Eur J Cancer Clin Oncol, Vol. 20, No. 10, pp. 1287–1293, 1984.
Printed in Great Britain.

0277-5379/84$03.00+0.00

Reduction of *cis*-Dichlorodiammineplatinum-induced Cell Inactivation by Benzaldehyde*

JOHN M. DORNISH,†‡ ERIK O. PETTERSEN, REIDAR OFTEBRO and JAN-EGIL MELVIK
Department of Tissue Culture, Norsk Hydro's Institute for Cancer Research, The Norwegian Radium Hospital, Montebello, Oslo 3, Norway

Abstract—*The inactivating effect of a combined treatment of human cells (NHIK 3025) in culture with* cis-*dichlorodiammineplatinum(II)* (cis-*DDP*) *and the protein synthesis inhibitor benzaldehyde was tested. Cell inactivation was measured as loss of colony-forming ability following drug treatment. While* 3.2 mM *benzaldehyde had no effect on the cell survival when given alone, it reduced the effect of* 10 μM cis-*DDP significantly when the two drugs were added simultaneously. Scheduling experiments indicate that benzaldehyde must be present immediately before addition of, or simultaneously with,* cis-*DDP for optimal reduction of cell inactivation. Benzoic acid, benzyl alcohol or the protein synthesis inhibitor cycloheximide did not reduce the inactivating effect of* cis-*DDP. Cells synchronized by mitotic selection were used to determine the variation in the responses throughout the cell cycle. It was found that concomitant 2-hr treatment of synchronized cells with* 3.2 mM *benzaldehyde and* 10 μM cis-*DDP at various times during the cell cycle resulted in a consistently greater surviving fraction of cells than* 10 μM cis-*DDP alone. Benzaldehyde thus reduced the inactivating effect of* cis-*DDP in all phases of the cell cycle. The effect of benzaldehyde in combination with two alkylating agents, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and nitrogen mustard (HN2), was also studied. Benzaldehyde was not found to influence the effects on cell survival induced by these drugs.*

INTRODUCTION

BENZALDEHYDE, an aromatic aldehyde identified as the anticancer agent present in figs [1, 2], has previously been shown to inhibit the growth of transformed mouse and simian cells [3–5]. Anticancer activity of benzaldehyde has also been demonstrated against a variety of human malignant neoplasms [6]. Previous reports from our laboratory have shown that benzaldehyde induces an inhibition of cell cycle traverse not dependent upon DNA synthesis but due to a decrease in the rate of protein accumulation [7, 8].

Since benzaldehyde seems to exert effects at the cellular level by mechanisms quite different from those of more established anticancer drugs, we found it interesting to test the effects of combining benzaldehyde with various chemotherapeutic agents. Synergistic or antagonistic effects might have implications not only on the clinical use of the drug combinations, but perhaps also on the nature of the underlying mechanism of action of each chemotherapeutic agent. In the present paper we report data on *cis*-DDP, BCNU and HN2 combinations with benzaldehyde.

BCNU and HN2 are considered to be typical alkylating agents and evidence indicates that *cis*-DDP also reacts with cellular DNA, forming both monoadducts and cross-links (interstrand, intra-strand, DNA–protein and bifunctional binding to one base [9–11]). Moreover, recent reports have been published that *cis*-DDP inhibits amino acid transport in L1210 cells [12]. It was suggested that *cis*-DDP may react with the plasma membrane, possibly with amino acid carrier molecules. In the case of benzaldehyde, Ishida *et al.* [13] have interpreted the enhancement of benzaldehyde toxicity in SV 40-transformed cells as compared with untransformed cells as an indication of selective benzaldehyde interactions with membrane proteins. Our studies indicate that the reduction in *cis*-DDP induced cell inactivation

Accepted 17 April 1984.
*This work was supported by the Norwegian Cancer Society.
†To whom all correspondence should be addressed.
‡Fellow of the Norwegian Cancer Society.

caused by simultaneous treatment with benzaldehyde may be attributed to membrane protein interactions by these two agents.

MATERIALS AND METHODS

Cell line and cell synchronization

Cells of the human line NHIK 3025 (a cervical carcinoma *in situ*) [14, 15] were used in this study. The cells were cultured as monolayers in medium E2a [16] supplemented with 20% human and 10% horse serum. Synchronized cells were obtained by shake-off of mitotic cells from populations in exponential growth [17] and all experiments utilizing synchronized cells were performed in a 37°C walk-in incubator room. Under growth conditions as used here the NHIK 3025 cells normally have a median cell-cycle duration of ~18 hr, with median G1 and S durations of ~7 and ~8 hr respectively and a duration of mitosis of about 50 min.

