined therapy · Hypoxic cytotoxins · Human xenografts · Murine tumors

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Introduction

One of the recognized microenvironmental features of solid tumors is the occurrence of regions of hypoxia which are resistant to ionizing radiation and chemotherapy and thus can negatively affect cure rates [4, 15, 20, 46]. However, tumor hypoxia also presents opportunities for the use of compounds that are selectively activated under hypoxic conditions, known as bioreductive drugs [50]. Although hypoxia-selective cytotoxins have been traditionally used in combination with radiation, numerous preclinical data suggest that enhanced antitumor activity and often a therapeutic benefit can be obtained when such compounds interact with certain conventional chemotherapeutic agents [6, 7, 12, 13, 21, 22, 23, 31, 34, 35, 36, 47, 48]. Recent clinical trials of the hypoxic cytotoxin tirapazamine (3-amino-1,2,4-benzotriazine-1,4-dioxide, SR-4233, TPZ) combined with cisplatin (cisDDP) have shown that a therapeutic gain can also be achieved in the clinic in terms of overall response rates and survival [27, 52, 54].

4-[3-(2-Nitro-1-imidazolyl)-propylamino]-7-chloroquinoline hydrochloride (NLCQ-1) is a 2-nitroimidaz-

ole-based hypoxia-selective cytotoxin, that does not fall into the category of classical nitroimidazole-containing bioreductive drugs because it possesses two unique characteristics. First, it binds to DNA through a weak intercalation [38]. This permits fast dissociation kinetics, extravascular diffusion, penetration to hypoxic regions of a tumor and thus effectiveness in vivo. Second, it demonstrates increasing hypoxic potency and selectivity with time, like the so-called 'bis-bioreductive agents', compounds bearing two reducible centers [16, 38]. Thus, in agreement with the above, NLCQ-1 synergistically enhances the effect of radiation against hypoxic cells in vitro and murine tumors in vivo [39]. Furthermore, it optimizes the effect of radioimmunotherapy in human xenografts [2]. Importantly, NLCQ-1 substantially enhances, in a schedule-dependent manner, the antitumor effect of alkylating agents, as well as 5-fluorouracil (5FU) and paclitaxel against murine tumors, without a concomitant enhancement in bone marrow or hypoxia-dependent retinal toxicity (unpublished results; [37, 40, 42, 43]). Moreover, studies at the NCI have shown that NLCQ-1 exhibits good stability in human plasma and favorable pharmacokinetics in mice [49].

Potentiation by nitroimidazole-based bioreductive drugs has been traditionally investigated with alkylating chemotherapeutic agents. However, as has been mentioned above, enhancement of tumor response is also observed by combining NLCQ-1 with the thymidylate synthase inhibitor 5FU or the antimitotic drug paclitaxel, against EMT6 and SCCVII tumors in mice [40, 42]. Paclitaxel, because of its different mechanism of action and its significant activity against a variety of cancers, including late-stage ovarian [26], advanced lung [10] and head and neck carcinomas [11], that is tumors with potential hypoxic regions, could be explored as a new opportunity for combination treatments with hypoxia-selective cytotoxins.

In our previous studies between NLCQ-1 and paclitaxel, using the clonogenic assay we demonstrated an optimal synergistic interaction when NLCQ-1 is administered 1–3 h after paclitaxel [40, 42]. In the present work we further investigated interactions between NLCQ-1 and paclitaxel against murine tumors and human xenografts using the tumor regrowth assay, and we demonstrated that tumor specificity and administration time are important factors for paclitaxel potentiation by NLCQ-1. The studies against murine tumors were performed in our laboratory and the studies against human xenografts were performed at the NCI under the RAID (Rapid Access to Intervention Development) Program.

