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ACTION OF cis-DICHLOROBIS (CYCLOPENTYLAMINE) PLATINUM(II) (cis-PAD) ON L5178Y CELLS OF TWO STRAINS INVERSELY CROSS-SENSITIVE TO X-RAYS AND UV-LIGHT

III. Differential effects of caffeine

I. Szumiel

Warszawa 1977

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DEPARTMENT OF RADIOBIOLOGY AND HEALTH PROTECTION

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III. DIFFERENTIAL EFFECTS OF CAFFEINE

DZIAŁANIE cis-DWUCHLORO-BIS(CYKLOPENTYLOAMINO)PLATYNY(II)(cis-PAD) NA KOMÓRKI L5178Y DWÓCH SZCZEPÓW KRZYŻOWO WRAŻLIWYCH NA PROMIENIOWANIE X i ŚWIATŁO NADFIOLETOWE

III. ZRÓŻNICOWANE ODDZIAŁYWANIE KOFEINY

ДЕЙСТВИЕ цис-ДИХЛОРБИС(ЦИКЛОПЕНТИЛАМИН) ПЛАТИНИ(II) (цис-ПАД) НА КЛЕТКИ L5178Y ДВУХ ШТАММОВ ПРОЯВЛЯЮЩИХ ПЕРЕКРЕСТНУЮ ЧУВСТВИТЕЛЬНОСТЬ К РЕНТГЕНОВСКИМ И УЛЬТРАФИОЛЕТОВЫМ ЛУЧАМ

III. РАЗЛИЧИЕ В ДЕЙСТВИИ КОФЕИНА

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Abstract

Two strains of L5178Y murine lymphoma, inversely cross-sensitive to X-rays and UV-light, were shown previously to respond to treatment with an antitumour platinum complex, cis-PAD, in a similar manner as to UV. The difference in sensitivity to cis-PAD, found in L5178Y-R and L5178Y-S cells is not caused by a difference in ability to bind platinum complex. Enhancement of chromosomal damage and potentiation of lethal effect of cis-PAD by 0.75 mM caffeine were found in cis-PAD and UV-light-resistant L5178Y-S strain but not in cis-PAD and UV-light -sensitive L5178Y-R strain. These results suggest that the extreme sensitivity of L5178Y-R strain to cis-PAD and UV-light is caused to some extent by deficiency in a caffeine-sensitive post-replication repair system.

Streszczenie

Jak wykazano uprzednio, dwa krzyżowo wrażliwe na promieniowanie X i światło nadfioletowe (UV) szczepy mysich komórek białaczkowych L5178Y reagują na traktowanie przeciwnowotworowym kompleksem platyny (cis-PAD) w podobny sposób jak na naświetlenie światłem UV. Różnica we wrażliwości na cis-PAD między komórkami szczepów L5178Y-R i L5178Y-S nie jest spowodowana odmienną zdolnością wiązania kompleksu platyny. W komórkach szczepu L5178Y-S (opornego na cis-PAD i UV) stwierdzono, że 0.75 mM kofeina potęguje letalne skutki cis-PAD i uszkodzenia chromosomów. Efektu takiego nie znaleziono w komórkach szczepu L5178Y-R, wrażliwego na cis-PAD i UV. Nasuwa to przypuszczenie, że niezwykle wysoka wrażliwość szczepu L5178Y-R na cis-PAD i UV spowodowana jest brakiem wrażliwego na kofeinę układu naprawczego typu poreplikacyjnego.

Резюме

Как было раньше обнаружено, два штамма клеток мышиной лейкемии L5178Y, проявляющие перекрестную чувствительность к рентгеновским и ультрафиолетовым лучам (УФ) отвечают на действие цис-ПАД подобным образом, как на УФ. Разница в чувствительности к цис-ПАД, найдена между штаммами L5178Y-R и L5178Y-S, не вызвана разной способностью к связыванию комплекса платины. Повышение повреждения хромосом и увеличение летального эффекта цис-ПАД под влиянием 0.75 мМ кофеина наблюдали в цис-ПАД и УФ резистентном штамме L5178Y-S но не в цис-ПАД и УФ-чувствительном штамме L5178Y-R. Эти наблюдения позволяют предположить, что чрезвычайная чувствительность штамма L5178Y-R к цис-ПАД и УФ вызвана недостатком чувствительной к кофеину пост-репликационной репаративной системы.

1. INTRODUCTION

Two strains of L5178Y murine lymphoma cells used in these studies [1,2] present a system unique among mammalian cells for its inverse cross-sensitivity to X-rays and UV-light [3,4]. It was shown previously [5,6] that the relatively X-ray-resistant, UV-light-sensitive L5178Y-R strain was also sensitive to an antitumour platinum complex, cis-dichlorobis(cyclopentylamine)platinum(II) (cis-PAD [7]), while the relatively X-ray-sensitive, UV-light-resistant L5178Y-S strain was resistant to cis-PAD. The similarity in sensitivity to cis-PAD and UV-light [5] could be explained on the assumption that both these agents induce such DNA lesions which are eliminated by the same type [5] of repair processes. Alternatively, differences in uptake and binding of cis-PAD could be responsible for the observed differences in sensitivity of both strains to the platinum complex; this possibility, however, was eliminated, on the ground of practically identical ability of both L5178Y strains to bind cis-PAD [6].