Cell survival

As benzaldehyde is a volatile compound, all cell survival experiments were performed using 25-cm^2 plastic flasks (Falcon 3013) instead of open Petri dishes. The selected mitoses existed in suspension immediately after shake-off. From this suspension controlled volumes were seeded directly into the plastic flasks in which the cells were to form colonies. Within 2 hr all the mitotic cells completed division and attached to the bottom of the flasks as doublets. Cell survival in experiments using synchronized cells thus had to be corrected for a cell multiplicity of two in order to represent the survival of single cells. In experiments where exponential growing (asynchronous) cells were used the cells were loosened from the bottom of the culture flask by a mild trypsin treatment (0.25% trypsin, Difco 1:250) and a known number of cells were seeded into the 25-cm^2 flasks as single cells and allowed to attach for 2 hr. Drugs were added to the exponentially growing cells 2 hr after the cells were seeded. To the synchronized cells the drugs were added at various times after seeding, but not earlier than 2 hr. The drugs were added by replacing the medium in the flasks with medium containing the desired concentration of the drugs. Following drug treatment, the flasks were rinsed with warm phosphate-buffered saline (8000 mg/l NaCl, 1150 mg/l $Na_2HPO_4 \cdot 2H_2O$, 200 mg/l KH_2PO_4, 200 mg/l KCl, 200 mg/l $MgCl_2 \cdot 6\ H_2O$, 100 mg/l $CaCl_2$) before fresh medium was added. Flasks were flushed with 5% CO_2 in air each time they were opened. After 12–14 days colonies of cells were fixed in ethanol and stained with methylene blue. Only colonies containing more than 40 cells were counted.

Cell cycle kinetics

The progression of the synchronized cells through the cell cycle (i.e. the time of entrance into S and the rate of DNA accumulation) was determined from DNA histograms of flow cytometric recordings at various times after mitotic selection as previously described [7, 8]. Cell division was recorded in a separate 25-cm^2 flask seeded immediately after cell synchronization where fields delineated at the bottom and circumscribing approximately 100 cells could be followed microscopically. The time of each cell division was recorded.

Drugs

Benzaldehyde was purchased from Koch-Light Laboratories Ltd., Colnbrook, Berks., U.K. and was vacuum-distilled and stored under N_2. *cis*-Dichlorodiammineplatinum (cisplatin) was from Farmitalia Carlo Erba, Barcelona, Spain. 1,3-Bis-(2-chloroethyl)-1-nitrosourea (carmustine) was produced by Bristol Laboratories, Syracuse, NY, U.S.A. Nitrogen mustard (methyl-bis(β-chloroethyl)amine hydrochloride) was a gift from Merck Sharp and Dohme Research Lab, Rahway, NJ, U.S.A. Benzoic acid and benzyl alcohol were purchased from EGA-Chemie GmbH., Steinheim, F.R.G. Cycloheximide was a product from Sigma Chemical Co., St. Louis, MO, U.S.A. Stock solutions of drugs were made in phosphate-buffered saline or Hanks' balanced salt solution and sterile-filtered. Drug dilutions and drug combinations were made in medium E2a immediately before use.

RESULTS

Figure 1 shows the surviving fraction of asynchronous (exponentially growing) NHIK 3025 cells following a 2-hr treatment with *cis*-DDP or *cis*-DDP in combination with 3.2 mM benzaldehyde as a function of the concentration of *cis*-DDP. As was also shown in our previous paper [7], 3.2 mM benzaldehyde alone has little or no effect on the survival of exponentially growing cells after such a short treatment period. From Fig. 1, however, the inactivating effect of *cis*-DDP is reduced considerably when the drug treatment includes 3.2 mM benzaldehyde. The data thus indicate a mitigating effect of benzaldehyde on cell inactivation by *cis*-DDP. To study how the reduction of *cis*-DDP cell inactivation depends upon the concentration of benzaldehyde we performed another experiment where exponentially growing cells were treated with a combination of a fixed concentration of 10 μM *cis*-DDP and benzaldehyde at various concentrations. The results are shown in Fig. 2 together with data showing the surviving fraction after

