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Effects of *N*-trifluoroacetyladriamycin-14-valerate (AD-32) on human bladder tumor cell lines

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Summary. We have compared the in vitro activity of *N*-trifluoroacetyladriamycin-14-valerate (AD-32) and doxorubicin hydrochloride (ADR) on the clonal growth of human bladder tumor cell lines (HBTCL). In order to determine the relative toxicity of ADR and AD-32 on hematopoietic stem cells, CFU-GM assays were set up using 10 normal human bone marrow samples. The mean lethal dose for 50% of the colonies (LD_{50}) for ADR was 1.6 ± 1.4 μ*M* and that for AD-32, 3.9 ± 4.9 μ*M* ($P < 0.55$), suggesting that these agents have similar bone marrow toxicity. Both drugs produced enhanced inhibition of clonal growth of HBTCL with increasing C × Ts. The spectrum of activity of the two drugs was similar against a panel of seven HBTCL. The activity of ADR was inhibited at 4 °C while the activity of AD-32 was unaffected by temperature. ADR was more effective against HBTCL in the log growth phase than the plateau phase while the reverse was found using AD-32. Verapamil was found to enhance the activity of both ADR and AD-32 against a HBTCL (T24), found to be resistant to both agents. The lipophilic properties of AD-32, along with its enhanced activity when used over prolonged periods of time and its activity against tumor cells in the plateau phase, suggest that AD-32 could be useful in the management of patients with superficial bladder cancer.

Introduction

Doxorubicin, administered systemically, has been shown to have limited activity in metastatic carcinoma of the bladder. The objective response rate in advanced disease is reported to be between 10%–25% (20, 27, 33, 35]. From 70%–80% of individuals with bladder cancer, however, have their initial tumor confined to the mucosa or lamina propria; at least one-half of them will develop a true recurrence or new occurrence despite resection of their initial tumor. Anticancer agents have been instilled directly into the bladder in an attempt to inhibit further growth of the tumor, reduce the requirement for repeated resections, and decrease the chance of subsequent invasive bladder cancer. ADR has been shown to be active intravesically in the treatment of superficial bladder cancer with response rates of 50%–70% [4, 22]. The high molecular weight of ADR and its polar character result in minimal systemic absorption and little systemic toxicity [8].

N-Trifluoroacetyladriamycin-14-valerate (AD-32) is a semisynthetic analogue of doxorubicin (ADR), differing from its parent compound in having a five-carbon straight-chain ester function at the 14-carbinol position and a trifluoroacetyl substituent on the amino group of the glycoside [12]. AD-32 was found to be superior to ADR in the L1210 and P388 leukemia models, both in terms of increasing the lifespan and in percentage of animals achieving long-term survival [12, 26]. AD-32 was also found to have superior activity to ADR in an ADR-resistant P388 subline. AD-32 has been shown to be active in the Ridgway osteogenic sarcoma, Lewis lung carcinoma, and B_{16} melanocarcinoma solid tumor models [13, 26, 32], thereby demonstrating the effectiveness of drug when administered at sites distant from the tumor mass. Certain mechanistic properties of this drug are markedly different from those of ADR. AD-32, for example, does not bind to double helical DNA [28] and shows drug-associated fluorescence in the cytoplasm, as opposed to the nuclear fluorescence seen with ADR [15]. Other properties, such as its ability to inhibit DNA and RNA synthesis [16] and to produce DNA lesions [17], are similar to those of ADR.

In early clinical trials, i.v. infusion of AD-32 showed activity against bladder cancer [7]. The high antitumor activity of this compound, and its lipophilic nature, which allows for easy tumor mass penetration, suggest that AD-32 may be of value as an intravesicular agent. In order to evaluate the potential usefulness of AD-32 in bladder cancer, we have compared its activity with ADR in vitro using a panel of human bladder tumor cell lines (HBTCL).

Material and methods

HBTCL. The following HBTCL were used for the in vitro studies: CUB-2 [6], CUB-3 [6], RT-4 [29], SCaBER [23], J82 [24], T24 [2], and 486P [5]. All cell lines were obtained from the laboratory of Dr. Jorgen Fogh (Sloan-Kettering Institute for Cancer Research, Rye, NY). These cell lines have been characterized by ultrastructural morphology, karyo-

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Abbreviations used: ADR, doxorubicin hydrochloride; AD-32, *N*-trifluoroacetyladriamycin-14-valerate; HBTCL, human bladder tumor cell lines; CFU-GM, colony forming units-granulocyte, macrophage; CFU-T, colony-forming units-tumor; C × T, concentration × time of drug exposure; HBSS, Hank's balanced salt solution; FCS, fetal calf serum

type, in vitro growth characteristics and tumorgenicity in animals; in addition they have been shown to be free of contamination by microorganisms or other cell lines [6].