It is known that rodent cells do not effectively excise pyrimidine dimers induced by UV-light [8-10] and that such DNA damage is dealt with by a caffeine-sensitive post-replicative repair system [11,12]. The same repair system was recently shown to operate in rodent cells damaged by a platinum complex [13-15]. Therefore, studies were undertaken in order to examine effects of post-treatment incubation in presence of caffeine on cis-PAD-treated L5178Y-R and L5178Y-S cells.

2. MATERIALS AND METHODS

2.1. Cell cultures

L5178Y-R and L5178Y-S cells were cultivated as suspensions in Fischer's medium containing 8 % of calf serum (Państwowa Wytwórnia Surowic i Szczepionek, Lublin, Poland) and 0.1 g/l neomycin. Doubling times of L5178Y-R and L5178Y-S cell cultures were 12-14 h and 10-11 h, respectively. A detailed description of cell culture methods and characteristics of both L5178Y strains were given previously [5].

Survival was determined by cloning in Fischer's medium supplemented with 0.19 % agar, as described in [5]. Surviving colonies were counted after 9 days (L5178Y-S cells) and 10-12 days (L5178Y-R cells).

2.2. Treatment with platinum complex

Platinum complex (cis-PAD) was kindly provided by Dr T.A.Connors and by Johnson Matthey and Co. Ltd. (London, UK). cis-PAD solution was prepared at a concentration of 2 mg/ml in dimethylsulphoxide (DMSO). The solution obtained was immediately diluted 1:4 with cold (+4°C) Fischer's medium and appropriate volumes of this solution were added as quickly as possible (ca 1 min) to a series of five 10 ml cultures containing $1x10^5$ cells per ml for determination of survival or to 50 ml cultures containing $2x10^5$ cells per ml (for analysis of chromosomal aberrations). Control cells were treated with DMSO diluted 1:4 with the Fischer's medium.

The cell suspensions were vigorously shaken after adding cis-PAD solution, exposed to the drug for 45 min at 37°C and subsequently transferred into test-tubes and centrifuged for 7 min at ca 500 g. Supernatants were discarded, the cell pellets were resuspended in warm (37°C) Fischer's medium and the centrifugation repeated. After the second change of medium, each cell culture was divided into two equal portions, one for caffeine treatment, the other left untreated.

A detailed description of cis-PAD treatment of L5178Y cells was given in [5].

2.3. Treatment with caffeine

Caffeine (Merck, Darmstadt, W.Germany) was applied at a final concentration of 0.75 mM, by adding a suitable volume of 75 mM solution in sterile saline. At this concentration caffeine did not affect cloning efficiency of L5178Y strains.

For survival determinations three types of experiments were carried out: 1) 7 h incubation in the presence of caffeine, change of medium and subsequent cloning of caffeine-treated and untreated cells; 2) 24 h incubation in the presence of caffeine, change of medium and subsequent cloning; 3) cloning immediately after completing the cis-PAD treatment, and caffeine solution pipetted into Fischer's medium used for the last dilution of cell suspension, before adding agar. These three procedures are shown diagrammaticaly in Fig.1.

For analysis of chromatid aberrations caffeine was added immediately after second change of medium following cis-PAD treatment and dividing the cell culture into 5 portions, designated "4h", "7h", "11h", "24h" and "51h"; colchicine (40 μg/ml)[16] was subsequently added to each sample 3h before cell harvest. A diagram for this procedure is shown in Fig.5.

In all types of procedures described above parallel samples incubated without caffeine were prepared.

2.4. Chromosome preparations

The procedure applied was described previously [5]. 50-100 metaphase cells were analyzed for each treatment schedule and time interval, except 7 h interval in which case mitotic index values were very low: 4-20 cells were analyzed and the data obtained were used merely to evaluate percentage of cells with abnormal chromosomes.

3. RESULTS

The effect of caffeine on survival of L5178Y-S cells was studied using three experimental schedules, outlined in Fig.1. At the concentration applied (0.75 mM) caffeine did not affect survival of L5178Y-S when the treatment time was 7 h (Fig.1a), and only slightly enhanced lethality at the highest cis-PAD dose (Fig.2) when the treatment time was 24h (Fig.1b). However, a pronounced effect was obtained (Fig.3), when L5178Y-S cells were cloned after cis-PAD treatment in soft agar containing 0.75 mM caffeine (Fig.1c). Thus, it follows that a prolonged (>24 h) treatment with caffeine is necessary to potentiate lethal effect of cis-PAD in L5178Y-S cells. This is in contrast with the usual observations that caffeine's presence during the first S phase after treatment is sufficient to potentiate lethal effect of UV-light or alkylating agents [17,18], UV-irradiated L5178Y-S cells being no exception in this respect [19].