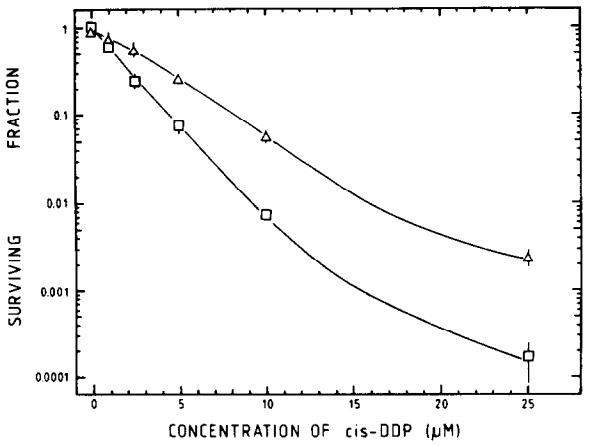

Fig. 1. Surviving fraction of asynchronous NHIK 3025 cells as a function of the concentration of cis-*DDP (□) or* cis-*DDP + 3.2 mM benzaldehyde (△). Single cells were plated in 25-cm² plastic flasks 2 hr before medium with the appropriate concentration of drugs was added. Following a 2-hr incubation period, the flasks were washed with phosphate-buffered saline then fresh medium was added. The experimental points represent the mean of 8 different parallel flasks. S.E. is represented by vertical bars when exceeding the size of the symbols.*

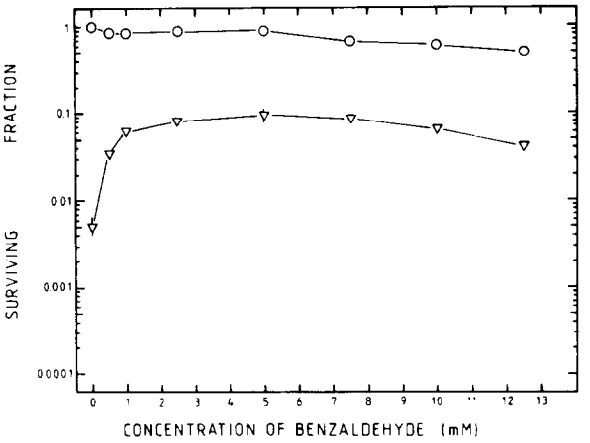

Fig. 2. Surviving fraction of asynchronous NHIK 3025 cells as a function of the concentration of benzaldehyde (○) or benzaldehyde + 10 μM cis-*DDP (▽). Experimental conditions as in Fig. 1.*

treatment with benzaldehyde alone. As demonstrated, cell inactivation induced by *cis*-DDP alone was significantly reduced by the simultaneous presence of benzaldehyde at concentrations above at least 0.5 mM. The reduction was, however, optimal for concentrations above about 2.5 mM. For concentrations above 5 mM, the inactivating effect of benzaldehyde alone was weak, as demonstrated by a slight reduction in the survival for cells treated with benzaldehyde alone at the highest concentrations. This effect is even reflected in the curve representing the combined treatment by a downward bending for the highest benzaldehyde concentrations. Thus the inactivating effect of benzaldehyde alone seems to be purely additive to that of *cis*-DDP.

In our previous study [8] we found that benzaldehyde at concentrations above 0.5 mM reduces the protein synthesis of our cells. The degree of reduction is the same in all stages of the cell cycle. It was earlier shown that cycloheximide has an effect on the protein synthesis of these cells which is very similar to that of benzaldehyde [8, 18]. However, the combination of 1 μM cycloheximide and 10 μM *cis*-DDP did not result in any reduction of *cis*-DDP cell inactivation (Table 1). Although the mechanisms of protein inhibition by benzaldehyde and cycloheximide may be different, it does not appear that protein synthesis inhibition in general influences *cis*-DDP cell inactivation.

Furthermore, neither benzoic acid nor benzyl alcohol, which represent oxidation and reduction products of benzaldehyde respectively, had any effect on cell inactivation by *cis*-DDP when these chemicals were tested in combination with *cis*-DDP (Table 1). These results indicate that the aldehyde moiety is important for a reduction of *cis*-DDP cell inactivation by benzaldehyde.

Table 1. Surviving fraction of NHIK 3025 cells following treatment with various chemicals singly or in combination with cis-*DDP*

Drug treatment (2 hr)	Surviving fraction	
	Single treatment	Combination with 10 μM *cis*-DDP
Control	1.00	0.014 ± 0.001
1.0 μM cycloheximide	1.06 ± 0.05	0.011 ± 0.001
3.2 mM benzoic acid	0.84 ± 0.13	0.014 ± 0.002
3.2 mM benzaldehyde	0.87 ± 0.09	0.170 ± 0.015
3.2 mM benzyl alcohol	0.99 ± 0.12	0.015 ± 0.002

Asynchronous NHIK 3025 cells attached to 25-cm² plastic flasks were treated for 2 hr with each drug or drug combination. Drugs were removed by washing the flasks in Hanks' balanced salt solution. Fresh medium was added to the flasks and incubation continued for 12–14 days with a medium shift on day 7. Colonies of cells were then fixed in absolute ethanol and stained with methylene blue. The surviving fraction represents the number of surviving cells giving rise to colonies of over 40 cells in relation to control flasks. Each experimental value represents the mean and standard error of counts from five replicate flasks.