Monolayer culture of HBTCL. All cell lines were maintained in monolayer culture in RPMI 1640 (GIBCO, Grand Island, NY) with 10% fetal calf serum (Flow Laboratories, Newbury Park, Calif) in 100-mm plastic petri dishes incubated at 37 °C in 6% CO_2 and 100% humidified atmosphere.

Tumor cells were harvested from monolayer culture by incubation for 10 min at 37 °C in 0.25% trypsin in Hank's balanced salt solution (HBSS). The resulting cell suspensions were counted in a hemocytometer; viability, as determined by trypan blue exclusion, was usually greater than 90%. Tumor cell suspensions produced in this manner were either drug tested immediately or subcultuured at a 1/10 dilution to monolayer culture for subsequent study.

Soft agar assay. After trypsinization, tumor cells were plated into the upper layer of a two-layer agar culture system. The underlayer consisted of McCoy's medium (Gibco, Grand Island, NY) with 15% fetal calf serum (FCS) and 0.5% agar. The overlayer consisted of CMRL 1066 medium (Gibco, Grand Island, NY) with 15% horse serum, 2 μg/ml insulin, 5 μg/ml transferrin, and 0.3% agar along with the tumor cells. Cultures were incubated at 37 °C in 6% CO_2 and 100% humidified atmosphere. Plates were examined with an Olympus CK inverted microscope. Final colony counts were made from 10–14 days after plating. Aggregates of 50 or more cells were scored as colonies.

CFU-GM assay. Bone marrow samples were aspirated into preservative-free heparinized syringes from patients with solid tumors and normal marrows. Marrow cells were passed through a 22 gauge needle, diluted 1:2 in HBSS and layered onto a Ficoll-Hypaque gradient (Sigma Diagnostics, Histopaque-1.077). Mononuclear cells were recovered from the interface, washed three times in HBSS and suspended in Iscove's Modified Dulbecco's medium (Gibco, Grand Islands, NY) containing 20% FCS. Quadruplicate cultures of 2×10^5 cells were plated into the upper layer of a two-layer agar system. The underlayer consisted of 1 ml of Iscove's medium supplemented with 30% FCS, 0.5% agar, and 20% human placental conditioned medium as a source of colony stimulating factor [3]. The upperlayer contained bone marrow cells in 1 ml Iscove's medium supplemented with 30% FCS, 0.3% agar, 100 Units penicillin, 200 μg streptomycin, and 2 m*M* of glutamine. Culture plates were incubated in a 6% CO_2 humidified incubator at 37 °C CFU-GM colonies (>40 cells) were scored after 7–8 days of culture.

Drug preparation. The chemotherapeutic drugs used in these experiments were ADR (doxorubicin hydrochloride), obtained from Adria Laboratories, Dublin, Ohio, and AD-32 (*N*-trifluoroacetyladriamycin-14-valerate), obtained from the Division of Cancer Treatment, NCI. ADR was diluted to the desired concentration in 0.9% NACl. AD-32 was put into solution with 1% DMSO and 15% FCS with plating medium. The drug was then further diluted to the desired concentration in 0.9% NaCl. Both drugs were aliquoted in 1.5-ml plastic Falcon centrifuge tubes and stored at −80 °C. Prior to use, drugs were thawed in a 37 °C water bath. Equimolar concentrations of drugs were used for all experiments, ranging from 0.01–20 μ*M*. Verapamil hydrochloride (Searle Pharmaceuticals) was prepared in the appropriate diluent just prior to each experiment and solutions of 1, 6 and 20 μ*M* were prepared for use in culture.

In vitro drug testing. For most experiments tumor cells were incubated in the presence of drug, along with a control tube, for 1 h in a shaking water bath at 37 °C. Cells were then centrifuged at 150 *g* for 10 min, washed twice in RPMI 1640 without horse serum and plated in culture. Control and treated cells were plated with four plates at each drug level and scored at 10–14 days. Continuous drug exposure was performed by plating the tumor cells with drug in agar without washing the drug off prior to incubation.

