Reduction by Caffeine of Adriamycin-induced Cell Killing and DNA Damage in Chinese Hamster Cells: Correlation with Modulation in Intracellular Adriamycin Content

George Iliakis and Wendy Lazar

ABSTRACT

The effect of caffeine, at concentrations between 10 \( \mu \text{M} \) and 20 mm, was studied on Adriamycin-induced cytotoxicity and DNA damage in exponentially growing Chinese hamster V79 cells. Simultaneous administration of caffeine with 0.4 \( \mu \text{g/ml} \) Adriamycin for 1 h resulted in a concentration-dependent reduction in cell killing. The surviving fraction increased from 0.001 for cells treated with Adriamycin alone to 0.14 for cells treated in the presence of 1 mm caffeine and to 0.8 for cells treated at caffeine concentrations higher than 6 mm. A significant reduction in Adriamycin-induced cell killing was also caused by caffeine at micromolar concentrations where the surviving fraction increased from 0.00076 to 0.0014 (2-fold) after treatment with 10 \( \mu \text{M} \), to 0.0038 (5-fold) after treatment with 20 \( \mu \text{M} \) and to 0.01 (13-fold) after treatment with 100 \( \mu \text{M} \) caffeine. Treatment of cells with caffeine for 1 h immediately after Adriamycin exposure (0.4 \( \mu \text{g/ml}, 1 \text{ h} \)) resulted in a dose-dependent increase in survival as well, but the effect was smaller than that observed after simultaneous administration (increase in the surviving fraction from 0.003 to about 0.8 at concentrations higher than 5 mm). The reduction in Adriamycin-induced cytotoxicity by caffeine was reflected by a decrease in the slope of the survival curve, and it was similar over the entire range of Adriamycin and caffeine concentrations examined. The ability of cells to accumulate Adriamycin was reduced by caffeine from 43 ng/10\(^6\) cells after treatment for 1 h in the presence of 0.5 \( \mu \text{g/ml} \) Adriamycin to 16 ng/10\(^6\) cells for cells treated in the presence of 2 mm and to 8 ng/10\(^6\) cells for cells treated in the presence of 40 mm caffeine. Induction of Adriamycin of DNA breaks, as assayed by the alkaline filter elution technique, was linear with concentration and was decreased in the presence of caffeine. The response to caffeine of Adriamycin-induced killing and DNA damage was similar, and it was only slightly different from the modulation induced in intracellular Adriamycin content. Compared to the effect of caffeine on cells exposed to ionizing radiations or other cytotoxic compounds, the results indicate an entirely different mode of caffeine action with anthracyclines. In addition, the results suggest caffeine-induced modulations in intracellular drug accumulation as an important determinant for the effect and may have useful implications in the clinical application of these compounds.

INTRODUCTION

Methylated xanthines, particularly caffeine, were shown to potentiate lethal and chromosome-damaging effects of a number of physical and chemical agents (see Ref. 1 for a review.) Caffeine was reported to increase in vitro cytotoxicity of neo-carzinostatin (2), of ellipticine, hycanthone, and actinomycin D (3). Furthermore, it was found to amplify the antitumor effect of a number of physical and chemical agents (see Ref. 1 for a review.) Potentiation of lethal and chromosome-damaging effects of a number of physical and chemical agents was reported after posttreatment incubation with caffeine of mouse leukemia L1210 cells following exposure to Adriamycin (3). Furthermore, 2 mm caffeine was found to reduce Adriamycin-induced killing in Chinese hamster V79 (7) and mouse leukemia P388 cells (8) when given simultaneously, and also, although to a lesser extent, when given after treatment. A similar reduction of the cytotoxic effect by caffeine was also observed with the Adriamycin analogue n-trifluoroacetyladriamycin-14-valerate (AD 32) (8), suggesting that this type of response may be a property of Anthracyclines in general. The calmodulin inhibitor trifluoperazine, reported to enhance killing of cell lines with acquired Adriamycin resistance (9, 10), was effective in compromising caffeine-mediated reduction in killing (7, 8).