In order to quantitatively express the difference between the survival curves (Fig.3) obtained for caffeine-treated and untreated L5178Y-S cells the dose-survival data were fitted to a linear-quadratic equation [20]:

$$S = \exp - p_c (k_1 C + k_2 C^2)$$

where S - survival,

p_c - mean probability that a cross-link formed by platinum complex with DNA leads to cell death,

C - concentration of cis-PAD used for 1 h treatment at 37°C,

$k_1 C$ - number of inter-stand cross links,

$k_2 C^2$ - number of intra-stand cross links, one on each of the two complementary strands on the DNA, relatively close to each other.

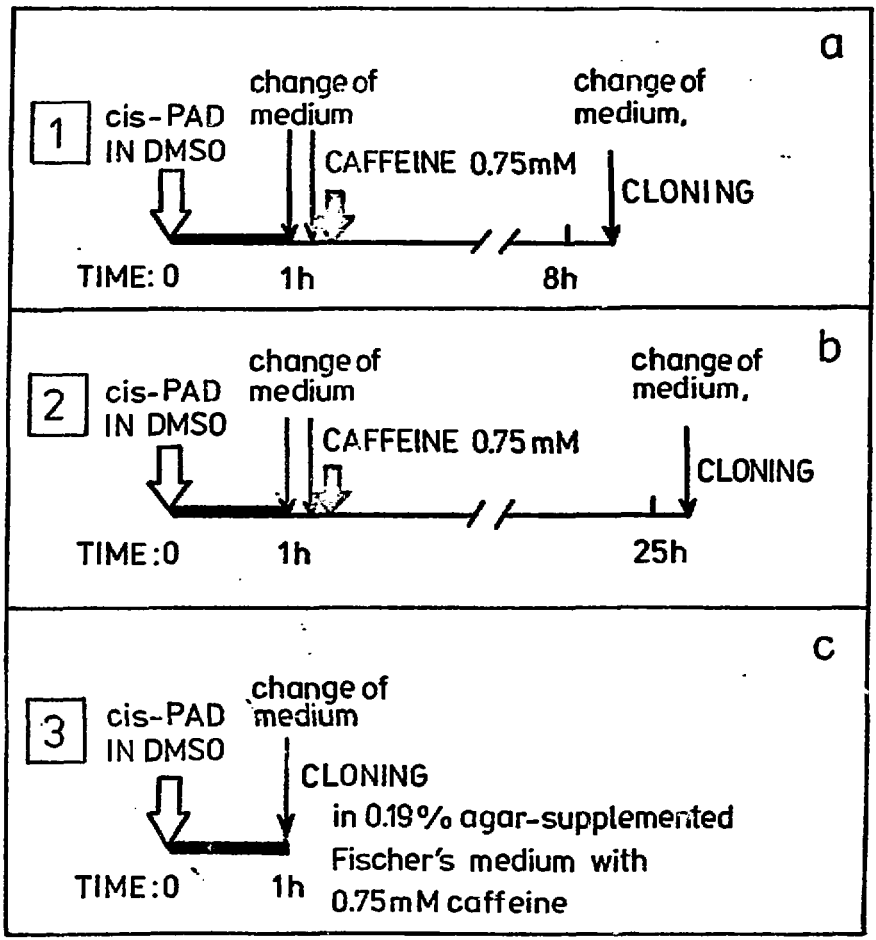

FIG.1. Experimental schedules used in examinations of the caffeine effect on survival of cis-PAD-treated L5178Y cells. Controls treated with DMSO only. Parallel samples incubated without caffeine.

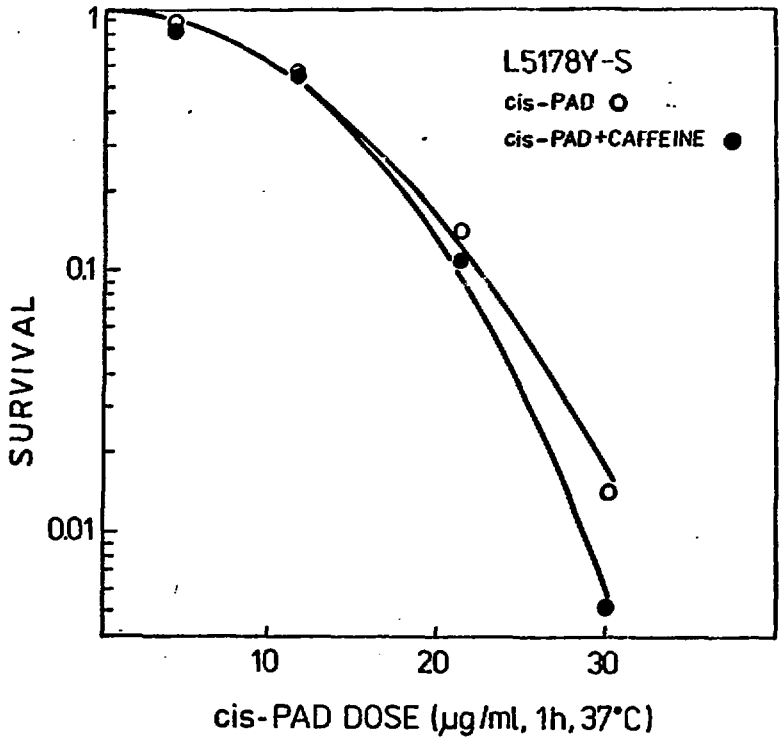

FIG. 2. Dose-survival relationship obtained for L5178Y-S cells treated with cis-PAD only (curve 1) and with cis-PAD followed by 0.75 mM caffeine (24 h incubation) according to schedule 2 (Fig. 1b) (curve 2). Data from 1 experiment.