In separate experiments, 1-h drug incubations were carried out in a 37 °C water bath and simultaneously at 4 °C in a refrigerator. To insure equivalent drug mixing during drug incubation, the tubes were shaken by hand at 10-min intervals.

Log verses plateau phase drug studies. Tumor cells were harvested in the log growth phase (2–3 days) and during the plateau growth phase (6–7 days) and exposed to each drug for 1 h prior to plating. The proliferative state of cells in both the log and plateau phase of growth were evaluated by DNA labeling with tritiated [methyl-^{3}H] thymidine (2 Ci/mm sp. act., Research Products International, Mt. Prospect, Ill). HBTCL in monolayer culture were trypsinized, counted using trypan blue exclusion as a marker for viability and cultured in quadruplicate in microtiter wells (Linbro Chemical Co., Hamden, Cann) at 1×10^5 cells/well. Culture medium consisted of RPMI 1640 supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, 2 m*M* *L*-glutamine, 2.5 mM Hepes buffer, and 10% newborn calf serum. Immediately after placing the cells in the microtiter wells, they were pulsed with 1 μCi of thymidine. Cells were harvested onto glass filter paper at various time intervals after pulsing using a 24-well semi-automated harvester (Microbiological Associates, Walkersville, Md). The paper disks were counted in Packard liquid scintillation counter.

Results

In order to determine the relative toxicity of ADR and AD-32 on hematopoietic stem cells, CFU-GM cultures were set up using bone marrow samples from 10 solid tumor patients with normal bone marrows. Figure 1 represents a colony survival curve formed by using the mean level of colony survival at each drug concentration from the ten CFU-GM assays. The mean LD_{50} of all ten CFU-GM assays for ADR was 1.6 ± 1.4 μ*M*, while the LD_{-50} of AD-32 was 3.9 ± 4.9 μ*M*. The difference between LD_{-50} levels was not statistically significant ($P < 0.17$). The LD_{-70} for each drug was 3.6 ± 4.5 μ*M* for ADR and 4.9 ± 4.6 μ*M* for AD-32 ($P < 0.55$).

We next compared the activity of the two drugs against a panel of HBTCL in a clonogenic assay using a drug exposure time of 1 h. Simultaneous assays were performed using continuous drug exposure to determine the effect of increasing the time of drug exposure on the clonal growth

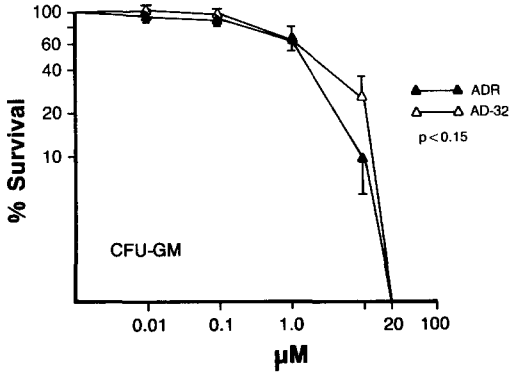

Fig. 1. Results of CFU-GM assays performed using bone marrow cells from patients with non small-cell lung cancer with no evidence of tumor in the marrow. The colony survival curve is formed by using the mean level of colony survival at each drug concentration from the 10 CFU-GM assays. Simultaneous drug assays were done using ADR and AD-32 at concentrations of 0.01–20 μ*M*. *Points* represent means and *bars*, SE in each study

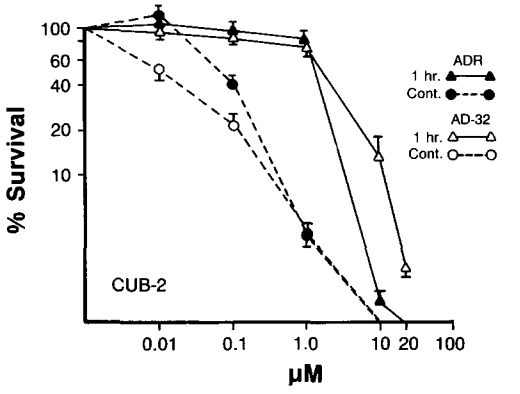

Fig. 2. Results of clonogenic drug assays using CUB-2. ADR and AD-32 were exposed to the bladder tumor cells for 1 h and continuously at concentrations of 0.01–20 μ*M*. Increasing the time of drug exposure enhanced the activity of both drugs

of HBTCL. A 1-h vs continuous drug incubation study using CUB-2 is depicted in Fig. 2. The LD_{-70} of each 1-h and continuous drug study is shown in Table 1. Using a 1-h drug exposure ADR was most active against two HBTCL (SCaBER, CUB-2) while AD-32 was most active against