Thus, a wide diversity is indicated in the effect of caffeine in combination with various chemotherapeutic agents. It is likely that better understanding of the molecular basis of this interaction may prove helpful, except for future improvements in the clinical application of these antitumor antibiotics, also for the elucidation of their mechanism of action per se. In the present study, we determined the effect of caffeine, administered over a range of concentrations (10 mm to 20 mm), on the cytotoxic effects of Adriamycin in proliferating Chinese hamster V79 cells. Both compounds were administered simultaneously in the majority of experiments and the effect was evaluated immediately after treatment. The parameters studied were: cell inactivation as measured by colony formation, damage to DNA (mainly induction of breaks as assayed by DNA alkaline filter elution), and intracellular Adriamycin accumulation. The results obtained indicated a concentration-dependent reduction by caffeine of the intracellular Adriamycin content accompanied by a reduction in killing and DNA damage.

MATERIALS AND METHODS

For experiments, a line of Chinese hamster V79 cells (S-171) was used (13). Cells were grown at 37°C in a humidified atmosphere of 5% CO\(_2\) in MEM\(^2\) supplemented with 15% fetal bovine serum (Hazelton, Denver, PA) and were subcultured every second day at a concentration of 10\(^6\) cells/75 cm\(^2\) flask (20 ml MEM). For experiments, cells obtained from these cultures were plated at a concentration of 1–2 × 10\(^4\) cells/dish (60 mm, 5 ml MEM) and were used the following day when the number of cells per dish had increased to about 4–7 × 10\(^4\). Caffeine and Adriamycin were obtained from Sigma Chemical Co. (St. Louis, MO). Adriamycin Exposure and Colony Forming Assay. Cells were exposed to various doses of Adriamycin (from a stock solution prepared in

\[\text{CANCER RESEARCH 47, 2224-2229, May 1, 1987}\]

Received 7/21/86; revised 11/10/86, 1/28/87; accepted 2/2/87.

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\(^1\) Supported in part by U.S.P.H.S. Grant 1RO1-CA42026 awarded by the National Cancer Institute, Department of Health and Human Services.  

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\[^\text{CANCER RESEARCH 47, 2224-2229, May 1, 1987}\]

2224
with 5 ml of phosphate buffered saline. Subsequently, 1 ml of 0.25% trypsin plus 0.1% EDTA solution was added and cultures were incubated at 37°C for 5-10 min. Following trypsinization, 2-5 ml of growth medium were added and cells were repeatedly pipetted to obtain a single cell suspension. The number of cells per milliliter in this suspension was determined with an electronic counter and was used to dilute and plate cells to form colonies, with the goal of obtaining 25-200 colonies per dish (60 mm, 5 ml MEM). Two to four replicates were prepared for each data point. The standard errors in the estimation of cell survival were, therefore, between 7 and 14% unless otherwise indicated. Colonies were stained with methylène blue (20% ethanol solution) before counting. Cell survival was calculated based on the cell counts obtained and the plating efficiency of the untreated controls. Survival curves shown were fitted to the data points by eye. Results derived from a single experiment are shown but the findings were reproduced in independent experiments. Survival results are described by \( D_0 \), defined as the inverse of the survival slope in the exponential part and corresponding to the Adriamycin dose required to decrease cell survival to 37% of the controls in this region, and \( D_h \) defined as the Adriamycin dose given by the intercept between the dose axis and a back extrapolation of the exponential part of the survival curve. The values of these parameters were determined by a computer best fit of the measurement points in the exponential region of the survival curve.

### Intracellular Adriamycin Accumulation

Cells were treated in MEM with 0.17-15 \( \mu \)g/ml Adriamycin for 1 h at 37°C in the presence of various concentrations of caffeine. Following treatment, cells were washed twice in ice-cold buffered saline, trypsinized for 5 min at 37°C, washed again to remove trypsin and medium, and centrifuged. The cell pellet was resuspended in 50% ethanol-0.3 N hydrochloric acid, mixed in a vortex mixer, and centrifuged again at 800 \( \times g \). The Adriamycin content in the supernatant was determined in a Turner spectrophotofluorometer (G. K. Turner Associates, Palo Alto, CA) as described previously (14). The excitation wavelength was at 470 nm and emission was collected at 585 nm. Calibration curves prepared with solutions of known Adriamycin concentration were used for the calculation of the intracellular Adriamycin content which is expressed as ng/10^6 cells. Using similar solutions supplemented with caffeine, it was established that the presence of this compound does not alter the fluorescence properties of Adriamycin.