Materials and methods

Drugs

NLCQ-1 (provided by the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute) and TPZ (provided by Sanofi-Winthrop, Malvern, Pa.) were dissolved in saline at 1 and 0.75 mg/ml, respectively. The vehicle for paclitaxel (Bristol-Myers Squibb, Princeton, N.J.) was 8% EtOH plus 8% Cremophor plus 84% saline (murine tumors) or 12.5% EtOH plus 12.5% Cremophor plus 75% saline (human xenografts). In the studies against murine tumors, all drugs were injected intraperitoneally (i.p.) on the basis of animal body weight and the total injected volume was ≤ 0.5 ml. A hyaluronidase solution (Wyeth Laboratories, Philadelphia, Pa.) was administered i.p. to the mice at 15 U/mouse in 0.05 ml saline immediately after paclitaxel administration, as a local adjuvant, to reduce paclitaxel ulcerative toxicity [8]. In the studies against human xenografts, paclitaxel was administered intravenously (i.v.) at 0.1 ml/10 g body weight and NLCQ-1 was given i.p. at 0.1 ml/10 g body weight.

Mice and tumors

FSaIIC tumor cells (2×10^6 cells in 0.2 ml; a gift from Dr. B. Teicher, Dana-Farber Cancer Institute, Boston, Mass.) [51] or SCCVII squamous carcinoma tumor cells (5×10^4 cells in 0.05 ml; a gift from Dr. D. Siemann, University of Florida, Gainesville, Fl.) [18] were inoculated subcutaneously into the leg of 18–20-g male or female C3H mice (Jackson Laboratories), respectively, housed under germ-free conditions. All studies were conducted according to the guidelines set by the Evanston Northwestern Healthcare Institutional Animal Care. Eight mice per group were used.

Subcutaneous human xenografts were established from PC-3 human tumor cell lines (NCI tumor repository at Frederick, Md.) which were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (HyClone). Cells were implanted subcutaneously (s.c.) (1.0×10^7 cells/0.1 ml/mouse) into male athymic nude mice (NCr *nu*/*nu*; obtained from the NCI Animal Program, Frederick, Md.) and on the rib cage near the axilla. Mice were housed in sterile polycarbonate filter-capped Microisolator cages (Laboratory Products), maintained in a barrier facility on a 12-h light/dark cycle, and provided with sterilized food and water ad libitum [44]. There were 10 animals in each treatment group and 20 animals in the control group. The growth of the solid tumors was monitored using in situ caliper measurements to determine tumor mass. Weights (milligrams) were calculated from measurements (millimeters) of two perpendicular dimensions (length and width) using the formula for a prolate ellipsoid and assuming a specific gravity of 1.0 g/cm^3 [14].

Interaction with paclitaxel

Murine tumors

In the studies against FSaIIC tumors, NLCQ-1 was given at 10 mg/kg (0.027 mmol/kg) and TPZ at 30 or 23 mg/kg (0.168 or 0.129 mmol/kg) which represent 28% [37] and 38% or 28% [7] of their single LD_{50} values, respectively. Each bioreductive drug was given once, 3 h or 1.5 h after a single paclitaxel dose. In the studies against SCCVII tumors, NLCQ-1 and TPZ were given twice a day 4 h apart at equitoxic doses of 10 and 23 mg/kg, respectively, at the optimal time intervals (determined previously) after paclitaxel, on day 0, or on days 0 and 9. To assess the response of the tumors to treatment, their size was measured every day or every other day using a vernier caliper. Tumor volumes were calculated using the formula $V=\pi(x\times y\times z)/6$, where x, y and z are the three measured orthogonal diameters minus folded skin thickness (1 mm). At the time of treatment, the mean volumes were in the ranges 225–292 mm^3 or 111–134 mm^3 (FSaIIC) and 58–88 mm^3 (SCCVII). The doubling time of untreated control tumors (Td) was calculated as the median of the time interval for individual tumors to increase in size from 200 to 400 mm^3 (usually a period of exponential growth), and was found to be 2.8 or 2.1 days (FSaIIC) and 2.1 or 2.8 days (SCCVII). Mean tumor volumes (V) were expressed as a fraction of their mean volume on the day of treatment (V_0). Tumor growth delay was determined at a relative tumor volume of 20 (FSaIIC) or 5 (SCCVII). Also, the net log cell kill corresponding to this growth delay was calculated from the formula:

$$\text{Log cell kill} = 0.301 \times [(T - C) - \text{duration of treatment}]/Td \quad (1)$$

where T and C are the median times in days for treated and control groups, respectively, to attain the specified size [44]. The duration of treatment was zero in the experiments with murine tumors. Synergism and additivity were calculated from the log cell kills [56]. Multiple comparisons between groups were performed using Student's *t*-test.

