Biochemical Pharmacology, Vol. 38, No. 18, pp. 3122–3123, 1989.
Printed in Great Britain.

0006-2952/89 $3.00 + 0.00

Correlation of resistance to nitrogen mustards in chronic lymphocytic leukemia with enhanced removal of melphalan-induced DNA cross-links

(*Received* 13 *January* 1989; *accepted* 15 *March* 1989)

Resistance to the nitrogen mustards, either *de novo* or acquired, is responsible for the failure of chemotherapy in the treatment of many types of cancer. The precise mechanism(s) involved in the development of resistance in the clinical situation to nitrogen mustards is not known. Ross *et al.* [1] have noted that persistence of DNA cross-links with time is an important factor in cytotoxicity. Hansson *et al.* [2] have found a correlation between the cytotoxicity of nitrogen mustards and the total area under the curve for DNA interstrand cross-links. Recently we reported that lymphocytes from untreated chronic lymphocytic leukemia (CLL) patients as compared to those obtained from resistant CLL patients demonstrate no significant differences in the kinetics of melphalan (MLN) transport, intracellular MLN accumulation, intracellular MLN inactivation or GSH levels. However, the lymphocytes from resistant patients demonstrate a decreased percentage of DNA interstrand cross-links after exposure to MLN followed by a 4-hr drug-free incubation to allow for maximal DNA cross-link formation [3]. To better define the kinetics of DNA interstrand cross-link formation and removal, we determined in the present study the percentage of DNA interstrand cross-links (C_t) following a 35-min incubation with 5 or 10 μM MLN and a variable drug-free incubation period.

Methods

Lymphocytes were isolated from patients with CLL as previously described [3]. There were eight untreated CLL patients and six treated resistant patients as previously defined [3]. Differences between the two groups were determined by Student's unpaired *t*-test.

The lymphocytes at 2×10^6 cells/ml were incubated at 37° with MLN for 35 min in PAG [phosphate-buffered saline (Dulbecco's modification), pH 7.4, with 0.1 mM albumin and 0.25% glucose]. The cell suspensions were centrifuged and resuspended in an equivalent volume of PAG for a variable period of drug-free incubation at 37° to allow for development of DNA cross-links. The percentages of DNA cross-links developed at 4 and 6 hr of drug-free incubation were similar for lymphocytes from two untreated CLL patients and thus a 4-hr time point was utilized. Lymphocyte viability was ≥95% by trypan blue exclusion at the end of the 24-hr drug-free incubation.

The DNA interstrand cross-links were detected by using an ethidium bromide fluorescence assay as described previously except that the pH of the ethidium bromide (EB) solution was adjusted so that the final pH of the solution resulting from the mixture of the cellular suspension and the EB solution was 11.5–11.9 in order to allow for optimal denaturation [3, 4].

Results

To better define the kinetics of the formation and removal of DNA cross-links, C_t values were determined at 0, 4, and 24 hr after a 35-min incubation with 5 or 10 μM MLN. Lymphocytes from eight untreated patients demonstrated a time-dependent increase in C_t without evidence of removal of DNA cross-links (Figs 1 and 2) except for one patient who removed approximately 50% of the DNA cross-links at 24 hr. In contrast, the lymphocytes from the six resistant CLL patients demonstrated the greatest C_t at 0 hr with removal of most of the DNA cross-links by 24 hr. At 0 hr, 10 μM MLN resulted in a significantly ($P < 0.01$)

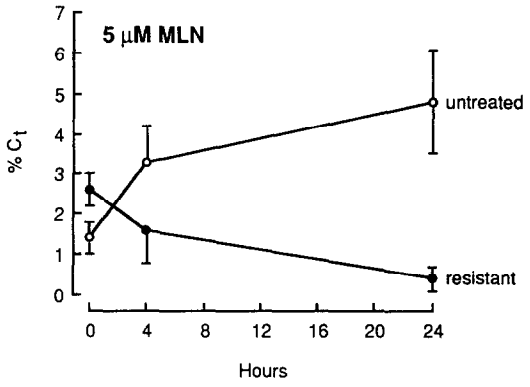

Fig. 1. Percentage of DNA crosslinks (%C_t) in lymphocytes from seven untreated (○) and six resistant (●) CLL patients. The cells were exposed to 5 μM melphalan in PAG for 35 min and then incubated in drug-free medium for 0, 4 and 24 hr. The data are expressed as the mean ± SE.

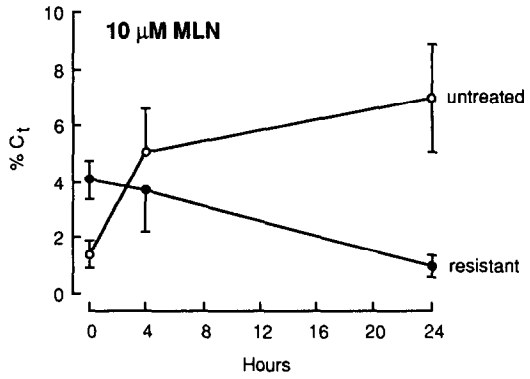

Fig. 2. Percentage of DNA crosslinks (%C_t) in lymphocytes from eight untreated (○) and six resistant (●) CLL patients. The cells were exposed to 10 μM melphalan in PAG for 35 min and then incubated in drug-free medium for 0, 4 and 24 hr. The data are expressed as the mean ± SE.

greater C_t with lymphocytes from resistant CLL patients than those from untreated CLL patients. At 0 and 4 hr following a 5 μM MLN incubation, there were no significant differences in C_t between these two groups. After 24 hr of drug-free incubation, following either a 5 or 10 μM MLN incubation, lymphocytes from untreated CLL patients had a statistically greater percentage of DNA cross-links ($P < 0.025$) than those from treated resistant patients.