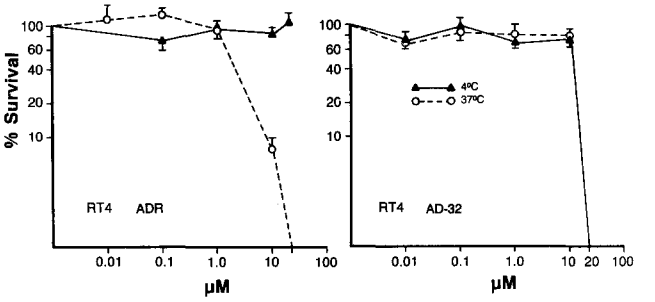

Fig. 3. Drug assays were performed on T24 with ADR and AD-32 using a 1-h drug exposure at 4 °C and 37 °C prior to plating. ADR was inactive at 4 °C while AD-32 was active at both 4 °C and 37 °C

CUB-2. The average shift in the LD_{-70} between a 1-h and continuous drug exposure was 6.2 μ*M* with ADR and 10.8 μ*M* with AD-32. Table 2 gives the comparison of the activity of ADR and AD-32 on HBTCL using a ratio of the LD_{-70} of CFU-T/CFU-GM to determine the relative activity of each drug for the tumor compared to bone marrow CFU-GM cells. The activity of both drugs against the tumor was equal to or greater than the activity against CFU-GM cells in SCaBER and CUB-2 using ADR, and CUB-2 using AD-32.

In order to determine whether drug activity was influenced by temperature, we exposed CUB-2 to ADR and AD-32 for 1 h at 37 °C and simultaneously at 4 °C. ADR was ineffective in inhibiting clonal growth at 4 °C but was active at 37 °C. The activity of AD-32 was not temperature dependent. The results of a representative temperature-dependent drug study using RT-4 is depicted in Fig. 3.

We evaluated whether the activity of ADR and AD-32 was dependent on the proliferative state of the tumor cells. CUB-2, was harvested from culture and two simultaneous monolayer cultures were set up with high- and low-density seedings. At 3 days the plates with high density seeding were confluent while the plates with low density seeding were less than two-thirds confluent. Tumor cells were then removed from monolayer culture, counted, drug tested in the clonogenic assay, and simultaneously labeled with tritiated thymidine. The results of a typical labeling experiment are shown in Table 3. During the first 1 h of labeling, log phase cells incorporated three times the amount of tritiated thymidine as did the plateau phase cells. Figure 4 re-

Table 1. Results of drug assays using seven HBTCL in a clonogenic assay. The LD_{-70} of each study has been calculated from the colony survival curves produced using ADR and AD-32 for 1 h and after continuous drug exposure. The range of in vitro drug dosages examined was 0.01–20 μ*M*

HBTCL	ADR		AD-32	
	1 h (μ*M*)	Continuous (μ*M*)	1 h (μ*M*)	Continuous (μ*M*)
J-82	12.5	1.8	>20.0	1.5
RT4	7.5	0.9	11.3	1.4
CUB-3	12.0	5.4	>20.0	11.0
486P	>20.0	11.3	>20.0	11.2
SCaBER	2.0	1.6	11.6	1.6
CUB-2	1.8	0.1	3.0	0.05
T24	14.2	5.4	>20.0	3.2

Table 2. Ratios[a] of the LD_{-70} of drug assays using a clonogenic assay (CFU-T) with ADR and AD-32 tested against 7 HBTCL compared with the mean LD_{-70} of 10 CFU-GM assays using bone marrow samples from patients with solid tumors

HBTCL	ADR		AD-32	
	LD_{-70} (CFU-T/CFU-GM	Ratio	LD_{-70} (CFU-T/CFU-GM	Ratio
J-82	12.5/3.6	3.5	20.0/4.9	4.1
RT4	7.5/3.6	2.1	11.3/4.9	2.3
CUB-3	12.0/3.6	3.3	20.0/4.9	4.1
486P	20.0/3.6	5.5	20.0/4.9	4.1
SCaBER	2.0/3.6	0.6	11.6/4.9	2.4
CUB-2	1.8/3.6	0.5	3.0/4.9	0.6
T24	14.2/3.6	3.9	20.0/4.9	4.1