Human xenografts

In the studies against PC-3 xenografts, NLCQ-1 was given i.p. at 10 mg/kg 1.5 h before an i.v. dose of paclitaxel for five consecutive days starting on day 11 after tumor implantation. Paclitaxel was given at three doses: 8, 5.4 and 3.6 mg/kg. On the first day of treatment, the median tumor weights ranged from 46 to 151 mg (early-stage s.c. models). The Td of PC-3 was 3.9 days for this experiment. Tumor size and body weights were obtained approximately twice per week. Antitumor activity was assessed by calculating optimal %T/C values from the formula:

$$\begin{aligned} \%T/C &= (\Delta T/\Delta C) \times 100 \quad \text{where} \quad \Delta T > 0 \quad \text{or} \\ &= (\Delta T/T_I) \times 100 \quad \text{where} \quad \Delta T < 0 \end{aligned} \quad (2)$$

where ΔT and ΔC are changes in tumor weight in treated and control groups, respectively, and obtained by subtracting the median tumor weight on the day of first treatment (staging day) from the median tumor weight on the observation day, and T_I is the median tumor weight at the start of treatment [44]. Tumor growth delay was determined at a relative tumor weight of 3 and net log cell kill was determined from the Eq. 1. Both drug-related deaths (DRDs) and maximum percent relative mean net body weight losses were determined [44].

Results

Two experiments were performed in mice bearing FSaIIC tumors. In the initial experiment (not shown) paclitaxel, NLCQ-1 and TPZ were given once at 20, 10 and 30 mg/kg, respectively, and each bioreductive compound was administered 3 h after paclitaxel. No signs of toxicity were observed in the groups treated with NLCQ-1 or TPZ alone. Unfortunately, 20 mg/kg paclitaxel was too toxic, resulting in > 50% deaths by day 8 in the groups receiving paclitaxel alone or in combination. Even though the extent of mortality at such an early stage did not allow definite conclusions, the results still suggested an additional tumor growth delay in the NLCQ-1 plus paclitaxel combination group compared to the paclitaxel-alone group.

When the paclitaxel dose was decreased to 12 mg/kg and NLCQ-1 and TPZ were administered once in FSaIIC tumor-bearing mice 1.5 h after paclitaxel at equitoxic doses of 10 and 23 mg/kg, respectively, paclitaxel-induced toxicity was still present. Thus, 37.5% deaths occurred in the paclitaxel-alone group and in the NLCQ-1 plus paclitaxel and TPZ plus paclitaxel groups by day 9, 8, and 10, respectively. However, no further lethality or any other type of toxicity occurred until the end of the experiment. The responses of FSaIIC tumors to paclitaxel with and without NLCQ-1 or TPZ treatment are summarized in Table 1. It is apparent that significant tumor growth delay occurred only in the NLCQ-1 plus paclitaxel group. This delay was about 6 days compared to the control group and corresponded to a 0.9 log cell kill, which is not insignificant considering that the paclitaxel was given only once and in an inactive dose.

Since paclitaxel was too toxic at 20 mg/kg and inactive at 12 mg/kg in the experiments described above, in our following experiment against SCCVII tumors paclitaxel was given at 2×8 mg/kg 4 h apart on day 0. NLCQ-1 or TPZ were given at 10 and 23 mg/kg 1.5 and 2 h after paclitaxel, respectively. No signs of toxicity or lethality were observed with this regimen up to day 20 after treatment. The deaths by day 20 were associated with large tumor volumes and occurred in the control group (25%) and the paclitaxel plus NLCQ-1 group (25%). The responses of SCCVII tumors to paclitaxel and paclitaxel plus NLCQ-1 or TPZ are shown in Table 2. Statistically significant tumor growth delay compared to the control group was observed in the paclitaxel-alone, TPZ-alone, paclitaxel plus NLCQ-1 and paclitaxel plus TPZ groups (Table 2). The most pronounced tumor growth delay occurred in the paclitaxel plus NLCQ-1 group (5.7 days or 0.82 log cell kill). Since NLCQ-1 alone did not produce any tumor growth delay, it is apparent that the delay observed in combination with paclitaxel was due to potentiation. Once again, the log cell kill of 0.82 was not very pronounced but it could be considered biologically significant because it occurred with only one treatment. TPZ alone was toxic against SCCVII tumors yielding 1.7 days tumor growth delay. However, in combination with paclitaxel, the corresponding delay was 4.4 days, similar to

