CELLULAR UPTAKE AND EFFLUX OF PEPLOMYCIN IN SENSITIVE AND BLEOMYCIN-RESISTANT SUBLINE OF MOUSE LYMPHOBLASTOMA L5178Y CELLS

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(Received for publication September 17, 1987)

Bleomycin (BLM) is clinically useful in the treatment of human squamous cell carcinomas, testicular carcinoma, and malignant lymphoma. The antibiotic is less effective for adenocarcinoma and sarcomas. The less effectiveness of BLM may be due to lowered BLM sensitivity of tumor cells. The determinant of BLM sensitivity is thought to be drug uptake, BLM hydrolase activity, enzymes involved in DNA breakdown and enzymes for DNA repair[1,2]. Therefore, to elucidate the mechanisms of cellular resistance to BLM is important in the treatment of neoplasms. MIYAKI *et al.*[3] reported that BLM-resistant rat ascites hepatoma cells show higher BLM hydrolase activity than BLM-sensitive hepatoma cells. AKIYAMA and KUWANO[4] isolated BLM-resistant mutants of Chinese hamster ovary (CHO) cell lines, whose resistance is due to higher BLM hydrolase activity. We obtained BLM-resistant mutant cell lines of murine lymphoma L5178Y, which showed similar level of BLM-inactivating activity as the parental cells[5]. Changes of membrane-associated enzyme activities in the resistant cells have been observed[6], which supports the hypothesis that the resistance is attributed to decreased uptake or retention of BLM in the resistant cells by alteration of plasma membrane. For the purpose of elucidating mechanisms of BLM resistance, we studied BLM uptake in the sensitive and resistant cells using [^{3}H]peplomycin (PEP), an antibiotic of BLM group, which contains 3-((*S*)-1-phenylethyl)aminopropyl amino group[7]. The results are presented in this publication. PEP and [^{3}H]PEP (phenyl-*m*-^{3}H, 250 μCi/mg)[8] were kindly provided by Dr. T. TAKITA, Nippon Kayaku Co., Ltd., Tokyo, Japan. The cells were grown in FISCHER's medium supplemented with 10% horse serum. The concentration for 50% inhibition of cell growth after 3 days' exposure to PEP was 3.4 μg/ml for the resistant cells, and 0.45 μg/ml for the parental cells. The degree of resistance was 7.7-fold. Cellular resistance was studied according to the method described previously[9]. Cells (10^7/ml) were incubated in the culture medium with [^{3}H]PEP (1.02 μg/ml) at 37°C for various periods. Cold PEP was added in order to elevate the extracellular concentration. The uptake was terminated by rapid sedimentation, using an Eppendorf centrifuge 5412. One ml of cell suspension was layered on 200 μl of an oil mixture, consisting of 80 parts of silicon oil SH500 and 20 parts of liquid paraffin. The mixture was sedimented at 15,000 rpm for 30 seconds. The cell pellet was solubilized with 0.5 ml of Protosol (New England Nuclear) by overnight incubation at 37°C. The radioactivity was determined in a liquid scintillation spectrometer, using scintillation fluid Dimilume-30 (United Technologies Packard).

As shown in Fig. 1, cellular uptake of [^{3}H]-PEP in the BLM-resistant cells was significantly lower than the parental cells. [^{3}H]PEP uptake at a concentration of 1.02 μg/ml for 20 minutes was 17.7 ng/10^7 parental cells and 11.2 ng/10^7 resistant cells. The uptake reached plateau level in 20 minutes. [^{3}H]PEP uptake for 20 minutes at higher extracellular concentration in the both cell lines was illustrated in Fig. 2. In the both cell lines, [^{3}H]PEP uptake for 20 minutes was proportionally increased according to elevating PEP concentration in the medium. [^{3}H]PEP uptake in the resistant cells at the extracellular concentration of 10 μg/ml of [^{3}H]PEP was 92.9 ng/10^7 resistant cells. On the other hand, the uptake of [^{3}H]PEP in the sensitive cells was 92.9 ng/10^7 sensitive cells at an extracellular concentration of about 7 μg/ml of PEP. For the same amount of [^{3}H]PEP uptake (*i.e.* 92.9 ng/10^7 cells), 1.4-fold higher PEP concentration in the culture medium was required for the resistant cells in comparison to the sensitive cells. Since the degree of PEP resistance was 7.7-fold, the BLM resistance may not be solely due to the decreased drug uptake. Nevertheless, the resistance is partly due to the

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Fig. 1. Cellular uptake of [^{3}H]PEP in the parental (●) and the BLM-resistant (○) cells, ([^{3}H]PEP; 1.02 μg/ml).