### DNA Strand Break Induction by Adriamycin

DNA strand break induction was determined by the alkaline filter elution technique. Details of this method have been published (15). Briefly, cells grown for 24 h in the presence of 0.1 \( \mu \)Ci/ml \([H]3\)-thymidine and 5 \( \mu \)M cold thymidine were treated for 1 h with Adriamycin in the presence or absence of caffeine. Following treatment, cells were washed and trypsinized as described and loaded on 2-\( \mu \)m porosity, polycarbonate filters (Nucleopore Corp., Pleasanton, CA) supported by Swinnex filter holders (Millipore Corp., Bedford, MA). Cells were rinsed with 10 ml phosphate buffered saline and lysed with 5 ml of a solution of 2% w/v sodium dodecyl sulphate (specially pure; BDH Chemical Ltd., Poole, England) and 0.025 M EDTA (acid form), pH 9.7. DNA was eluted through the filters at a constant flow rate of about 2 ml/h (Minipuls 2; Gilson Medical Electronics Inc., Middleton, WI) with a solution of 0.025 M EDTA, 0.1% w/v sodium dodecyl sulphate, and sufficient tetra-\( \nu \)-propyl ammonium hydroxide for a final pH of 12.9. Fractions (90 min) were collected and weighed. Aliquots from each fraction were neutralized with 0.4 \( \mu \)l HCl and counted in a scintillation counter. The fraction of the total activity remaining on the filter was plotted as a function of the elution time, after each particular treatment. In certain experiments, aliquots of cells used for the experiment were exposed to 250 kVp X-rays (2Gy) and DNA was eluted for comparison.

### RESULTS

The effect of various concentrations of caffeine, in the range between 0.5 and 20 mM, on the survival of Chinese hamster V79-cells exposed to 0.4 \( \mu \)g/ml Adriamycin for 1 h is shown in Fig. 1. The closed circles depict the modulation in survival observed when Adriamycin and caffeine were given simultaneously, whereas the open circles depict the modulation in survival induced when cells were incubated with caffeine for 1 h after exposure to Adriamycin. As evident from the results, cotreatment with caffeine resulted in a dramatic reduction in cell killing. For example, treatment with Adriamycin alone resulted in a surviving fraction of 0.001 but the presence of 0.5 mM caffeine caused an increase to 0.08 and of 2 mM to 0.45. Concentrations higher than 6 mM caffeine resulted in surviving fractions no higher than 0.8. Incubation of cells with caffeine alone in the concentration range and the treatment time intervals examined did not affect plating efficiency.

Since a rapid increase in cell survival was observed with caffeine in the range between 0 and 0.5 mM, we studied, in a separate experiment, the effect of caffeine in the range between 0 and 100 \( \mu \)M on Adriamycin-induced killing. The results obtained are shown in the Fig. 1, insert. Caffeine (100 \( \mu \)M) caused an increase in survival by one order of magnitude, and an increase in survival by a factor of two was observed at caffeine concentration as low as 10 \( \mu \)M.

The effect of caffeine in the modulation of cell survival when given after treatment with Adriamycin was smaller than that observed after cotreatment and resulted in an increase in survival from 0.0032 to a maximum of about 0.055 for caffeine concentrations higher than 6 mM. Therefore, later experiments were confined to cotreatment protocols.

To better evaluate the effect of caffeine on Adriamycin-induced killing, a set of cultures was treated with various doses of Adriamycin in the range between 0 and 1.8 \( \mu \)g/ml in the presence of caffeine at various concentrations and \( D_h \) and \( D_0 \) were calculated from the exponential part of the survival curves, as described in "Materials and Methods." The results are shown in Fig. 2, left. It is evident that the reduction in killing observed in Fig. 1 is reflected by an increase in both \( D_h \) and \( D_0 \), whose values are plotted in Fig. 2, right, as a function of caffeine concentration. The effect of caffeine was essentially independ-

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*Fig. 1.* Survival of cells exposed to various concentrations of caffeine for 1 h either during (B) or after (C) treatment with Adriamycin (0.4 \( \mu \)g/ml, 1 h). Insert, cell survival as a function of caffeine concentration at micromolar concentrations after treatment with Adriamycin (0.4 \( \mu \)g/ml, 1 h).
Effect of caffeine on Adriamycin cytotoxicity

Fig. 2. Left, survival curves of cells exposed to various amounts of Adriamycin in the presence of various caffeine concentrations as indicated. Right, $D_A$ and $D_C$, (see "Materials and Methods"), calculated from the survival curves at right, as a function of caffeine concentration.