Table 1 Time for the FSaIIC tumors to grow to 20 times their original volume (111–134 mm^3), tumor growth delay relative to saline-treated control, and corresponding log kill. Paclitaxel, NLCQ-1 and TPZ were given at 12, 10 and 23 mg/kg, respectively, once on day 0. Eight mice per group were used. The median tumor doubling time was 2.1 days

Treatment group	Time (days, mean ± SD)		Tumor growth delay (days)	Log kill
		P value		
Control (saline)	15.6 ± 3.7		0	–
Paclitaxel	16.5 ± 3.7	> 0.05 vs control	0.9	0.13
NLCQ-1	14.2 ± 2.0	> 0.05 vs control	–1.4	–0.20
TPZ	16.1 ± 2.6	> 0.05 vs control	0.5	0.07
Paclitaxel plus NLCQ-1 1.5 h later	21.9 ± 1.5	< 0.02 vs paclitaxel alone	6.3	0.90
Paclitaxel plus TPZ 1.5 h later	14.1 ± 3.5	> 0.05 vs control	–1.5	–0.22

Table 2 Time for the SCCVII tumors to grow to five times their original volume (62–78 mm^3), tumor growth delay relative to saline-treated control, and corresponding log cell kill. Paclitaxel, NLCQ-1 and TPZ were given twice a day 4 h apart on day 0 at 8, 10 and 23 mg/kg, respectively. Eight mice per group were used. The median tumor doubling time was 2.1 days

Treatment group	Time (days, mean ± SD)		Tumor growth delay (days)	Log kill
		P value		
Control (saline)	7.6 ± 0.6		0	–
Paclitaxel	10.6 ± 1.2	< 0.001 vs control	3	0.43
NLCQ-1	7.7 ± 1.1	> 0.05 vs control	0.1	0.01
TPZ	9.3 ± 0.6	< 0.001 vs control	1.7	0.24
Paclitaxel plus NLCQ-1 1.5 h later	13.3 ± 2.0	< 0.02 vs paclitaxel alone	5.7	0.82
Paclitaxel plus TPZ 2 h later	12.0 ± 0.5	< 0.05 vs paclitaxel alone	4.4	0.63

the delay that would be expected from an additive effect between paclitaxel and TPZ (Table 2).

In a second experiment performed against SCCVII tumors, a regimen similar to that above was followed with the exception that the treatment was repeated on day 9 after the first treatment. Paclitaxel was well tolerated when it was given twice a day 4 h apart on days 0 and 9. Thus, 12.5% lethality (one of eight mice died) was observed by day 14 after treatment in the paclitaxel-alone, the NLCQ-1 plus paclitaxel and the TPZ plus paclitaxel groups, but lethality did not increase thereafter. A death was also observed by day 13 in the TPZ-alone group. However, no other signs of toxicity were observed and the mean mouse weights in all groups were not significantly different. The responses of SCCVII tumors to paclitaxel and NLCQ-1/TPZ plus paclitaxel treatments are shown in Fig. 1. It is apparent that tumor volume decreased significantly after the first treatment in the NLCQ-1 plus paclitaxel group. Thus, on day 6 after treatment the mean tumor volume in the paclitaxel plus NLCQ-1 group was 28% of its initial value (day 0). Similarly, the tumor volume in the same group had decreased after the second treatment (day 9) to 53% of its initial value (day 0). The only other group in which tumor volume loss was seen after the first treatment was the TPZ plus paclitaxel group. Thus, on day 3, the tumor volume in this group had decreased to 85% of its initial value (day 0).

