Antitumor Activity of Quinocarmycin (KW2152) against Various Cultured Leukemia and Lymphoma Cell Lines in Vitro

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ABSTRACT

Growth inhibitory activity of quinocarmycin citrate (KW2152) against 25 human cultured cell lines derived from leukemias and lymphomas was assessed quantitatively by regrowth assay. EC50 values (drug concentration required for 50% growth inhibition of treated cells) measured at 1-h exposure to the drug in vitro were more than 16 μg/ml in five of six T-cell lines derived from T-lymphoma/leukemia, hence we were insensitive to KW2152. On the other hand, four of six B-cell lines derived from B-lymphoma and three of four cell lines derived from non-T, non-B acute lymphoblastic leukemia were sensitive to KW2152 with EC50 values of 0.3 to 2.2 μg/ml at 1-h exposure. Six myelomonocytoid cell lines derived from acute myelogenous leukemia were also sensitive with EC50 values of 1.8 to 3.0 μg/ml on 1-h exposure, but two myeloid cell lines derived from chronic myelogenous leukemia and one cell line derived from erythroleukemia were insensitive with EC50 values of more than 16 μg/ml.

INTRODUCTION

Quinocarcin (DC52) has been reported to be a unique anticancer agent with isoquinoline structure (Fig. 1), but its antitumor activity against animal tumors. But this was done not been done since it is not stable in aqueous solution (1–3). Quinocarmycin citrate (KW2152: C14H23N2O2·C6H4O2Cl) is a citrate salt of quinocarcin and highly stable in soluble form even at 37°C, has recently been isolated in crystalline form from the culture broth of Streptomycetes melanovinaceus nov. sp. (4). This drug is a novel anticancer agent with isoquinoline structure (Fig. 1), but its antiproliferative activity against cultured human leukemia and lymphoma cell lines has not yet been examined. This paper describes its growth inhibitory activity against cultured human leukemia and lymphoma cells.

MATERIALS AND METHODS

Drug. Quinocarmycin citrate (KW2152; M, 522.5) was obtained from Kyowa Hakko Kogyo Co. Ltd., Tokyo. It was dissolved in a culture medium at an appropriate concentration just before use.

Cell Lines. 25 human cultured cell lines derived from various hematological malignancies were used. The characteristics of these cell lines are summarized in Table 1. The cells were cultured in a growth medium, RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal calf serum (GIBCO, lot 27N8433), 100 μg/ml aminobenzyl penicillin, and 20 μg/ml gentamicin, in a floating state at 37°C under a humidified atmosphere in a 5% CO2 incubator (Forma Scientific, model 3156). The cells in the exponentially proliferating phase were used for the experiments.

Quantitative Measurement of Antiproliferative Activity of KW2152.

The experiments were carried out according to the quantitative growth assay of Alexander (5) with slight modifications (6, 7). The cells were suspended in fresh growth medium at an appropriate concentration in a glass test tube (LT-15100, Telmo Co., Ltd.). KW2152, which was diluted with the growth medium at an appropriate concentration, was added to the glass test tube containing the cells. The cells were incubated until the drug concentration for 1-h exposure. In insensitive cell lines, on the other hand, exponential cell kill in proportion to drug concentration was not observed. The difference in the sensitivity of the drug, as shown in Fig. 2, revealed that there are at least two classes of cell lines in terms of sensitivity. In sensitive cell lines, cells could be killed exponentially by increasing the concentration of the drug for 1-h exposure. In insensitive cell lines, on the other hand, exponential cell kill in proportion to drug concentration was not observed. The difference in the sensitivity of cell lines was also clearly shown by the EC50 value measured after 1-h exposure to the drug (Table 1). According to the EC50 values, P67/SHI cells were the most sensitive to KW2152. The EC50 values of four B-cell lines (Daudi, Ohno, A5/TAK, and P67/SHI), three non-T, non-B acute lymphoblastic leukemia cell lines (KOPN-1, Reh, and P30/OHK), and six myelomonocytoid cell lines (P39/TSU, ML-1, U937, HL-60, THP-1, and KG-1) were less than 3.0 μg/ml of KW2152. Three myeloid cell lines (KYO-1, K562, and HEL), five T-cell lines (ATL-1K, JM, MOLT-3, P12/IC, and HPB-ALL) and one B-cell line (Raji) were insensitive, as evidenced by their EC50 values of far more than 16 μg/ml (Table 1) and no decrease in surviving cells in the concentration-response curves even at high concentrations of the drug, as shown in Fig. 2.

On the other hand, EC50 values measured after 24-h exposure...
to the drug were not proportional to the respective EC₉₀ values measured at 1-h exposure (Table 1). In most cell lines, EC₉₀ values measured at 24-h exposure were quite low and in the 0.02 to 0.06 µg/ml range. This indicates that KW2152 is active not only against highly sensitive cell lines with shorter exposure but is also broadly active against most cell lines with longer exposure.

Mode of Growth Inhibitory Action of KW2152. Two insensitive (ATL-1K and P12/ICH) and two sensitive (A5/TAK and U937) cell lines were treated in vitro with graded concentrations of KW2152 for various periods as representative cell line. The growth inhibitory activity of KW2152 against two cell lines (ATL-1K and P12/ICH), which were insensitive to KW2152 at 1-h exposure, was minimum with short exposure times, but increased as the exposure time increased. According to the concentration-response curves of a representative cell line (ATL-1K) as shown in Fig. 3, the rate of population reduction deviated from a first-order rate even with longer exposure time and the concentration-response curve seems to approach an asymptote which represents the maximum degree of population reduction. The time-response curves revealed that the growth inhibitory action of KW2152 occurred at a first-order rate against exposure time. These results clearly indicate that KW2152 has time-dependent action, but not concentration-dependent action.