However, the combination of several straight chain aldehydes, butyraldehyde and glyceraldehyde with *cis*-DDP did not result in any modification of *cis*-DDP-induced cell inactivation (data not shown). It appears that the aldehyde must also possess aromatic character.

The ability of benzaldehyde to modify *cis*-DDP cell inactivation with respect to the duration of drug exposure is presented in Figure 3. In these experiments NHIK 3025 cells were exposed continuously to 10 μM *cis*-DDP either alone or in combination with 3.2 mM benzaldehyde for up to 4 hr. On the one hand, benzaldehyde treatment alone at 3.2 mM resulted in almost 100% survival for all treatment times. On the other hand, *cis*-DDP treatment alone at 10 μM resulted in a decreasing survival with increasing treatment times. The simultaneous presence of 10 μM *cis*-DDP and 3.2 mM benzaldehyde resulted in a reduced effect as compared with that after *cis*-DDP alone. From the data 1 hr treatment with *cis*-DDP alone resulted in the same survival as 4 hr treatment with *cis*-DDP and benzaldehyde in combination.

In the experiments described thus far *cis*-DDP and benzaldehyde were added and removed simultaneously for combined treatment. We have also studied the combined effects where the treatment period for the two drugs were separated or overlapped only partially. The data in Fig. 4 represents such an experiment where 10 μM *cis*-DDP was present for 2 hr (horizontal line marking from 0 to 2 hr) and benzaldehyde was present as 2 hr pulses either before, during or after the *cis*-DDP treatment period. Survival is plotted as a function of the time when benzaldehyde was added. Cells treated with 10 μM *cis*-DDP alone (□) or with 3.2 mM benzaldehyde alone (○) are also shown.

From Fig. 4, benzaldehyde (3.2 mM) given simultaneously with 10 μM *cis*-DDP resulted in cell survival about 25 times greater than with *cis*-DDP alone. When benzaldehyde was added 1 hr later than *cis*-DDP and thus overlapped the pulse of *cis*-DDP by only 1 hr, cell survival was only four times greater than with *cis*-DDP alone. Benzaldehyde treatment immediately following the *cis*-DDP pulse resulted in nearly identical survival as with *cis*-DDP alone. Further separation of benzaldehyde treatment after *cis*-DDP resulted in no further change in survival.

Figure 4 also shows that even when benzaldehyde treatment preceded that of *cis*-DDP by 7 hr significantly greater cell survival than after *cis*-DDP alone was measured. The modifying effect of *cis*-DDP cell inactivation by benzaldehyde increased as the treatment period for benzaldehyde approached that of *cis*-DDP. This indicates that benzaldehyde is binding to some cell constituent which hinders the inactivating effect of *cis*-DDP.

To determine whether the reduction of *cis*-DDP cell inactivation by benzaldehyde was specific to any particular phase of the cell cycle we treated synchronized cell populations with 2-hr pulses with 10 μM *cis*-DDP alone or in combination with 3.2 mM benzaldehyde at various times after

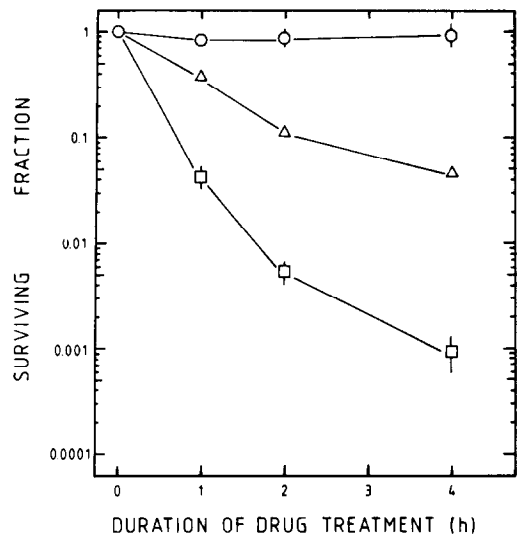

Fig. 3. Surviving fraction of asynchronous NHIK 3025 cells as a function of the duration of drug treatment with 3.2 mM benzaldehyde (○), 10 μM cis-DDP (□) or 3.2 mM benzaldehyde + 10 μM cis-DDP (△). Single cells attached to 25-cm² plastic flasks were treated with each drug or drug combination for the time indicated in the figure. Drug treatment was terminated by removal of the drug-containing medium, and thereafter the cells were washed in phosphate-buffered saline and reincubated in fresh medium for colony formation. Data from four replicate flasks were averaged for each experimental point and S.E. is represented by vertical bars.