Tumor growth delays and corresponding log cell kills are shown in Table 3. Even though some tumor growth delay was observed in the paclitaxel-alone, the NLCQ-1-alone and the TPZ-alone treated groups compared to the untreated control group, there were no significant differences between each of these groups and the control group ($P > 0.5$). On the contrary, there was significant tumor growth delay between the NLCQ-1 plus paclitaxel and the paclitaxel-alone groups ($P < 0.01$), and between the TPZ plus paclitaxel and the paclitaxel-alone groups

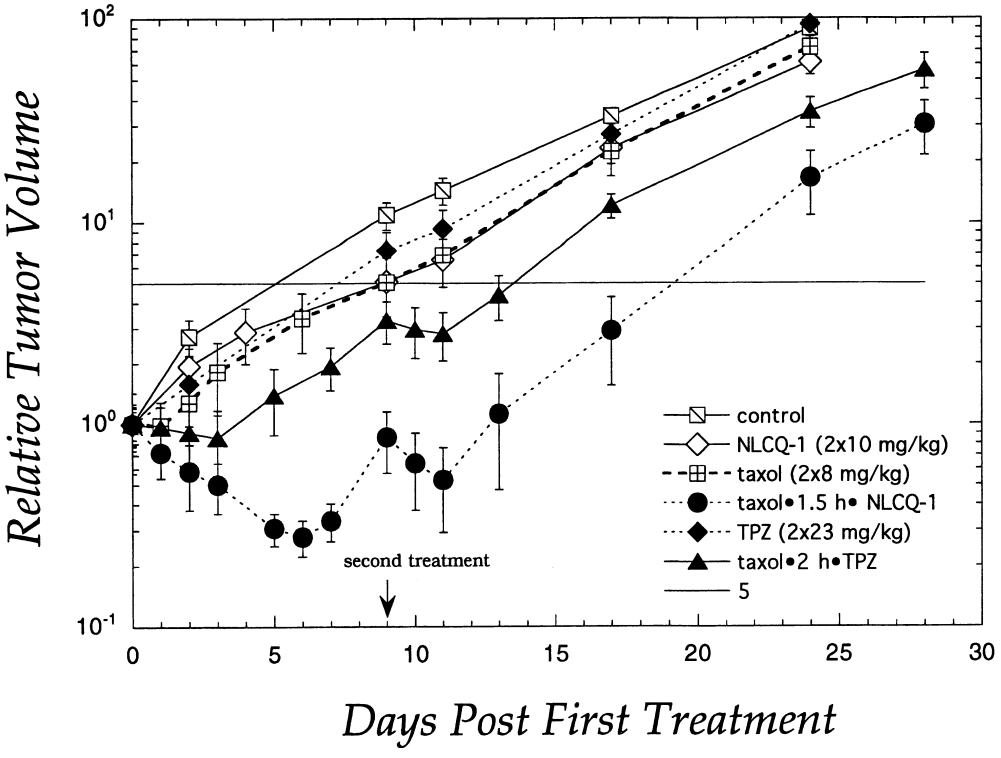

Fig. 1 Response of SCCVII tumors to paclitaxel (*Taxol*), NLCQ-1, TPZ, and paclitaxel plus NLCQ-1 or TPZ. Relative mean tumor volumes are plotted as a function of time after first treatment. NLCQ-1 and TPZ were given at 10 and 23 mg/kg (equitoxic doses) 1.5 or 2 h, respectively, after paclitaxel twice a day 4 h apart on days 0 and 9. Tumor growth delay was evaluated at fivefold the original tumor volume. Initial mean tumor volumes in the groups were: control 68 ± 11 mm^3, NLCQ-1 alone 80 ± 18 mm^3, paclitaxel alone 73 ± 19 mm^3, paclitaxel plus NLCQ-1 72 ± 15 mm^3, TPZ alone 58 ± 25 mm^3, and paclitaxel plus TPZ 88 ± 18 mm^3. Eight mice per point were used (*bars* SD)

Table 3 Time for the SCCVII tumors to grow to five times their original volume (58–88 mm^3), tumor growth delay relative to saline-treated control, and corresponding log cell kill. Paclitaxel, NLCQ-1 and TPZ were given twice a day 4 h apart on day 0 and day 9 at 8, 10 and 23 mg/kg, respectively. Eight mice per group were used. The median tumor growth delay was 2.8 days

