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

Satoshi Inaba and Masanori Shimoyama
Hematology-Oncology and Clinical Cancer Chemotherapy Division, National Cancer Center Hospital, I-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104

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 90% 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 they 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. The EC50 values of most cell lines decreased as exposure time increased, and those measured at 24-h exposure were similarly low and mostly in the 0.02 to 0.06 μg/ml range. The kinetics analysis of growth inhibitory activity of KW2152 revealed that the drug showed time-dependent action. These in vitro results, as correlated with in vivo results reported elsewhere (K. Fujimoto, T. Oka, and M. Morimoto, Cancer Res., 47:1516-1522, 1987), suggest that daily consecutive or continuous dose therapy as well as single or intermittent large-dose therapy would be worthy of testing in the clinical trial of KW2152.

INTRODUCTION

Quinocarcin (DC52) has been reported to be a unique antibiotic which has antitumor activity against animal tumors. But further characterization and evaluation of this compound including clinical trials have not been done since it is not stable in aqueous solution (1-3). Quinocarmycin citrate (KW2152: C18H22N2O4CoHsO7), which is a citrate salt of quinocarcin and highly stable in soluble form even at 37°C, has been recently isolated in crystalline form from the culture broth of Streptomyces melanovinaceus nov. sp. (4). This drug is a novel anticancer agent with inosinoline 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 regrowth 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 initial cell density of each cell line was adjusted from 1.5 x 10^6/ml to 3.5 x 10^6/ml according to the growth rate and cell density required for exponential growth in each cell line throughout the experiment.

The cells were incubated with an appropriate concentration of KW2152 in the growth medium for a given period at 37°C in the CO2 incubator, then washed three times, resuspended, and cultured in the growth medium without KW2152 for the regrowth assay. The growth medium was changed at least every 3 days until exponential growth of surviving cells was observed. The size distribution curves and cell number of cultured cells in each experimental group were determined every 2 to 3 days with a Coulter counter model ZB C-1000 (Coulter Electronics, Inc.). The percentage of reduction of cell number compared to the control culture was calculated at the exponential growth phase of each experimental group. The EC50 value was calculated as the drug concentration which was required for 90% reduction of number of treated cells as compared to that of control cells, and was used as an indicator of the drug sensitivity. All experiments were carried out in duplicate.

RESULTS

Growth Inhibitory Effect of KW2152. The effect of KW2152 on the growth of 25 human cultured cell lines was assessed by regrowth assay. The concentration-response curves at 1-h exposure to the drug (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 (KOYO-1, K562, and HEL), five T-cell lines (ATL-1K, JM, MOLT-3, P12/ICH, 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...
ANTITUMOR ACTIVITY OF QUINOCARMycin

Fig. 1. The structure of quinocarmycin (KW2152).