Table 1 Dose modifying factor

<table>
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<tr>
<th>Survival</th>
<th>Caffeine*</th>
<th>0.5 mM</th>
<th>1 mM</th>
<th>2 mM</th>
<th>5 mM</th>
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</thead>
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<td>0.1</td>
<td>1.6</td>
<td>2.4</td>
<td>3.5</td>
<td>6.0</td>
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</tr>
<tr>
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<td>1.6</td>
<td>2.3</td>
<td>3.4</td>
<td>5.9</td>
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<td>2.2</td>
<td>3.2</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>0.005</td>
<td>1.6</td>
<td>2.1</td>
<td>3.1</td>
<td>5.4</td>
<td></td>
</tr>
</tbody>
</table>

Modification of intracellular ADR content

* Results obtained at 10 mM caffeine were excluded from this comparison due to the limited availability of survival measurements.

It was previously reported that caffeine can affect the ability of cells to accumulate Adriamycin (7, 8). In a set of experiments we measured, therefore, the amount of Adriamycin accumulated in cells after a 1-h treatment with 0.5 μg/ml Adriamycin, as a function of the caffeine concentration. The results obtained, expressed as ng of Adriamycin per 10⁶ cells, are shown in Fig. 3. A reduction in the amount of Adriamycin accumulated in cells was observed with increasing caffeine concentration.

The effects of Adriamycin on DNA, either alone or in combination with caffeine, are summarized in Fig. 4. DNA elution patterns are shown in Fig. 4, left, obtained with cells treated for 1 h with 0.6 μg/ml Adriamycin (0 mM caffeine) together with those of untreated controls. For comparison the elution pattern of DNA obtained from cells exposed to 2 Gy X-rays is shown. An estimate of the amount of DNA damage induced by Adriamycin, (both single- and double-strand breaks) as measured under the elution conditions employed, can be obtained by calculating the slope of the first, fast component of the elution curve. For this purpose, fractions obtained between 1.5 and 6.0 h or longer, depending on the shape of the individual curve, were used, and linear regression analysis was performed. The values of the slopes obtained this way are shown in Fig. 4, top right, expressed as elution rate in h⁻¹, for a series of 1-h exposures with various concentrations of Adriamycin, given alone. A nearly linear increase was observed in the amount of DNA damage induced as a function of Adriamycin concentration. Fig. 4, bottom right, shows the modification in the elution rate induced by caffeine at various concentrations, obtained from DNA elution patterns of cells exposed to Adriamycin in the presence of caffeine as shown in Fig. 4, left. Caffeine given alone did not affect the elution pattern of cells that were not exposed to Adriamycin. Similar to the effect on cell survival, a reduction in the DNA elution rate was observed with increasing concentration of caffeine, indicating a reduction in the amount of DNA damage induced.

The results presented so far show a caffeine concentration-dependent reduction of Adriamycin-induced cell killing and...
DNA damage accompanied by a reduction in intracellular Adriamycin accumulation. To enable a comparison of these caffeine-induced modulations of Adriamycin effect of the mentioned endpoints, the effect observed was normalized to that of untreated controls and the values obtained are shown in Fig. 5. Fig. 5, top, shows the relative modification induced by caffeine in the intracellular Adriamycin accumulation in cells treated for 1 h with 0.17, 0.5, or 1.5 µg/ml. A similar reduction in the intracellular Adriamycin levels was found at all concentrations used, which are within the range where cell survival was measured (see Fig. 2). A similar response was also observed at a concentration of 15 µg/ml, but a tendency was noted for the intracellular Adriamycin amount to plateau at slightly higher values. The relative effect of caffeine on Adriamycin-induced cell killing and DNA damage is compared with the modulation induced in the intracellular drug accumulation in Fig. 5, bottom. The effect on cell survival is expressed as the relative modification in the slope of the survival curve (1/DA). The effect on DNA is expressed as the relative modification in the elution rate. The effect on the modulation of intracellular Adriamycin accumulation is depicted by the broken line which has been transferred from the data shown in Fig. 5, top; data points have been omitted for clarity. Cell killing and DNA damage showed the same kinetics of reduction with increasing caffeine concentration. The reduction in intracellular Adriamycin accumulation showed similar kinetics up to concentrations of 2 mM caffeine but became slightly slower at higher concentrations. The same tendency is also indicated by a comparison of the DMF shown in Table 1 and the modulation induced in the intracellular Adriamycin content shown in the last line of the table (obtained from Fig. 3). The results indicate a close relationship between DMF and intracellular Adriamycin accumulation up to caffeine concentrations of 2 mM. At higher concentrations DMF values larger than the corresponding modulation in intracellular Adriamycin content were observed. This comparison suggests that the reduction in intracellular Adriamycin content in the presence of caffeine, may be a major determinant in the mechanism of action with anthracyclines. However, additional effects cannot be excluded and are partly suggested by the results presented.