Treatment group	Time (days, mean ± SD)		Tumor growth delay (days)	Log kill
		P value		
Control (saline)	5.1 ± 3.2		0	–
Paclitaxel	9.0 ± 4.6	> 0.05 vs control	3.9	0.42
NLCQ-1	9.0 ± 3.0	> 0.05 vs control	3.9	0.42
TPZ	7.2 ± 4.6	> 0.05 vs control	2.1	0.23
Paclitaxel plus NLCQ-1 1.5 h later	19.3 ± 5.2	< 0.01 vs paclitaxel alone	14.2	1.53
Paclitaxel plus TPZ 2 h later	13.6 ± 1.5	< 0.05 vs paclitaxel alone	8.5	0.91

($P < 0.05$). Thus, 14.2 and 8.5 days delay, compared to the control, were seen in the NLCQ-1 plus paclitaxel and the TPZ plus paclitaxel groups, which correspond to 1.53 and 0.91 log cell kills, respectively.

Finally, the responses of PC-3 human xenografts to paclitaxel and paclitaxel plus NLCQ-1 treatments are shown in Fig. 2 and Table 4. All mice had developed tumors by the day of treatment. No significant tumor growth delays were observed at the lower paclitaxel doses alone or in combination with NLCQ-1. Similarly, NLCQ-1 alone did not cause any tumor response. However, paclitaxel at 8 mg/kg daily for 5 days was responsible for 11.7 days of tumor growth delay compared to vehicle treatment. Furthermore, by combining the higher paclitaxel dose with NLCQ-1, 17.3 days of tumor growth delay was achieved. The corresponding log cell kills were 0.52 (paclitaxel alone) and 0.95 (combination treatment). Tumor growth delay and log cell kill were calculated at threefold the original tumor size (Table 4).

Optimal %T/C values were also calculated for all treated groups and are shown together with toxicity parameters in Table 4. Even though this was an early stage experiment, %T/C values were determined as $\%\Delta T/\Delta C$ values because the median tumor weight in the control group was 46 mg, that is < 63–200 mg [44]. No drug-related deaths were observed in any of the treated groups. Maximum percent relative mean net weight losses were similar in the paclitaxel-alone and the paclitaxel plus NLCQ-1 groups, and were also comparable to that of the control group (PC-3-related weight loss).

Discussion

NLCQ-1 represents a newer class of nitroimidazole-based hypoxia-selective cytotoxins that bind noncovalently to DNA through weak intercalation [32, 33, 38]. Such binding offers the advantage of mobility around