In the case of two other cell lines (A5/TAK and U937) which were sensitive to KW2152 at 1-h exposure, the growth-inhibitory effects were observed for longer exposure times. The concentration-response curves showed that the growth inhibitory action of KW2152 was concentration-dependent and time-dependent. The time-response curves revealed that the growth inhibitory action of KW2152 occurred at a first-order rate against exposure time. These results clearly indicate that KW2152 has time-dependent action, but not concentration-dependent action.

In Table 1, the EC₉₀ values of KW2152 against various cultured cell lines are presented. The EC₉₀ values were measured at both 1-h and 24-h exposures. The EC₉₀ values were higher for the 1-h exposure compared to the 24-h exposure in most cell lines. This indicates that KW2152 is active not only against highly sensitive cell lines with shorter exposure but is also broadly active against most cell lines with longer exposure.

To summarize, KW2152 shows broad-spectrum antitumor activity against various cultured cell lines, and its growth inhibitory action is time-dependent and concentration-dependent. The time-response curves revealed that the growth inhibitory action of KW2152 occurred at a first-order rate against exposure time, indicating that KW2152 has time-dependent action, but not concentration-dependent action.
tion rate followed first-order kinetics at least up to approximately 99% inhibition in both concentration- and time-response curves, regardless of the length of the exposure time, indicating that KW2152 has both time- and concentration-dependent growth inhibitory actions on these cell lines. Typical concentration- and time-response curves of A5/TAK are shown in Fig. 4.

DISCUSSION

KW2152 is a novel antibiotic which possessed more antitumor activity against human cancer xenografts in nude mice than murine transplantable tumors (4). The present study revealed that KW2152 had time-dependent growth-inhibitory activity, which is also characteristic of Vinca alkaloids and antimetabolites (13–15). It is clear that KW2152 seems to be basically a time-dependent drug rather than a concentration-dependent drug. In sensitive cell lines, however, concentration-dependent growth inhibitory action was observed at least up to 99% cell kill even under short exposure conditions. However, this is not contradictory, because it has been reported that time-dependent drugs usually have concentration-dependent action in the initial phase against highly sensitive cells, and there are no concentration-dependent drugs which do not show concentration-dependent action, even though cells might be insensitive (13–15).

The difference in drug sensitivity levels of the cell lines measured under shorter exposure conditions disappeared at longer exposure. The EC50 values of the cell lines measured under longer exposure conditions were equally low and mostly in the range of 0.02 and 0.06 μg/ml. Some cell lines were insensitive to KW2152 under shorter exposure conditions, hence, their EC50 values were too high to be measured under this condition. However, their EC50 values decreased drastically as exposure time increased. A previous report (4) clearly showed that the activity of KW2152 was schedule-dependent and daily administration was the most effective in in vivo studies with murine tumor systems. In addition, the order of in vitro efficacy of KW2152 against murine tumors correlated with the order of the sensitivity on the i.p.-i.p. system of these tumors (4). The in vitro results presented in this report as well as in vivo data reported elsewhere (4) suggest that daily consecutive or continuous dose therapy, as well as single or intermittent large dose therapy, may be scheduled for clinical trials. It is suggested that intermittent large dose therapy may be effective in highly sensitive tumors, and continuous dose therapy may be effective against various types of tumor, if the clinical efficacy of these therapies are not limited by toxicity. Also, it is assumed that the drug may not be effective against hematological malignancies unless a serum concentration of 0.3 μg/ml, preferably 1.2 μg/ml, for more than 1 h or of at least more than 0.03 μg/ml, preferably 0.06 μg/ml, for more than 24 h could be maintained safely in clinical Phase I trials. Under this condition, some B-lymphoma and non-T, non-B acute lymphoblastic leukemia may be candidates as tumors sensitive to KW2152.

Recently, many human cancer cell lines are available for in vitro as well as in vivo sensitivity tests of new investigational drugs. Since pharmacokinetic data and toxicity data will be available from Phase I study, the determination of drug sensitivity levels of human cancer cell lines to new investigational drugs may be useful for the selection of effective target tumors in subsequent Phase II studies (7). This approach could also be useful for determination of the optimal administration schedule of the drugs. Therefore, the quantitative measurement of drug sensitivity of various human cancer cell lines in vitro as well as in vivo to new investigational drugs may be referred to as a nonclinical Phase II study. The results of the nonclinical Phase II study may indicate what target human cancers would be suitable for clinical trials, and unprofitable clinical trials may be avoidable based on the nonclinical Phase II study data.
However, much effort must be made in order to establish efficient and reliable methods and guidelines for such nonclinical Phase II study, i.e., how many cell lines, or what kinds of cell lines should be used for such studies. Also, further studies for each new investigational drug are necessary to determine whether the sensitivity of their cell lines in vitro parallels the order of sensitivity to the drug from in vivo studies and whether in vitro results correlate with optimal scheduling in vivo. So far, there are no definite answers to these questions, but it is now necessary to accumulate data of this sort, and to analyze whether or not the study would be truly useful for this purpose.

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