A HBTCL is considered to be sensitive with a ratio of 1.0 or less

Table 3. Results of DNA-labeling experiments performed with CUB-2 either in the log or plateau growth phase

Time post pulse	Growth phase		Log/plateau ratio of thymidine incorporation
	Plateau (cpm)	Log (cpm)	
1 h	1162.3	3236.5	2.8
2 h	1603.7	3144.0	1.9
3 h	1502.3	2708.5	1.8
4 h	1429.7	4262.0	2.9

Each value represents mean counts per minute (cpm) of seven experiments

presents the colony survival curves from drug assays performed simultaneously in the clonogenic assay using a 1-h drug exposure prior to plating. ADR was more active against log phase cells with an LD_{-70} of 0.6 μM compared with an LD_{-70} of 2.6 μM using cells in the plateau phase. In contrast, AD-32 was more active against cells in the plateau phase with an LD_{-70} of 2.5 μM compared to an LD_{-70} of 12.1 μM for cells in the log growth phase.

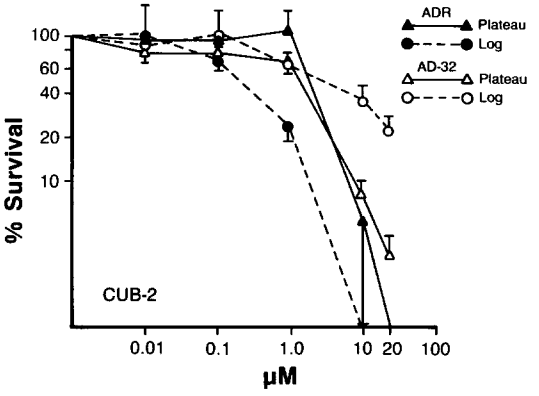

Fig. 4. This graph depicts the colony survival curves resulting from a 1-h drug exposure to CUB-2 tumor cells in the log and plateau growth phases. ADR was found to inhibit clonal growth to a greater degree in cells in the log growth phase than in those in the plateau growth phase. The same differential activity depending on the proliferative state of the cells was not found with AD-32

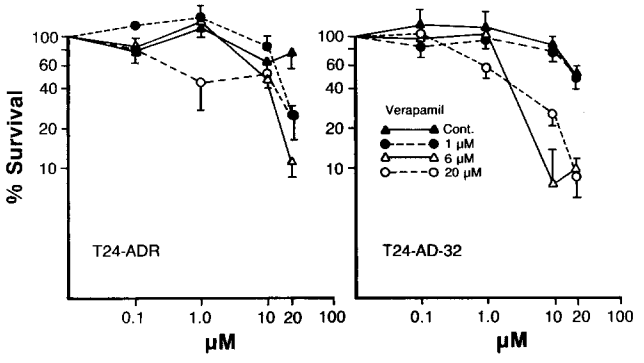

Fig. 5. These graphs depicts the results of clonogenic assays performed with ADR and AD-32 in the presence of increasing concentrations of verapamil. The resistant cell line T24 was exposed to drugs for 1 h prior to plating. The resulting colony survival curves show that the activity of ADR and AD-32 was progressively enhanced in the presence of 1–20 μM of verapamil. Colony survival was not influenced by the presence of verapamil alone at concentrations of 1–20 μM

We also examined the effect of the calcium channel blocker, verapamil, on the activity of ADR and AD-32 against a relatively resistant cell line and a sensitive cell line. No enhancement in activity was seen with either drug using CUB-2, which was the most sensitive cell line to both drugs. The activity of ADR and AD-32 was enhanced with verapamil against the resistant cell line, T24. Figure 5 depicts the results of experiments with T24. Increasing concentrations of verpaamil from 1–20 μM resulted in enhanced activity of ADR with a shift of the LD_{-70} from >20 μM to 14 μM. The enhanced activity of AD-32 was more pronounced with a shift of the LD_{-70} from >20 to 6 μM. Verapamil alone was not found to inhibit colony formation of the HBTCL in the range of 1–20 μM.