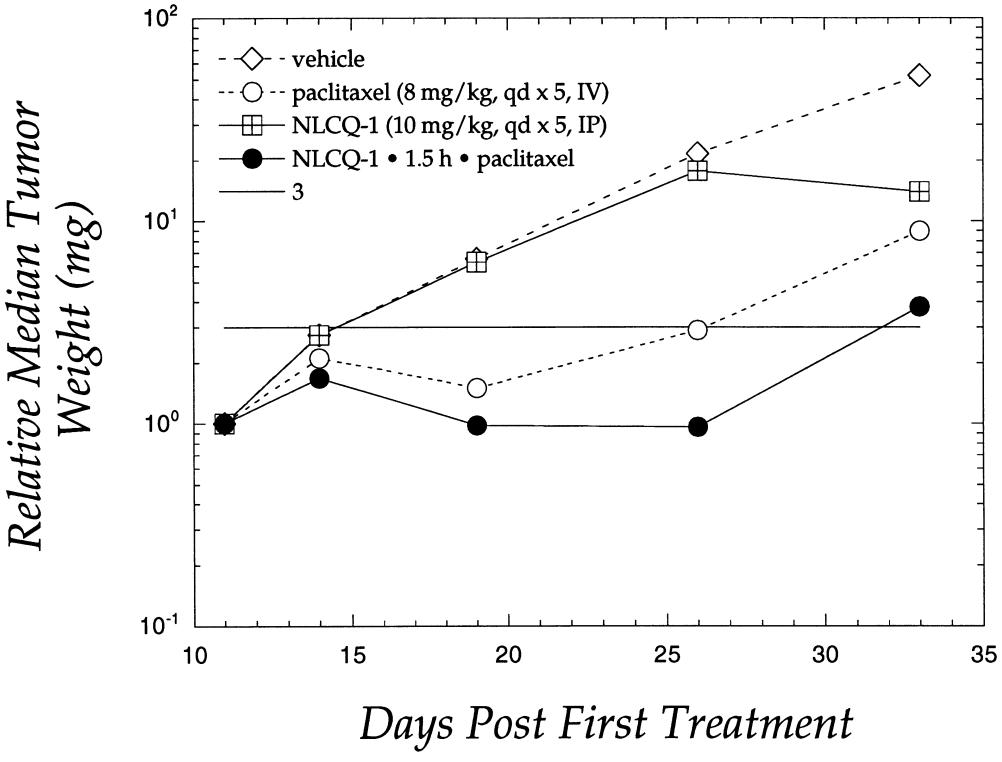

Fig. 2 Response of PC-3 human xenografts to paclitaxel, NLCQ-1, and paclitaxel plus NLCQ-1. Relative median tumor weights are plotted as a function of time. NLCQ-1 was given i.p. daily for 5 days 1.5 h before paclitaxel. Paclitaxel was given i.v. daily for 5 days. Treatment was initiated on day 11. Tumor growth delay was evaluated at threefold the original tumor weight. Initial median tumor weights in the groups were: vehicle 46 mg, paclitaxel alone 146 mg, NLCQ-1 alone 103 mg, and NLCQ-1 plus paclitaxel 151 mg. In the control and treated groups, 20 and 10 mice per point were used, respectively

Table 4 Time for the PC-3 human xenografts to grow to three times their original weight (46–151 mg), and other parameters regarding the effect of the treatment. Treatment was administered daily for 5 days and was initiated on day 11 after xenograft inoculation. Paclitaxel was given i.v. in the vehicle (12.5% EtOH, 12.5% Cremophor, 75% saline). NLCQ-1 was given i.p. and, in combination with paclitaxel, 1.5 h before each paclitaxel dose. Ten mice were used in the treated groups and 20 mice in the control group. The median tumor growth delay was 3.9 days

Treatment group	Median time (days)	Tumor growth delay (days)	Log kill	Optimal %T/C (day)
Control (vehicle)	14.5	–		
Paclitaxel alone (mg/kg)				
8	26.2	11.7	0.52	28 (19)
5.4	16.0	1.5	–0.27	83 (26)
3.6	15.7	1.2	–0.29	99 (19)
NLCQ-1 (10 mg/kg)				
Alone	14.5	0.0	–0.39	57 (33)
Plus paclitaxel 8 mg/kg	31.8	17.3	0.95	–4 (26)
Plus paclitaxel 5.4 mg/kg	16.8	2.3	–0.21	79 (26)
Plus paclitaxel 3.6 mg/kg	16.0	1.5	–0.27	75 (33)

the site of action (DNA) and offers improved hypoxic selectivity because it prevents the hindering of topoisomerases, polymerases or other DNA-interacting enzymes [32] which could cause a nonbioreduction-related toxicity. In addition, binding through weak intercalation may allow better extravascular diffusion as has been shown in the paradigm of 5-nitraquine versus nitracrine [5, 55]. Better penetration translates into greater effectiveness in vivo, provided that the bioreductive compound has an optimal metabolic rate constant [17]. Our present study showed once again that NLCQ-1 can indeed be effective in vivo as a chemosensitizer. From the results against the murine tumors, we can conclude that the interaction between NLCQ-1 and paclitaxel was synergistic [56]. Thus, the log tumor-cell kill in the NLCQ-1 plus paclitaxel group was always greater than the sum of the log cell kills produced by NLCQ-1 and paclitaxel alone (Tables 1, 2, and 3). In the case of the TPZ plus paclitaxel combination treatment, the effect was seemingly additive (see log cell kill in Table 2) or slightly synergistic (Table 3).