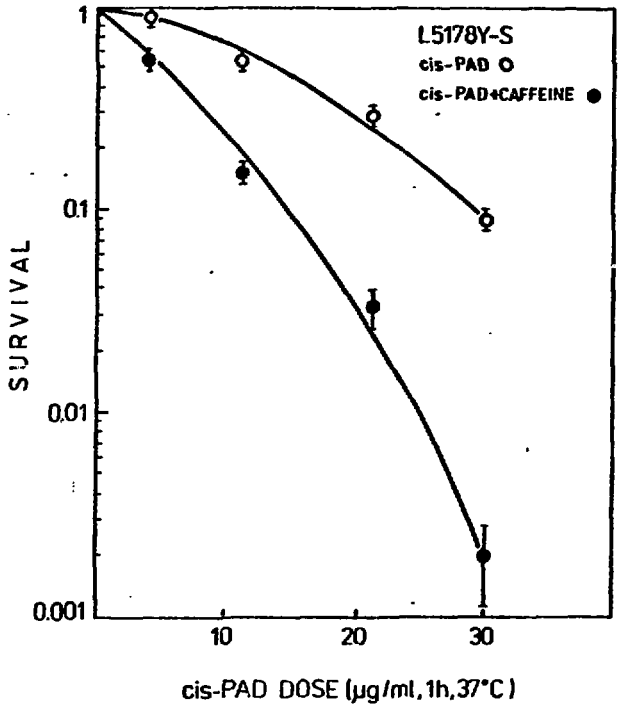

FIG.3. Dose-survival relationship obtained for L5178Y-S cells treated with cis-PAD only (curve 1) and with cis-PAD followed by 0.75 mM caffeine (until scoring of surviving colonies) according to schedule 3 (Fig.1c) (curve 2). Mean values from 3 experiments, standard error indicated.

The following values of $p_c k_1$ and $p_c k_2$ were obtained: 3.3×10^{-2} and 1.4×10^{-3} for caffeine-untreated cells and 7.5×10^{-2} and 4.7×10^{-3} for caffeine-treated cells. Thus caffeine treatment caused an increase of $p_c k_1$ by a factor of 2.3 and $p_c k_2$ by a factor of 3.4.

L5178Y-R cells were treated with cis-PAD and caffeine only according to the third procedure (Fig.1c). The dose-survival relationship obtained is presented in Fig.4.