Discussion

AD-32 has been proposed for use as an intravesical anticancer agent for patients with superficial bladder cancer. This drug has the attractive property of being lipophilic, resulting in the potential for deeper tumor penetration. This same lipophilic property could also result in more drug being absorbed into the systemic circulation. The administration of thio-TEPA results in a significant amount of systemic toxicity compared to ADR which penetrates normal and neoplastic transitional cell epithelium to a lesser degree [1, 8, 10, 14]. Due to the potential for greater systemic absorption we examined the effect of AD-32 compared to ADR on CFU-GM cells. The major limiting effect to dose escalation with most anticancer drugs is the myelosuppressive effect of the agents on the bone marrow. Although much work has been done defining the inhibitory effects on the clonal growth of tumor cells, only recently have investigators attempted to define the in vitro effects of drugs on normal tissues as an indicator of host toxicity. Lohrmann et al. [18] have reported that the in vitro inhibition of granulocyte-macrophage colony formation predicted the in vivo myelosuppression of patients receiving chemotherapy for breast cancer. Park et al. [25] used the CFU-GM assay as a method of standardizing the in vitro drug levels used for in vitro drug testing. He found that the ratio of drug effect on the bone marrow progenitor cells, as measured in the CFU-GM assay, compared to the activity of the same drugs against human leukemia stem cells strongly correlated with the ability of patients with acute leukemia to obtain a complete remission. Subsequently, a number of investigators have begun to explore the use of a CFU-GM assay for determination of optimal in vitro drug levels for human tumor drug testing. Hug et al. [11] have reported that a comparison of the CFU-GM assay and CFU-T assay for breast cancer predicted response to ADR clinically. Taetle et al. [30] reported that such an in vitro comparison predicted the activity of ADR in human tumors drug tested in a nude mouse. Using a similar CFU-T/CFU-GM comparison we found CUB-2 to be sensitive to the in vitro anticancer effect of both ADR and AD-32, while SCaBER was only sensitive to AD-32. Using these HBTCL we were unable to identify a significant difference in the spectrum of activity between the two drugs. We have noted that AD-32 is comparable to ADR in its inhibition of clonal growth of bone marrow CFU-

GM cells and therefore would be predicted to be no more myelosuppressive than its parent compound, if significant systemic absorption did occur from intravesical chemotherapy.

Several investigators have shown that the concentration × time of drug exposure (C × T) is important when evaluating the inhibition of clonal growth of animal and human tumors by various anticancer drugs. Hill et al. [9] demonstrated that the inhibition of clonal growth of MNB/P_L murine neuroblastoma cells by ADR was enhanced by increasing the exposure time from 1 to 24 h. Likewise, Wu et al. [34] demonstrated that the inhibition of clonal growth of the Raji cell line of Burkitt's lymphoma by ADR was enhanced by continuous exposure to the drug as apposed to a 1-h drug exposure. We have previously demonstrated [21] an enhanced activity of ADR against a variety of murine bladder tumor cell lines with increasing C × Ts. In the present study the activity of both ADR and AD-32 against the clonal growth of HBTCL was enhanced with prolonged drug exposure. This enhanced activity of AD-32 could have a direct bearing on its use in superficial bladder cancer, since much higher C × Ts are usually achieved with the intravesical administration of anticancer drugs than with systemic administration.

The mechanisms of uptake between ADR and AD-32 appear to be different. Krishan et al. [15] have shown that the uptake of ADR into CCRF-CEM human lymphoblasts and in a WI-38 human fibroblast cell line is slow and temperature-dependent. Intracellular drug appears to be localized to the nucleus. AD-32 is totally taken up after a 1-min exposure and all the drug-associated fluorescence localizes to the cytoplasm. Meriwether et al. [19] also have shown that the uptake of ADR is temperature-dependent. They demonstrated that the uptake of ADR into L1210 cells was almost totally inhibited at 4 °C. It had been previously demonstrated that the activity of AD-32 in human lymphoblasts or fibroblast cell lines was not inflenced by temperature. We have found that the uptake of ADR and AD-32 by HBTCL is different at 4 °C. The activity of ADR is almost totally inhibited at 4 °C while the activity of AD-32 is unaltered by lower temperatures. This difference suggests that ADR may require an energy-dependent process for uptake while AD-32 almost certainly enters the cell by simple diffusion.

We have found that ADR and AD-32 differ in their activity depending on the proliferative state of the cell. It has been previously shown that ADR is more active against rapidly proliferating cells than against cells in the plateau growth phase. Krishan et al. [16] showed that CCRF-CEM cells exposed to ADR in monolayer culture killed more cells in the log growth phase than in the plateau phase; a similar difference was not apparent with AD-32. We have found that ADR is more effective against the clonal growth of HBTCL cells in the log growth phase while AD-32 is more effective against plateau than log phase cells. The activity of AD-32 against slow growing cells could be an advantage for the treatment of bladder cancer, which is a slow-growing tumor.