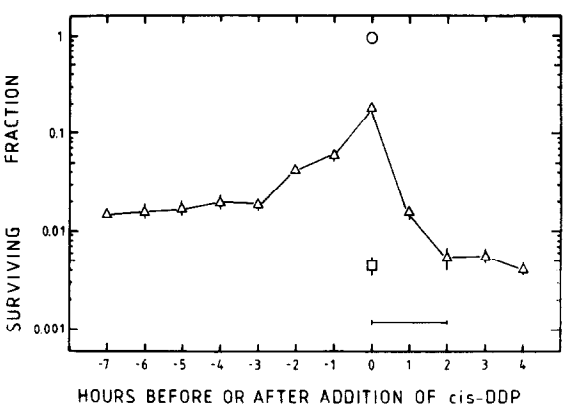

Fig. 4. Surviving fraction of asynchronous NHIK 3025 cells treated with 10 μM cis-DDP as a function of scheduling of 2-hr pulses of 3.2 mM benzaldehyde (△). (○) and (□) represent cell survival after a 2-hr pulse of 3.2 mM benzaldehyde alone or 10 μM cis-DDP alone respectively. The horizontal bar represents the treatment period for cis-DDP and data points are plotted from the time at which drug incubation began. Each experimental point represents the mean of 4–5 replicate flasks. Vertical bars represent S.E.

mitotic selection. The results are shown in Fig. 5. From these data a reduction in *cis*-DDP cell inactivation by benzaldehyde is present and of about the same magnitude in all phases of the cell cycle.

Since *cis*-DDP is a chemotherapeutic agent often grouped together with nitrogen mustard and other DNA cross-linking agents, the effect of benzaldehyde in combination with alkylating agents was also studied. The survival of asynchronous NHIK 3025 cells to 1 hr treatment with either BCNU or HN2 is shown in Fig. 6. The simultaneous presence of 3.2 mM benzaldehyde during the drug incubation period did not affect cell survival as compared with that measured after BCNU or HN2 alone.

DISCUSSION

Analysis of the data in Fig. 1 has shown that the two curves have a similar shape but differ with a dose-modifying factor of 1.7. Thus benzaldehyde reduces the inactivating effect of *cis*-DDP to about the same degree for all concentrations of *cis*-DDP (Fig. 1). The optimal degree of reduction of *cis*-DDP cell inactivation is found for benzaldehyde concentrations above about 1 mM (Fig. 2), but a significant effect is also found at 0.5 mM. Thus the range of concentrations of benzaldehyde giving rise to reduced *cis*-DDP cell inactivation corresponds with the range of concentrations that was earlier found to induce inhibition of protein synthesis in these cells [8]. Another similarity is that both the inhibition of protein synthesis and the *cis*-DDP-modifying effect of benzaldehyde seem non-specific with respect to cell-cycle phase. Although we found, as did Robert and Fraval [19], that cells treated with *cis*-DDP revealed maximum sensitivity in G1, the modifying effect of benzaldehyde (Fig. 5) was nearly uniform throughout the cell cycle. It is therefore tempting to suppose that the two effects of benzaldehyde—inhibition of protein synthesis on the one hand and reduction of *cis*-DDP cell inactivation on the other—might have some common outspring. From the results in Table 1 one can, however, rule out the possibility that an inhibition of protein synthesis generally reduces the inactivating effect of *cis*-DDP. In that case 1 μM cycloheximide would have reduced the effect of *cis*-DDP to about the same extent as 3.2 mM benzaldehyde. It is still possible that the mechanism by which benzaldehyde reduces the protein synthesis is the same as that by which it reduces the effect of *cis*-DDP.