DISCUSSION

The search for ways to potentiate the effect of various antitumor drugs by various physical (e.g., ionizing radiations) or chemical (e.g., caffeine) agents has been a topic of particular interest due to the possibilities it offers for improvement in the treatment of cancer. In this respect, screening of various sensitizing compounds and analysis of the underlying mechanism is likely to reveal the determinants, whose controlled modulation may lead to the desired goal. Methylated xanthines, and particularly caffeine, are potential candidates in this context due to their efficacy in potentiating lethal and chromosome-damaging effects after various physical and chemical treatments (1). Caffeine, at concentrations between 1 and 10 mM, was found to potentiate the lethal effects of ionizing radiations (11, 12), and nitrogen mustard (16) in vitro, and was also found effective in potentiating the antitumor effect of various compounds in vivo as for example phleomycins and bleomycins (6) and nitrosoureas (4, 6, 17). The molecular mechanism of this effect is not known but is thought to be correlated with the inhibitory effect caffeine exerts on certain DNA repair processes. Caffeine was found effective in inhibiting DNA repair replication in rodent, but also in excision repair-deficient human cells (1, 18). In combination with anthracyclines, however, caffeine exerts an entirely different mode of action dramatically reducing, in a concentration-dependent way, Adriamycin cytotoxicity. This difference in the mode of action was first reported in LI210 cells treated with Adriamycin and subsequently incubated in caffeine-containing medium for colony formation (3). In later studies it was established that similar effects can be also observed in a variety of cell lines and that the reduction in killing induced by caffeine is at maximum when given simultaneously with the anthracycline under study (7, 8).

The effect of caffeine on the cytotoxicity of anthracyclines differs from the enhancement of killing observed with other compounds and treatments in various ways. For example, as already mentioned, the effect is at maximum when caffeine is given during treatment and it is observed for treatments as short as a few minutes (30–60 min) (7, 8); this is in contrast to the requirement for several hours or days long incubation for maximum enhancement in killing as observed after exposure to X-rays (11, 12) or other antitumor drugs (3). Furthermore, a significant reduction in Adriamycin-induced killing can be observed at caffeine concentrations in the range of 10–100 mM (Fig. 1), whereas for potentiation in radiation-induced killing usually concentrations above 1 mM are necessary (11, 12).

The mechanism by which caffeine reduces Adriamycin cytotoxicity is not understood, but the results presented here suggest that modulation in the intracellular drug accumulation may be an important determinant. It is interesting that reduced cellular Adriamycin accumulation has also been proposed as the reason for diminished cytotoxicity in Adriamycin-resistant tumors (19–21). The reduction by caffeine of the intracellular Adriamycin accumulation may have chemical or biochemical reasons. For example, it is possible that direct association between
cafeine and Adriamycin results in a reduction of free Adriamycin in the medium and leads to the formation of molecular complexes which may be transported through cell membrane at a reduced rate and/or show reduced toxicity at the cellular targets. Supporting evidence for this possibility is the observation that Adriamycin has affinity for association with cafeine, with various DNA-derived bases, nucleotides, and nucleosides as well as with other compounds, as indicated by measurements of partition coefficients in water (22). On the other hand, complicated, transport-related biochemical processes cannot be excluded and are suggested by the observation that caffeine-mediated reduction in Adriamycin-induced killing and drug accumulation can be reversed by the calmodulin inhibitor trifluoperazine (7, 8), reported to sensitize in vitro strains with acquired Adriamycin resistance by increasing intracellular drug accumulation (9).

As pointed out, an effect of cafeine on cell survival after treatment with Adriamycin can be observed at concentrations as low as 10 ìM and approaches a plateau at concentrations higher than about 6 ìM; only about 10% of the cells are inactivated at this concentration range compared to 99.999% inactivated when Adriamycin (0.4 ìg/ml, 1 h) is given alone. The reduction in Adriamycin cytotoxicity observed at caffeine concentrations in the micromolar range may have implications in the clinical application and efficacy of anthracyclines when administered to individuals with caffeine blood levels in this range. For comparison, it is mentioned that approximately 100 mg cafeine is present in a cup of coffee which, when completely absorbed, will result in maximum blood caffeine levels of about 100 ìM, a concentration well in the range where significant modulation in cell killing was observed.