Table 1. EC90 values of KW2152 against various cultured cell lines

<table>
<thead>
<tr>
<th>Cell linea</th>
<th>Originb</th>
<th>EC90 (µg/ml) 1 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATL-1K</td>
<td>ATL</td>
<td>&gt;16.0</td>
<td>0.3</td>
</tr>
<tr>
<td>JM</td>
<td>ALL</td>
<td>&gt;16.0</td>
<td>ND</td>
</tr>
<tr>
<td>MOLT3</td>
<td>ALL</td>
<td>&gt;16.0</td>
<td>0.04</td>
</tr>
<tr>
<td>P12/ICH</td>
<td>ALL</td>
<td>&gt;16.0</td>
<td>0.04</td>
</tr>
<tr>
<td>HPB-ALL</td>
<td>ALL</td>
<td>&gt;16.0</td>
<td>0.2</td>
</tr>
<tr>
<td>SKW-3</td>
<td>CLL</td>
<td>4.5</td>
<td>0.04</td>
</tr>
<tr>
<td>B-cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raji</td>
<td>A-Burkitt</td>
<td>&gt;16.0</td>
<td>ND</td>
</tr>
<tr>
<td>Daudi</td>
<td>A-Burkitt</td>
<td>2.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Ohno</td>
<td>NHL</td>
<td>1.2</td>
<td>0.03</td>
</tr>
<tr>
<td>A5/TAK</td>
<td>NHL</td>
<td>1.2</td>
<td>0.03</td>
</tr>
<tr>
<td>P30/SHI</td>
<td>NHL</td>
<td>0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>A10/SAS</td>
<td>NHL</td>
<td>6.5</td>
<td>0.04</td>
</tr>
<tr>
<td>Non-T, non-B ALL cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reh</td>
<td>ALL</td>
<td>1.0</td>
<td>0.03</td>
</tr>
<tr>
<td>KOPN-1</td>
<td>ALL</td>
<td>0.8</td>
<td>0.03</td>
</tr>
<tr>
<td>NALM-6</td>
<td>ALL</td>
<td>6.0</td>
<td>0.06</td>
</tr>
<tr>
<td>P30/OHK</td>
<td>ALL</td>
<td>1.2</td>
<td>0.03</td>
</tr>
<tr>
<td>Myelomonocytoid cell lines</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HL-60</td>
<td>APL</td>
<td>1.8</td>
<td>0.06</td>
</tr>
<tr>
<td>ML-1</td>
<td>AML</td>
<td>2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>U-937</td>
<td>Histocytic</td>
<td>2.5</td>
<td>0.04</td>
</tr>
<tr>
<td>THP-1-0</td>
<td>AMoL</td>
<td>1.8</td>
<td>ND</td>
</tr>
<tr>
<td>KG-1</td>
<td>AML</td>
<td>2.0</td>
<td>ND</td>
</tr>
<tr>
<td>P39/TSU</td>
<td>AMoL</td>
<td>3.0</td>
<td>0.06</td>
</tr>
<tr>
<td>KYO-1</td>
<td>CMLbx</td>
<td>&gt;16.0</td>
<td>0.2</td>
</tr>
<tr>
<td>K562</td>
<td>CMLbx</td>
<td>&gt;16.0</td>
<td>ND</td>
</tr>
<tr>
<td>HEL</td>
<td>Erythroleukemia</td>
<td>&gt;16.0</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*a Original papers for the cell lines except HPB-ALL (10), SKW-3 (11), and KG-1 (12) were listed in our previous report (8, 9).

**ATL, adult T-cell leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; A-Burkitt, African Burkitt’s lymphoma; NHL, non-Hodgkin’s lymphoma; APL, acute promyelocytic leukemia; AML, acute myelogenous leukemia; AMoL, acute monocytic leukemia; AMMoL, acute myelomonocytic leukemia; CMLbx, chronic myelocytic leukemia in blast crisis; ND, not done.

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-inhibi-
tion rate followed first-order kinetics at least up to approxi-
mately 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 concentra-
tion- and time-response curves of A5/TAK are shown in Fig.
4.

DISCUSSION

KW2152 is a novel antibiotic which possessed more antitu-
mor activity against human cancer xenografts in nude mice
than murine transplantable tumors (4). The present study re-
vealed 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 concen-
tration-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 EC_{50} values of the cell lines measured
under longer exposure conditions were equally low and mostly
in the range of 0.02 and 0.06 \mu g/ml. Some cell lines were
insensitive to KW2152 under shorter exposure conditions,
hence, their EC_{50} values were too high to be measured under
this condition. However, their EC_{50} 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 \textit{in vivo} studies with
murine tumor systems. In addition, the order of \textit{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
\textit{in vitro} results presented in this report as well as \textit{in vivo} data
reported elsewhere (4) suggest that daily consecutive or contin-
uous 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 sen-
sitive 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 malignan-
cies unless a serum concentration of 0.3 \mu g/ml, preferably 1.2
\mu g/ml, for more than 1-h or of at least more than 0.03 \mu g/ml,
preferably 0.06 \mu g/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 \textit{in
vitro} as well as \textit{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 sensi-
tivity 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 \textit{in vitro} as well as
\textit{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.

ACKNOWLEDGMENTS

The authors are grateful to Dr. J. Patrick Barron of St. Marianna University School of Medicine for revising the manuscript.

REFERENCES

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

Satoshi Inaba and Masanori Shimoyama


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/21/6029

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.