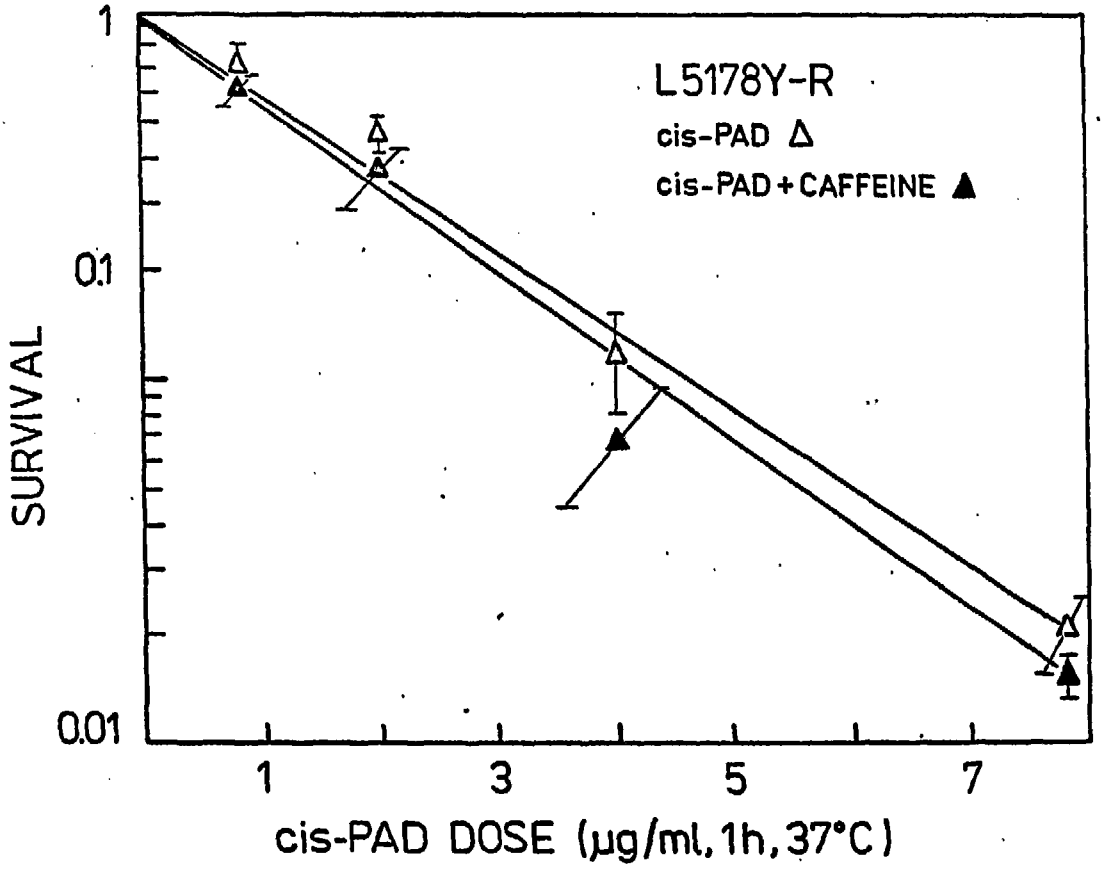

FIG.4. Dose-survival relationship obtained for L5178Y-R cells treated with cis-PAD only (curve 1) and with cis-PAD followed by 0.75 mM caffeine according to schedule 3 (Fig.1c) (curve 2). Mean values from 4 experiments; standard error indicated.

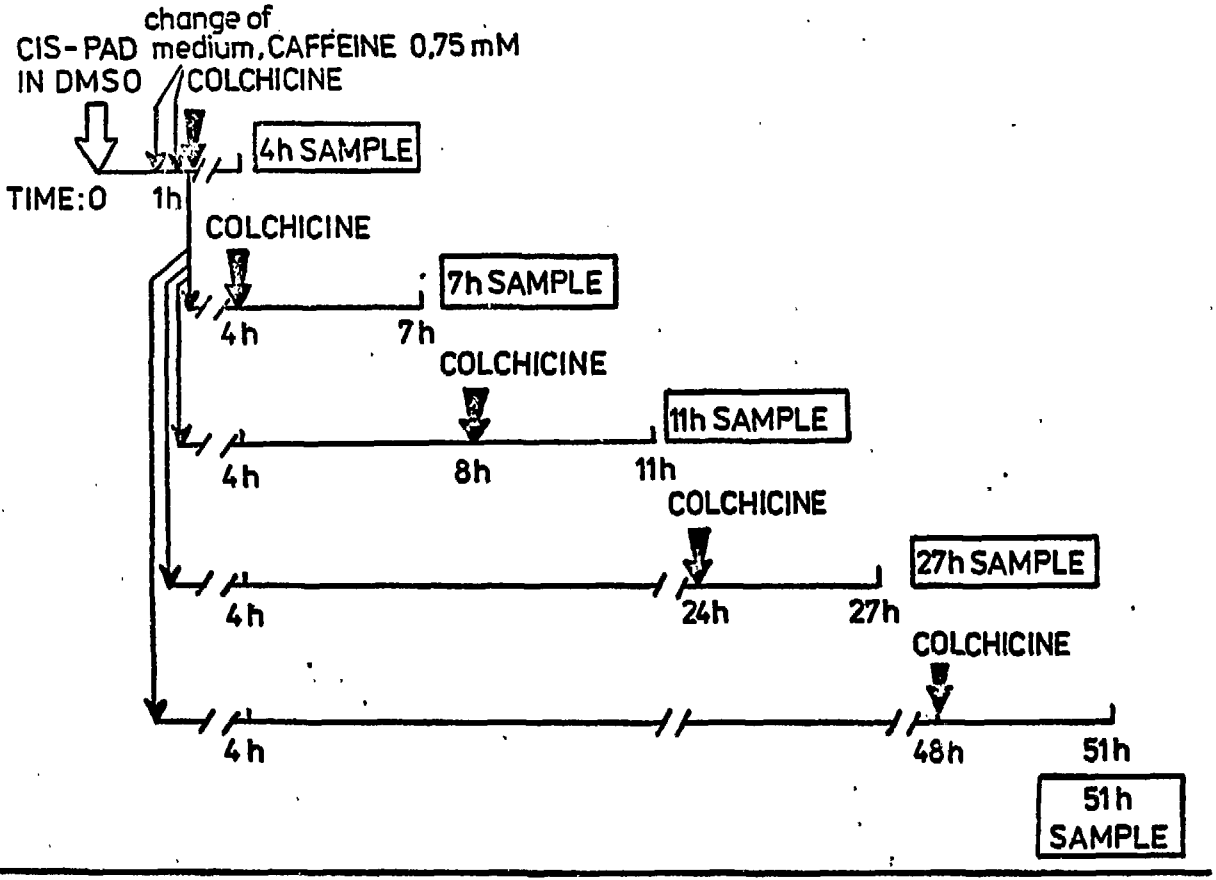

FIG.5. Diagram of L5178Y cell treatment for studies on chromatid aberrations. The concentrations of cis-PAD used were 15.4 µg/ml for L5178Y-R cells and 42.8 µg/ml for L5178Y-S cells, the respective surviving fractions - 2.3×10^{-3} and 4.5×10^{-3}.
Control treated with DMSO only. Parallel samples incubated without caffeine.