Most patients with bladder cancer have tumors that are resistant to the anticancer drug effect of ADR. The mechanisms of drug resistance to ADR are not completely understood. It has been shown that in some P388/ADR-resistant sublines that the accumulation of ADR is actively transported outside the cell resulting in low intracellular drug levels and therefore low cytotoxicity. Tsuruo et al. [31] reported that the calcium channel blocker, verapamil, augmented the cytotoxic effect of ADR on ADR-resistant P388 cell lines by enhancing the intracellular accumulation of ADR in ADR-resistant cell lines. We have found that the activity of both ADR and AD-32 are enhanced in a resistant HBTCL by simultaneous exposure to verapamil.

In summary we have found that AD-32 differs from its parent compound in its effects on the clonal fraction of cells in HBTCL. The lipophilic properties of AD-32, along with the enhanced activity of this compound when used over prolonged periods of time and its activity against cells in the plateau phase, suggest that AD-32 could be useful in the management of patients with superficial bladder cancer.

References

1. Bessman JD, Johnson RK, Goldin A (1975) Permeability of normal and cancerous rat bladder to antineoplastic agents. Urology 6: 187
2. Bubenik J, Baresoma M, Viklicky V, Jakoubkova J, Sainerova H, Donner J (1973) Established cell line of urinary bladder carcinoma (T24) containing tumor-specific antigen. Br J Cancer 11: 765
3. Burgess AW, Wilson EM, Metcalf D (1977) Stimulation by human placental conditioned medium of hematopoietic colony formation by human marrow cells. Blood 49: 573
4. Edsmyr F, Berlin T, Bomen J, Duchek M, Esposti PL, Gustafsson H, Wijkstrom H, Collste LG (1980) Intravesical therapy with adriamycin in patients with superficial bladder tumors. Eur Urol 6: 132
5. Elliott AY, Bronson DL, Cervenka J, Stein N, Fraley EE (1977) Properties of cell lines established from transitional cell cancers of the human urinary tract. Cancer Res 37: 1279
6. Fogh J (1978) Cultivation, characterization, and identification of human tumor cells with emphasis on kidney, testis, and bladder tumors. Natl Cancer Inst Monogr 49: 5
7. Garnick MB, Griffin JD, Sack MJ, Blum RH, Israel M, Frei E III (1982) Phase II evaluation of *N*-trifluoroacetyladriamycin-14-valerate (AD-32). In: Muggia FM, Young CW, Carter SK (eds) Anthracycline antibiotics in cancer therapy. Martinus Nijhoff, The Hague, p 451
8. Garnick MB, Schade D, Israel M, Richie JF (1984) Prophylactic use of intravesical adriamycin in the management of superficial bladder cancer. J Urol 131: 43
9. Hill BT, Whelan RDH, Rupniak HT, Dennis CY, Rosholt MA (1981) A comparative assessment of the in vitro effects of drugs on cells by means of colony assays or flow microfluorimetry. Cancer Chemother Pharmacol 7: 21
10. Hollister D Jr, Coleman M (1980) Hematologic effects of intravesical thiotepa therapy for bladder cancer. JAMA 244: 2065
11. Hug V, Thames H, Blumenschein GR, Spitzer G, Drewinko B (1984) Normalization of in vitro sensitivity testing of human tumor clonogenic cells. Cancer Res 44: 923
12. Israel M, Modest EJ, Frei E III (1975) *N*-Trifluoroacetyladriamycin-14-valerate, an analog with greater experimental antitumor activity and less toxicity than adriamycin. Cancer Res 35: 1365
13. Israel M, Seshadri R, Kumar N (1982) *N*-Trifluoroacetyladriamycin-14-O-hemiadipate (AD-143), a promising new experimental antitumor agent. Proc 13th Int Cancer Congr 13: 407
14. Jakse G, Hofstadter F, Marberger H (1981) Intracavitary doxorubicin hydrochloride therapy for carcinoma in situ of the bladder. J Urol 125: 185