One obvious possibility to be considered is that benzaldehyde might in some way reduce the amount of *cis*-DDP entering the cells. There are

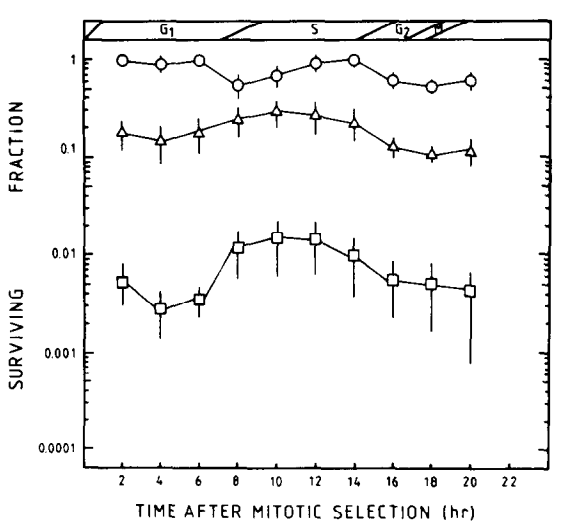

Fig. 5. Surviving fraction of synchronized NHIK 3025 cells treated for 2 hr *with* 10 μM cis-*DDP* (□), 3.2 mM *benzaldehyde* (○) *or* 10 μM cis-*DDP* + 3.2 mM *benzaldehyde* (△) *as a function of the time after mitotic selection. Experimental points from* 1 *typical experiment are plotted from the time at which drug incubation was begun. The duration of the various cell cycle stages for control cells is shown at the top of the figure. Each experimental point represents the mean colony count from* 4 *replicative flasks. The vertical bars represent S.E.*

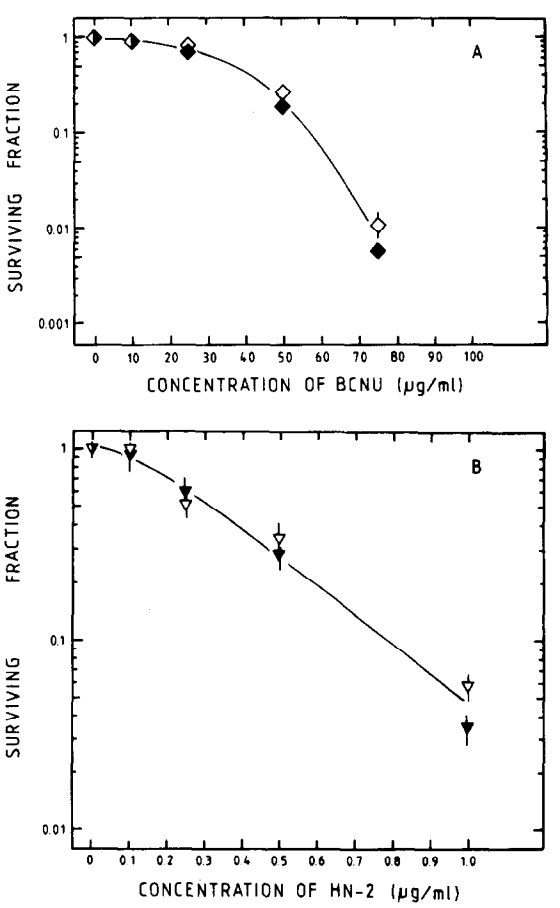

Fig. 6. Surviving fraction of asynchronous NHIK 3025 *cells as a function of the concentration of* (*A*) *BCNU* (◇) *or BCNU* + 3.2 mM *benzaldehyde* (◆), *and* (*B*) *HN2* (▽) *or HN2* + 3.2 mM *benzaldehyde* (▼). *Drug incubation was for* 1 hr; *otherwise conditions were as in Fig.* 1.

mainly two possible ways this could happen, and in the following text each of these are discussed separately.

(i) Benzaldehyde and cis-*DDP could react to form a non-toxic product*

There are several examples of chemicals which react with *cis*-DDP and form products with inactivating effects different from that of *cis*-DDP alone. Pyrimidines form complexes together with *cis*-DDP, many of which are highly potent cytotoxic agents [20]. Thus *cis*-DDP-induced cell inactivation is, in many cases, potentiated by combinations with pyrimidines and not diminished, as is the case with benzaldehyde. Two drugs, however, tend to reduce the inactivating effect of *cis*-DDP. Diethyldithiocarbamate has been shown to counteract the inactivation by *cis*-DDP of Chinese hamster (HA-1) cells *in vitro* [21] and also to counteract the toxicity of *cis*-DDP *in vivo* [22]. Evans *et al.* [21] have indicated that this reduction in *cis*-DDP cell inactivation is due to platinum chelation by diethyldithiocarbamate, thus not representing true protection but a type of rescue form *cis*-DDP cell inactivation. Benzaldehyde, however, is not a chelating agent. Furthermore, it does not react with *cis*-DDP in a manner similar to pyrimidine compounds (data not shown). Thiourea has also been shown to protect cells from the inactivating effect of *cis*-DDP [23]. The mechanism of thiourea protection appears to be the prevention of DNA cross-link formation by *cis*-DDP, apparently by reacting with DNA monoadducts [23].