A slight, statistically insignificant difference was observed between effects of the two treatments.

The survival data suggest that L5178Y-R cells are deficient in caffeine-sensitive post-replication repair which is known to operate in rodent cells [11,12,21]. In order to gain further support for this conclusion the effects of caffeine on cis-PAD-treated L5178Y-R and L5178Y-S cells were examined at the chromosomal level.

The diagram in Fig.5 shows the design of the respective experiment, which was repeated twice. The mitotic index changes and percentage of cells with chromatid aberrations are shown in Fig.6. In accordance with the survival data (Fig.3 and 4) in L5178Y-R strain neither mitotic index nor proportion of aberration containing cells are affected by caffeine, while both these parameters are altered in L5178Y-S cells. The proportion of L5178Y-S cells with damaged chromosomes is ca 20 percent higher after cis-PAD + + caffeine treatment than after cis-PAD treatment alone; mitotic index is lower at 27 and 51 h after combined treatment than after treatment with cis-PAD alone. Control cells of both L5178Y strains were free from detectable chromosomal damage and the mitotic index values were not influenced by caffeine treatment.

The histograms of chromatid aberration yields in both cell strains are shown in Fig.7. From these data it can be concluded that not only the proportion of aberration-containing cells but also the severity of chromosomal damage induced by cis-PAD are enhanced in L5178Y-S strain by caffeine treatment. While the number of scorable aberrations is frequently lower in cis-PAD + caffeine - treated cells than in those treated with cis-PAD only, this is more than compensated for by a considerable increase in the number of cells with "degenerated" chromosomes, that is, with multiple breaks and exchanges or with "shattered" chromosomes [14]. This effect of caffeine is more pronounced 27 h than 51 h after treatment; no difference in chromosomal damage is seen in cells harvested 11 h after treatment.

The differences between caffeine-treated and untreated cis-PAD damaged L5178Y-R cells are much less pronounced than these found in L5178Y-S cells and, as shown previously (cf Fig.4) , are of small importance for survival. The only exception is the decrease in chromatid exchange yield 27 h after combined treatment with cis-PAD and caffeine; this, however, is compensated for by a slight increase in the number of cells with the "degenerated" chromosomes.