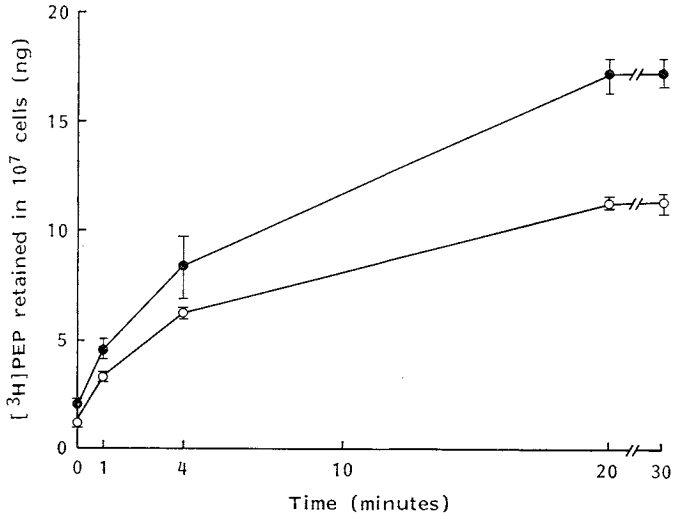


Fig. 2. Plateau level of [^{3}H]PEP uptake in the parental (●) and the resistant (○) cells.

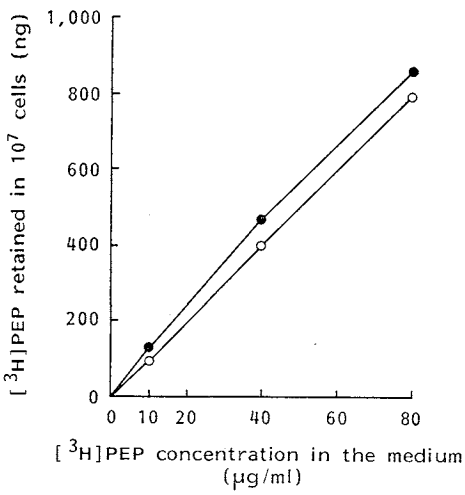
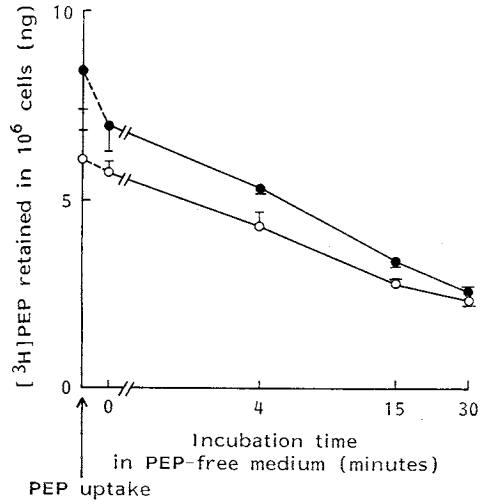


Fig. 3. Efflux of [^{3}H]PEP from the parental (●) and the resistant (○) cells.



significantly lowered PEP uptake in the resistant cells.

To determine whether the lowered cellular uptake of [^{3}H]PEP in the resistant cells was attributed to increased rate of [^{3}H]PEP efflux, the efflux of [^{3}H]PEP from the cells was examined, following the method previously described[10]. Cells (10^6/ml) were incubated with [^{3}H]PEP (0.9 μg/ml) for 30 minutes at 37°C in the culture medium. Then a part of the cells was sedimented for determining the radioactivity incorporated as described above. The residual cells were washed 3 times by centrifugation, resuspended in the fresh medium (10^6/ml), and incubated for 30 minutes at 37°C. The radioactivity remaining in the cells, before and after incubation, was analyzed as described above. The results are shown in Fig. 3. Efflux of [^{3}H]PEP from the sensitive cells and that from the resistant cells was almost comparable. The [^{3}H]PEP retained after 30 minutes' incubation in PEP-free medium was 2.58 ng PEP/10^6 cells, which was similar to that in the resistant cells (2.40 ng PEP/10^6 cells). The results do not

indicate that the decreased [^{3}H]PEP uptake in the resistant cells is attributed to increased rate of [^{3}H]PEP efflux.

In so-called pleiotropic drug-resistant phenotype such like resistance to anthracyclines and vinca alkaloids, decreased drug uptake and increased energy dependent efflux of drug have been demonstrated[11,12]. On the contrary, the BLM-resistant cells in this study did not show any elevated efflux of PEP. ROY and HORWITZ[13] studied association of BLM A_2 with HeLa cells and showed that interaction of the drug with cells is temperature sensitive but is unaffected by metabolic poisons like sodium azide and sodium cyanide, suggesting that this process is not energy dependent.

As illustrated in Fig. 3, even after elaborate washing the cells which had incorporated [^{3}H]-PEP for 30 minutes, significant difference in the retention of [^{3}H]PEP between the parental and the resistant cells was observed. Therefore, the BLM-resistant cells may possess a certain mechanism to be determined for decreased uptake of PEP. Since BLM causes DNA strand scission, studies on DNA repair and breakdown of DNA in the sensitive and resistant cells as well as the transport system would be of interest.

Acknowledgments

The current work was supported in part by a Grant-in-Aid for cancer research from the Ministry of Education, Science and Culture, Japan. The authors express their deep thanks to the late Prof. HAMAO UMEZAWA, Institute of Microbial Chemistry, for his generous advice and cooperation.

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