Both BCNU and HN2 are alkylating agents which also form monoadducts and cross-link DNA [24–26]. Zwelling *et al.* [23] reported that thiourea protected L1210 cells from inactivation by nitrogen mustard. Our results with benzaldehyde (Fig. 6) in combination with BCNU or HN2 do not show modification of the inactivating effects of these two alkylating agents. We conclude, therefore, that benzaldehyde does not protect cells by a thiourea-type mechanism (i.e. reaction with monoadducts).

In addition, the polyamines putrescine and spermine have also been shown to reduce cell inactivation by *cis*-DDP but not by bleomycin in experiments with V79 cells cultured *in vitro* [27]. However, in contrast to what is known about benzaldehyde, the polyamines may alter the effect of *cis*-DDP by stabilizing the secondary structure of DNA [27].

Thus the modifying effect of benzaldehyde on cell inactivation by *cis*-DDP is different from the cases illustrated above. It is not yet clear whether we can call this effect a true protection against *cis*-DDP if, by protection, we mean direct interference with the damaging reactions of *cis*-DDP with cellular molecules. Apparently, benzaldehyde must be present simultaneously with, or directly before addition of, *cis*-DDP in order for the modifying effect to be optimal. Postincubation of NHIK 3025 cells with benzaldehyde following a *cis*-DDP pulse did not increase the surviving fraction from that of *cis*-DDP alone (Fig. 4).

(ii) Benzaldehyde might inhibit the uptake of cis-*DDP*

Although *cis*-DDP appears to enter cells quite freely, it cannot be ruled out that benzaldehyde inhibits the uptake of *cis*-DDP. Benzaldehyde does bind to membrane proteins [2, 5, 15] and has been shown to inhibit the uptake of nucleosides and 2-deoxy-D-glucose into SV 40-transformed cells [28]. It is therefore possible that benzaldehyde inhibits the uptake of *cis*-DDP, either directly by inhibiting some transport mechanism or indirectly by membrane distortion due to benzaldehyde–protein binding. BCNU and HN2, which are not influenced by the presence of benzaldehyde, may enter cells by an entirely different mechanism.

REFERENCES

1. Takeuchi S, Kochi M, Sakaguchi K, Nakagawa K, Mizutani T. Benzaldehyde as a carcinostatic principle in figs. *Agric Biol Chem* 1978, **42**, 1449–1451.
2. Sakaguchi K, Miyakawa T, Takeuchi S, Nakagawa K, Hayase E. Interaction of benzaldehyde to the membrane protein of *Escherichia coli*. *Agric Biol Chem* 1979, **43**, 1775–1777.
3. Nambata T, Terada N, Mizutani T, Takeuchi S. Effect of benzaldehyde on mouse embryo cells *in vitro*. *Gann* 1980, **71**, 572–573.
4. Nambata T, Terada N, Mizutani T, Takeuchi S. Characteristics of C3H/He mouse embryo cell lines established by culture with or without benzaldehyde. *Gann* 1982, **73**, 592–599.
5. Miyakawa T, Zundel J-L, Sakaguchi K. Selective inhibitory effect of benzaldehyde on the growth of simian virus 40-transformed cells. *Biochem Biophys Res Commun* 1979, **87**, 1024–1030.