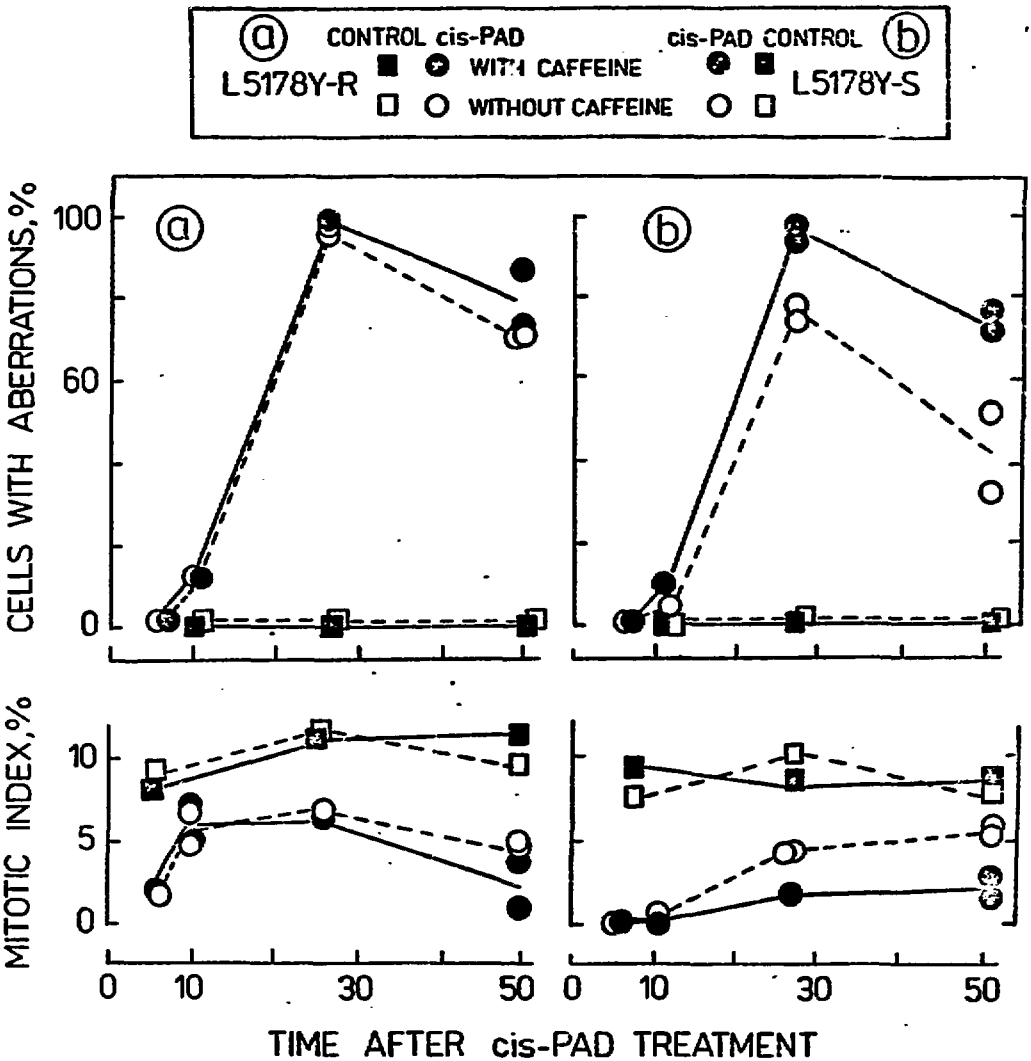

FIG.6. Mitotic indices and percentage of cells with chromatid aberrations after treatment with cis-PAD alone and in combination with 0.75 mM caffeine; a - L5178Y-R cells, b - L5178Y-S cells. Experimental schedule is outlined in Fig.5.

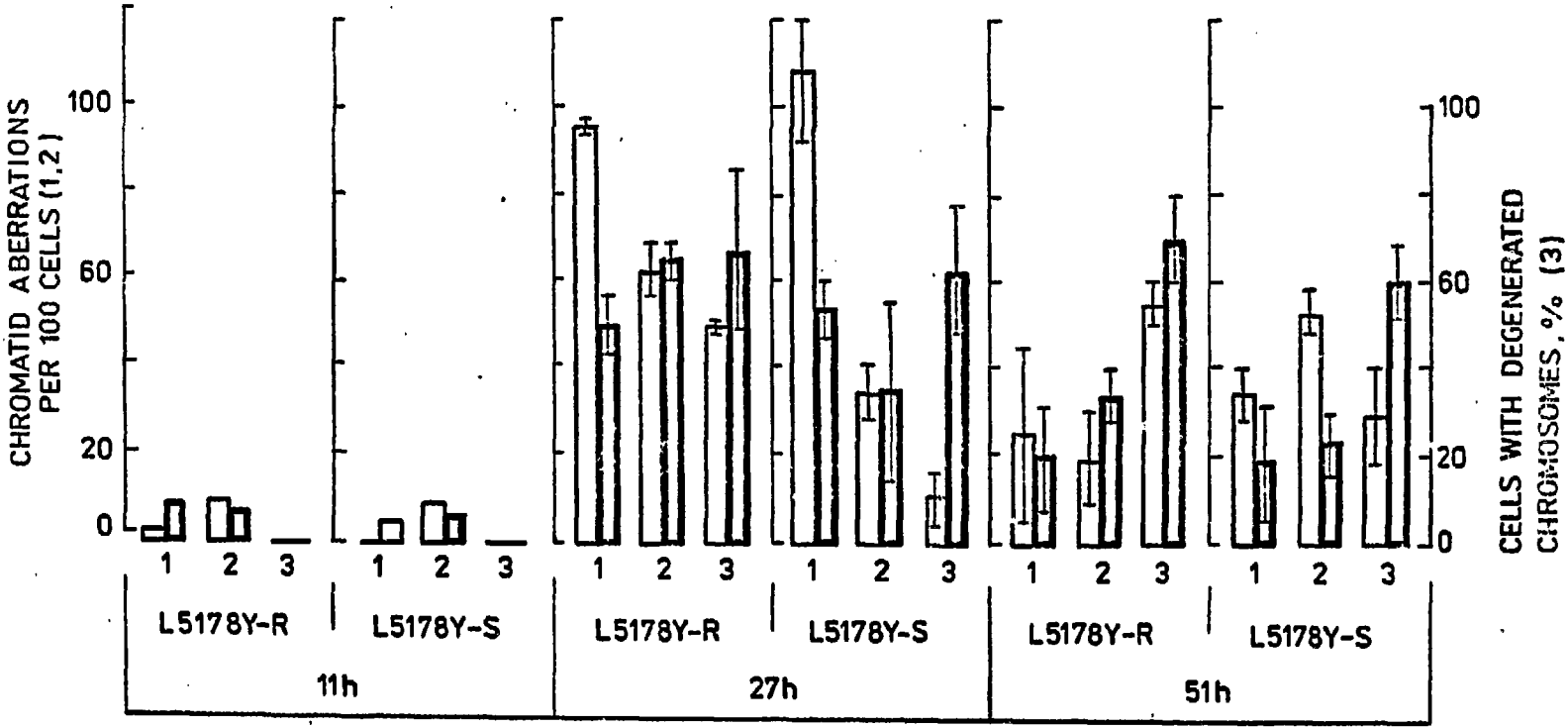

FIG.7. Effect of post-treatment incubation in the presence of 0.75 mM caffeine on chromatid aberrations in cis-PAD-treated L5178Y-R and L5178Y-S cells. Experimental schedule is outlined in Fig.5.