In general, the effects of NLCQ-1 and TPZ alone against the murine tumors were insignificant or minimal (Tables 1, 2, and 3), perhaps because of the small initial tumor volumes which could not guarantee sufficient hypoxic fractions. It is known that FSaIIC tumors do not develop significant hypoxia unless they grow above 200 mm^3 [30]. Similarly, SCCVII tumors usually contain about 15–25% radiobiologically hypoxic cells at volumes ranging from 200 to 600 mm^3 [1, 9], whereas the initial tumor volume in our present study ranged from 58 to 88 mm^3. Paclitaxel alone at the nontoxic doses used was also ineffective in the experiments against murine tumors. Thus, the maximum obtained log tumor-cell kill was not more than 0.43 (Table 2). However, in combination with NLCQ-1 this log cell kill was improved to up to 1.53, which is biologically meaningful. A lesser effect (a log cell kill up to 0.91) was observed in combination with TPZ (Table 3). Multiple dosing with sufficient time between doses to allow recovery from paclitaxel-induced systemic toxicity and reestablishment of tumor hypoxia may be the best way to explore the synergistic interaction between paclitaxel and NLCQ-1 in the clinic (Fig. 1).

In the experiment against PC-3 human xenografts, NLCQ-1 alone did not have any effect, even though it was administered at 10 mg/kg for five consecutive days. In this case, the initial small median tumor size (103 mg) in the NLCQ-1-alone group may have been responsible for limited hypoxia, which was taken care of by the initial NLCQ-1 treatments. According to the literature, there is no correlation between stage of breast, cervical or lung cancer and status of oxygenation [19, 45, 53]. However, Brizel et al. have reported a positive correlation between hypoxic fractions and size in sarcomas [3], and Lartigau et al. have reported a significantly lower mean pO_2 in N3 neck nodes than in N2 nodes [24]. There is no information in the literature for an analogous correlation in prostate tumors, and specifically in PC-3 xenografts. However, the presence of hypoxia has been demonstrated in prostate cancer [29] and a study in PC-3 xenografts with an average size of about 750 mm^3 revealed a hypoxic fraction of 52.3 [25].

Paclitaxel alone at the higher dose (8 mg/kg) inhibited tumor growth (%T/C 28 <40) whereas in combination with NLCQ-1 caused tumor stasis (%T/C <0 to –50) from day 19 to day 26 and tumor growth inhibition thereafter (Table 4 and Fig. 2) without prolonged systemic toxicity (mean body loss $\leq 14.4\%$). The interaction between paclitaxel and NLCQ-1 against PC-3 xenografts was synergistic, since the log tumor cell kill produced with the combination treatment (0.95) was greater than the sum of the log cell kills of the two individual drugs (Table 4). With regard to the administration schedule, NLCQ-1 was chosen to be delivered i.p. 1.5 h ahead of paclitaxel (given i.v.) since no optimal administration data were available for this particular tumor. Even though in our previous experiments against murine tumors, enhancement in the tumor cell kill was seen when NLCQ-1 was administered exclusively after paclitaxel [40, 42], to our surprise, administration of NLCQ-1 before paclitaxel in this case proved to be beneficial. This suggests that tumor specificity may be

also important for a synergistic interaction to occur between NLCQ-1 and paclitaxel.

With regard to mechanisms explaining the beyond additivity tumor growth delay, preliminary in vitro results show that enhancement in apoptosis (i.e. increased caspase 3 activation and nucleosome formation), unrepairable DNA damage and persistent inhibition of DNA, RNA and protein synthesis are some of the mechanisms involved in the synergism seen between NLCQ-1 and paclitaxel, when NLCQ-1 was given under hypoxic conditions to V79 cells previously exposed aerobically to paclitaxel [41]. Moreover, since the same schedule-dependent potentiation seen in vitro exists in vivo, at least with regard to murine tumors [42], it is quite possible that the above mechanisms may account in part for the potentiation of paclitaxel in vivo. It is known that paclitaxel causes mitotic arrest and apoptosis, but only the apoptosis and not the mitotic arrest is correlated with the antitumor effect of paclitaxel [28]. It is speculated that NLCQ-1/TPZ may trigger apoptosis in cells arrested in the G_2M-phase.

In conclusion, a therapeutic advantage could be achieved by combining the hypoxia-selective cytotoxin NLCQ-1 with nontoxic paclitaxel doses against murine tumors or human xenografts in vivo, and further studies with various human cancers are therefore warranted.

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