The reduction in Adriamycin-induced killing by cafeine given posttreatment is smaller in magnitude but shows a response similar to that observed with simultaneous incubation; the initial rapid increase in survival observed up to concentrations of 2 ìM is followed by a plateau at concentrations higher than 6 ìM (Fig. 1). This indicates an effect of cafeine on cellular Adriamycin, which is found mainly bound on chromatin. It may be due to the potent solubilizing activity of cafeine which could enhance Adriamycin dislodging from the DNA (23), and it may be reflected in the faster kinetics observed in the rate of decay of Adriamycin-related fluorescence in the presence of caffeine (7). Thus, it can be assumed that cafeine alters the binding affinity of Adriamycin to chromatin, probably by competing for binding sites at the DNA, thus, reducing the concentration gradient driving the diffusion process causing the intracellular drug accumulation (24). This model can be used to explain both the reduced Adriamycin accumulation observed after cotreatment with cafeine as well as the reduced retention of Adriamycin after posttreatment incubation with cafeine.

It is generally accepted that the binding of Adriamycin to nucleic acids and the subsequent inhibition of DNA and RNA synthesis are the pertinent effects leading to the observed cytotoxicity (25), but the existence of other cellular targets as well has been proposed (26). It is well established that Adriamycin induces DNA strand breaks and protein-associated breaks in mammalian cells (3, 27, 28). The experiments reported here indicate a linear increase in the amount of DNA breaks induced as a function of Adriamycin dose. Presence of cafeine during treatment was found to reduce DNA damage in a way similar to which cell killing was reduced with increasing cafeine concentration. Thus, the reduction of Adriamycin cytotoxicity in the presence of cafeine was also reflected by a reduction in DNA damage, suggesting a cause-effect relationship between these phenomena. However, since induction of DNA breaks by antitumor drugs does not seem to parallel their cytotoxic effects (3), the results obtained with cafeine should await identification of the critical lesions for a more conclusive interpretation.

The results presented here complement and confirm previously reported results obtained with the same cell system (7). However, they differ in several ways from results obtained with P388 mouse leukemia cells (8). In the latter cell system and for the experimental conditions employed, cafeine reduced cell killing to a larger extent at low than at high Adriamycin concentrations. On the other hand cafeine reduced intracellular drug accumulation at a measurable extent only at high Adriamycin concentrations. The reasons for these differences are not presently known. However, it is possible that they are, at least partly, due to differences in the biological systems used. P388 mouse leukemia cells are more sensitive to Adriamycin than Chinese hamster V79 cells. It is likely that factors affecting cell sensitivity to Adriamycin may also affect its response to cafeine, thus resulting in the observed differences in response. In addition, the trypsinization step (see "Materials and Methods"), required for Chinese hamster cells before collection and measurement, for Adriamycin accumulation may affect intracellular drug concentration especially if drug efflux is affected by precipitation with cafeine. Further experimentation, directly comparing the response of both cell lines under similar experimental conditions, is necessary to conclusively elucidate this point.

In summary, cafeine was found to affect Adriamycin-induced cytotoxicity at micromolar concentrations and to reverse Adriamycin-induced killing and DNA damage at millimolar concentrations. A possible determinant for this effect may be the reduced ability of cells to accumulate Adriamycin in the presence of cafeine, but the mechanism of this phenomenon remains unknown.

ACKNOWLEDGMENTS

The authors are indebted to Dr. P. Mojaverian of the Department of Clinical Pharmacology and P. Green of the Department of Endocrinology at Thomas Jefferson University for their permission to use the spectrofluorometers, to S. B. Boybock for preparing the graphs, and to S. M. Douthart for typing the manuscript. Special thanks go to Dr. Dennis B. Leeper for helpful discussions during the preparation of the manuscript.

REFERENCES

8. Ganapathi, R., Grabowski, D., Schmidt, H., Yen, A., and Iliakis, G. Modulation of Adriamycin and N-trifluoroacetyl adriamycin-14-valerate (AD32) induced effects on cell cycle traverse and cytotoxicity in P388 mouse leukemia
EFFECT OF CAFFEINE ON ADRIAMYCIN CYTOTOXICITY


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