6. Kochi M, Takeuchi S, Mizutani T, Mochizuki K, Matsumoto Y, Saito Y. Antitumor activity of benzaldehyde. *Cancer Treat Rep* 1980, **64**, 21–23.
7. Pettersen EO, Nome O, Rønning ØW, Oftebro R. Effects of benzaldehyde on survival and cell cycle kinetics of human cells cultivated *in vitro*. *Eur J Cancer Clin Oncol* 1983, **19**, 507–514.
8. Pettersen EO, Rønning ØW, Nome O, Oftebro R. Effects of benzaldehyde on protein metabolism of human cells cultivated *in vitro*. *Eur J Cancer Clin Oncol* 1983, **19**, 935–940.
9. Rosenberg B. Anticancer activity of *cis*-dichlorodiammineplatinum(II) and some relevant chemistry. *Cancer Treat Rep* 1979, **63**, 1433–1438.
10. Zwelling LA, Kohn KW. Mechanism of action of *cis*-dichlorodiammineplatinum(II). *Cancer Treat Rep* 1979, **63**, 1439–1444.
11. Zwelling LA, Kohn KW. Effects of cisplatin on DNA and the possible relationships to cytotoxicity and mutagenicity in mammalian cells. In: Prestayko AW, Crooke ST, Carter SK, eds. *Cisplatin: Current Status and New Developments*. New York, Academic Press, 1980, 21–35.
12. Scanlon KJ, Safirstein RL, Thies H, Gross RB, Waxman S, Guttenplan JB. Inhibition of amino acid transport by *cis*-diamminedichloroplatinum(II) derivatives in L1210 murine leukemia cells. *Cancer Res* 1983, **43**, 4211–4215.
13. Ishida A, Miwa N, Mizuno S. Differential enhancement of cytotoxicity by combination of the carcinostatic agent benzaldehyde and hyperthermia in simian virus 40-transformed and normal cell lines. *Cancer Res* 1983, **43**, 4126–4220.
14. Nordbye K, Oftebro R. Establishment of four new cell strains from human uterine cervix. I. *Exp Cell Res* 1969, **58**, 458.
15. Oftebro R, Nordbye K. Establishment of four new cell strains from human uterine cervix. II. *Exp Cell Res* 1969, **58**, 459–460.
16. Puck TT, Cieciura SJ, Fisher HW. Clonal growth *in vitro* of human cells with fibroblastic morphology. *J Exp Med* 1957, **106**, 145–165.
17. Pettersen EO, Bakke O, Lindmo T, Oftebro R. Cell cycle characteristics of synchronized and asynchronous populations of human cells and effect of cooling of selected mitotic cells. *Cell Tissue Kinet* 1977, **10**, 511–522.
18. Rønning ØW, Lindmo T, Pettersen EO, Seglen PO. The role of protein accumulation in the cell cycle control of human NHIK 3025 cells. *J Cell Physiol* 1981, **109**, 411–418.
19. Robert JJ, Fraval HNA. Repair of *cis*-platinum(II) diammine dichloride-induced DNA damage and cell sensitivity. In: Prestayko AW, Crooke ST, Carter SK, eds. *Cisplatin: Current Status and New Developments*. New York, Academic Press, 1980, 57–77.
20. Davidson JP, Faber PJ, Fischer RG Jr *et al.* Platinum-pyrimidine blues and related complexes: a new class of potent antitumor agents. *Cancer Chemother Rep* 1975, **59**, 287–300.
21. Evans RG, Engel C, Wheatley C, Nielsen J. Modification of the sensitivity and repair of potentially lethal damage by diethyldithiocarbamate during and following exposure of plateau-phase cultures of mammalian cells to radiation and *cis*-diamminedichloroplatinum(II). *Cancer Res* 1982, **42**, 3074–3078.
22. Gale GR, Atkins LM, Walker EM Jr. Further evaluation of diethyldithiocarbamate as an antagonist of cisplatin toxicity. *Ann Clin Lab Sci* 1982, **12**, 345–355.
23. Zwelling LA, Filipski J, Kohn KW. Effect of thiourea on survival and DNA cross-link formation in cells treated with platinum(II) complexes, *L*-phenylalanine mustard, and bis(2-chloroethyl)methylamine. *Cancer Res* 1979, **39**, 4989–4995.
24. Ewig RAG, Kohn KW. DNA damage and repair in mouse leukemia L1210 cells treated with nitrogen mustard, 1,3-bis(2-chloroethyl)-1-nitrosourea, and other nitrosoureas. *Cancer Res* 1977, **37**, 2114–2122.
25. Ewig RAG, Kohn KW. DNA-protein cross-linking and DNA interstrand cross-linking by haloethylnitrosoureas in L1210 cells. *Cancer Res* 1978, **38**, 3197–3203.
26. Ross WE, Ewig RAG, Kohn KW. Differences between mephalan and nitrogen mustard in the formation and removal of DNA cross-links. *Cancer Res* 1978, **38**, 1502–1506.
27. Roizin-Towle L. Studies with *cis*-diamminedichloroplatinum II and exogenous polyamines using mammalian cells in culture. *Br J Cancer* 1981, **43**, 378–383.
28. Watanuki M, Sakaguchi K. Selective inhibition by benzaldehyde of the uptake of nucleosides and sugar into simian virus 40-transformed cells. *Cancer Res* 1980, **40**, 2574–2579.