15. Krishan A, Israel M, Modest EJ, Frei E (1976) Differences in cellular uptake and cytofluorescence of adriamycin and *N*-Trifluoroacetyladriamycin-14-valerate. Cancer Res 36: 2114
16. Krishan A, Dutt K, Israel M, Ganapathi R (1981) Comparative effects of adriamycin and *N*-trifluoroacetyladriamycin-14-valerate on cell kinetics, chromosomal damage, and macromolecular synthesis in vitro. Cancer Res 41: 2745
17. Levin M, Silber R, Israel M, Goldfeder A, Khetarpal VK, Potmesil M (1981) Protein-associated DNA breaks and DNA-protein crosslinks caused by DNA non-binding derivatives of adriamycin in L1210 cells. Cancer Res 41: 1006
18. Lohrmann HP, Schroml W, Lang M, Betxler M, Fliedner TM, Heimpel H (1978) Changes of granulopoiesis during and after adjuvant chemotherapy of breast cancer. Br J Haematol 40: 369
19. Meriwether WD, Bachur NR (1972) Inhibition of DNA and RNA metabolism by daunorobucin and adriamycin in L1210 mouse leukemia. Cancer Res 32: 1137
20. Merrin C, Cartagena R, Wajsman Z, Baumgartner G, Murphy GP (1975) Chemotherapy of bladder carcinoma with cyclophosphamide and adriamycin. J Urol 114: 884
21. Niell HB, Soloway MS, Wood CA (1985) Effect of concentration and time of drug exposure on clonal growth of murine bladder cancer. Urology 25: 267
22. Niijima T (1979) Intravesical therapy with adriamycin and new trends in the diagnostics and therapy of superficial urinary bladder tumors. In: Montedison Laekermedol AD (ed) Diagnostics and treatment of superifical urinary bladder tumors. Stockholm, WHO collaborating centre for research and treatment of urinary bladder Cancer, p 37
23. O'Toole C, Nayak S, Price Z, Gilbert WH, Waisman J (1976) A cell line (Scaber) derived from squamous cell carcinoma of the human urinary bladder. Int J Cancer 17: 707
24. O'Toole C, Price LH, Ohnuki Y, Unsguard B (1978) Ultrastructure, karyology and immunology of a cell line originated from a human transitional-cell carcinoma. Br J Cancer 38: 64
25. Park CH, Amare M, Savin MA, Goodwin JW, Newcomb MM, Hoogstraten B (1980) Prediction of chemotherapy response in human leukemia using an in vitro chemotherapy sensitivity test on the leukemic colony forming cells. Blood 55: 595
26. Parker LM, Hirst M, Israel M (1978) *N*-Trifluoroacetyladriamycin-14-valerate: additional mouse antitumor and toxicity studies. Cancer Treat Rep 62: 119
27. Pavone-Macaluso M, EORTC Genitourinary Tract Cooperative Group A (1976) Single drug chemotherapy of bladder cancer with adriamycin, VM-26, or bleomycin. A phase II multicentric cooperative study. Eur Urol 2: 138
28. Pearlman LF, Chuang RY, Israel M, Simpkins H (1986) The interaction of three second-generation anthracyclines with polynucleotides, RNA, DNA and nucleosomes. Cancer Res 46: 341
29. Rigby CC, Franks LN (1970) Human tissue culture cell line from a transitional cell tumor of the urinary bladder: growth, chromosome pattern and ultrastructure. Br J Cancer 24: 746
30. Taetle R, Howell SB, Giuliani FC, Koziol J, Koessler A (1982) Comparison of the activity of doxorubicin analogues using colony-forming assays and human xenografts. Cancer 50: 1455
31. Tsuruo T, Lida H, Tsukagoshi S, Sakurai Y (1982) Increased accumulation of vincristine and adriamycin in drug-resistant P388 tumor cells following incubation with calcium antagonists and calmodulin inhibitors. Cancer Res 42: 4730
32. Vecchi A, Cairo M, Mantovani A, Sironi M, Spreafico F (1978) Comparative antineoplastic activity of adriamycin and *N*-trifluoroacetyladriamycin-14-valerate. Cancer Treat Rep 61: 111
33. Weinstein SH, Schmidt JD (1976) Doxorubicin in advanced transitional cell carcinoma. Urology 8: 336
34. Wu P-C, Ozol RF, Hatanaka M, Boone CW (1982) Anticancer drugs: effect on the cloning of Raji lymphoma cells in soft agar. JNCI 68: 115
35. Yagoda A, Watson RC, Whitmore WF, Grabstald H, Middleman MP, Krakoff IH (1977) Adriamycin in advanced urinary tract cancer. Cancer 39: 279

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