Discussion

The kinetics of the formation and removal of DNA cross-links in lymphocytes from the untreated CLL patients demonstrated that the maximal development of DNA cross-links occurred 4–24 hr after the initial drug incubation as previously described [1] and, in general, there was no evidence of removal of DNA cross-links. In contrast, with lymphocytes from resistant CLL patients, maximal DNA cross-links occurred immediately following the MLN incubation and almost all the DNA cross-links were removed by 24 hr. The results in the present study suggest that lymphocytes from resistant CLL patients may have an altered chromatin alkylation which allows for a more efficient repair of DNA and/or an enhanced DNA repair enzymatic system that removes the DNA cross-links. Altered chromatin structure in the presence of elevated topoisomerase II activity has been associated with resistance to nitrogen mustard [5]. Examination of the patterns of DNA alkylation and the chromatin structure in lymphocytes from these two groups of patients may indicate important differences.

The involvement of DNA repair enzymes in the development of resistance to the nitrogen mustards in CLL is supported by the accelerated removal of interstrand DNA cross-links in lymphocytes from resistant CLL patients. It is possible that DNA cross-links induced by nitrogen mustards are repaired as are other bulky chemical adducts or UV-induced damage. Two human genes (ERCC-1 and ERCC-2) have been implicated in the excision of lesions induced by ultraviolet light, *cis*-platinum and mitomycin C [6–8]. The activity of DNA polymerase β was found to be elevated 5-fold in a P388 cell line resistant to cisplatin [9]. Alternatively, it is also possible that repair of nitrogen mustard-induced DNA cross-links is via specific glycosylases such as 7-methylguanine-DNA glycosylase [10]. Elucidation of the enzymatic component(s) involved in the repair of DNA cross-links in lymphocytes from CLL patients, including possible gene amplification and/or increased expression of the above mentioned repair genes, could be informative.

Acknowledgements—The authors gratefully acknowledge R. Rosenzweig for typing this manuscript. This work was funded by the Cancer Research Society (Montreal), the U.S. National Institute of Neurological and Communicative Disorders and Stroke Grant R01-NSC22230, and a private donation from Mr Tibor Schiff.

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REFERENCES

1. Ross WE, Ewig RAG and Kohn KW, Differences between melphalan and nitrogen mustard in the formation and removal of DNA crosslinks. *Cancer Res* **38**: 1502–1506, 1978.
2. Hansson J, Lewensohn R, Ringborg U and Nilsson B, Formation and removal of DNA cross-links induced by melphalan and nitrogen mustard in relation to drug-induced cytotoxicity in human melanoma cells. *Cancer Res* **47**: 2631–2637, 1987.
3. Panasci L, Henderson D, Torres-Garcia SJ, Skalski V, Caplan S and Hutchinson M, Transport, metabolism, and DNA interaction of melphalan in lymphocytes from patients with chronic lymphocytic leukemia. *Cancer Res* **48**: 1972–1976, 1988.
4. De Jong S, Zijlstra JG, Timmer-Bosscha H, Mulder NH and De Vries EGE, Detection of DNA crosslinks in tumor cells with the ethidium bromide fluorescence assay. *Int J Cancer* **37**: 557–561, 1986.
5. Tan KB, Mattern MR, Boyce RA, Hertzberg RP and Schein PS, Elevated topoisomerase II activity and altered chromatin in nitrogen mustard-resistant human cells. *NCI Monogr* **4**: 95–98, 1987.
6. Westerveld A, Hoeijmaker HJ, Van Duin M, De Wit J, Odijk H, Pastink A, Wood RD and Bootsma D, Molecular cloning of a human DNA repair gene. *Nature* **310**: 425–429, 1984.
7. Zdzienicka MZ, Roza L, Westerveld A, Bootsma D and Simons JWIM, Biological and biochemical consequence of the human ERCC-1 repair gene after translocation into a repair deficient CHO cell line. *Mutat Res* **183**: 69–74, 1987.
8. Weber CA, Salazar EP, Stewart SA and Thompson LH, Molecular cloning and biological characterization of a human gene, ERCC-2, that corrects the nucleotide excision repair defect in CHO UV5 cells. *Mol Cell Biol* **8**: 1137–1146, 1988.
9. Kraker AJ and Moore CW, Elevated DNA polymerase beta activity in a *cis*-diaminedichloroplatinum(II) resistant P388 murine leukemia cell line. *Cancer Lett* **38**: 307–314, 1988.
10. Gallagher PE and Brent TP, Further purification and characterization of human 3-methyladenine-DNA glycosylase. *Biochim Biophys Acta* **782**: 394–401, 1984.