□ – cis-PAD
■ – cis-PAD+CAFFEINE 0.75 mM
Dose of cis-PAD:
15.4 μg/ml, 1 h, 37° L5178Y-R
42.8 μg/ml, 1 h, 37° L5178Y-S

1 - CHROMATID EXCHANGES PER 100 CELLS
2 - CHROMATID BREAKS PER 100 CELLS
3 - PERCENTAGE OF CELLS WITH DEGENERATED CHROMOSOMES

4. DISCUSSION

Enhancement by caffeine of cis-PAD-induced cytotoxicity and chromatid aberration formation in L5178Y-S but not in L5178Y-R strain is particularly interesting in the light of data indicating that post-replication repair is inhibited by methylxanthines [11,12,21]. As was mentioned, such repair mechanism is known to operate in UV-irradiated cells as well as in cells treated with certain chemicals [18,21-23]. Platinum complexes inflict damage repaired by post-replicative mechanism, as recently shown by Van den Berg and Roberts in experiments with Chinese hamster V79-379A cells [15]. These authors found that inter- and intra--strand cross-links formed by platinum complexes with DNA delay the replicating machinery. However, any discontinuities formed in newly synthesized DNA are rapidly sealed. In the presence of caffeine, DNA synthesis takes place at the normal rate, but the gaps left in the newly synthesized DNA remain unsealed. These findings made at the molecular level comply with the observations on caffeine-potentiated lethality and chromosome damage in cis-DDP--treated cells [14].

There is a controversy concerning the number of post-replication repair systems and their nature [21] and more facts are needed in order to elucidate this problem. In any case, the data presented show that L5178Y-R cells are deficient in caffeine-sensitive, thus, presumably a post-replication repair. This is clearly seen from the lack of effect of caffeine on survival and chromatid aberrations of cis-PAD-treated L5178Y-R cells (Figs 4 and 6). In contrast with the L5178Y-R strain, L5178Y-S strain is effectively sensitized by caffeine to cis-PAD, as seen from the survival (Fig.3) and chromatid aberration data (Figs 6 and 7). These facts indicate a similarity between repair processes following treatment with cis-PAD and cis--DDP [14].

The lack of caffeine-sensitive post-replication repair in L5178Y-R strain obviously contributes to its extreme sensitivity to cis-PAD; in L5178Y-S strain, due to the presence of this type of repair, a higher proportion of cells survive the treatment with cis-PAD than in L5178Y-R strain, and less damage is expressed at the chromosomal level [5]. The question remains unanswered, whether the difference in sensitivity between L5178Y-R and L5178Y-S strains is caused by lack of one or more repair systems in the first strain; caffeine treatment was carried out at one concentration only and, possibly, higher concentrations would sensitize L5178Y-S cells in a still more

pronounced way, yielding eventually an exponential dose-survival curve with D_o close to that obtained for L5178Y-R cells. Although further experiments along these lines are planned, caffeine toxicity at higher concentrations must be taken into consideration, as a limiting factor.

It was reported previously, that many common features could be found in the response of L5178Y strains to cis-PAD and UV-light [5]. Therefore, it could be expected that caffeine would enhance lethal effects of UV-irradiation on the L5178Y-S strain, while having no influence on the response of the L5178Y-R strain. This problem was recently examined by Walicka [19] and the expected results of interaction of UV and caffeine have been experimentally demonstrated.

5. CONCLUDING REMARKS

Particular suitability of this experimental system for studies on the post-treatment phenomena emerges not only from differences described in this series including the pronounced difference in caffeine-sensitive repair. The two cell strains show also marked differences in efficiency of repair processes operating after exposure of L5178Y-R and L5178Y-S cells to X-rays. Among others, various rates and efficiencies of single strand break rejoining were found for L5178Y-R and L5178Y-S cells [24]. Thus, it seems that a number of problems emerging from the observations on platinum complexes or radiation action on mammalian cells could be conveniently approached with the use of these two cell strains.

A number of unsolved intriguing questions concerns the basic mechanism of formation of intracellular lesions. The most interesting questions concern the problem of endonuclease activity, specificity and their relations to the formation of double strand breaks in DNA of cis-PAD treated cells. Van den Berg and Roberts [15] postulated existence of an endonuclease which could produce incisions next to the inter-strand cross-links formed by platinum complexes. Partial degradation of these sites results in formation of gaps replicated during S phase of the cell cycle. Such gaps can give rise to chromatid aberrations scorable in the first mitosis after treatment [25]. Contribution of this mechanism to formation of the aberrations seems to be of minor importance in cis-PAD treated CHO cells [20,26]. However, this problem requires further studies in other

cell systems, in particular involving cell labelling and examination of label segregation and aberration formation.

L5178Y-R cells are deficient in the caffeine-sensitive repair system. In this connexion it would be interesting to know whether this particular kind of repair is completely absent in L5178Y-R cells or if its efficiency is only partially reduced. If caffeine-sensitive repair was completely absent in these cells it would be important to know which kinds of repair processes do operate in L5178Y-R cells and at which level of efficiency. It seems that this information might contribute to our general knowledge of repair processes, their occurrence and their significance as defence mechanisms.

Comparisons of effects of platinum complexes, UV-light and ionizing radiation open most promising perspectives in studies on relations between molecular lesions, repair of them and ultimate effects, of harmful agents. Elucidation of these relations might additionally aid understanding of effects of combined treatment of cells with platinum complexes and radiation.

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