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CAMPTOTHECIN ANALOG (CPT-11)-SENSITIVE HUMAN PANCREATIC TUMOR CELL LINE QGP-1N SHOWS RESISTANCE TO SN-38, AN ACTIVE METABOLITE OF CPT-11

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SUMMARY: In the course of our study to determine the cross-sensitivity between CPT-11 and its active metabolite, SN-38, we found a SN-38-resistant human pancreatic tumor cell line, QGP-1N, which shows sensitivity to CPT-11. The IC_{50} of SN-38 was 152 times greater for QGP-1N than for SUIT-2, also a human pancreatic tumor cell line, whose IC_{50} of CPT-11 was similar to that for QGP-1N. The uptakes of CPT-11 and SN-38 and the intracellular conversion of CPT-11 to SN-38 could not explain the difference in sensitivity. DNA synthesis of QGP-1N cells was inhibited by CPT-11 which did not affect that of SUIT-2, while SN-38 inhibited the DNA synthesis of SUIT-2 at lower concentrations than that of QGP-1N. The inhibition test of topoisomerase I catalytic activity by CPT-11 or SN-38 revealed no difference in the biochemical properties of the topoisomerase I enzymes to the compounds between these two cell lines. These results indicate that CPT-11 should have its own inhibitory effect on DNA synthesis through a yet unknown mechanism in QGP-1N cells, although SN-38 plays an essential role in the antitumor activity of CPT-11 in SUIT-2 cells. In some cases, the antitumor effect of CPT-11 might be consequent not only on SN-38 but also on CPT-11 itself.

Camptothecin(CTP) was first extracted from Camptotheca acuminata(1) and has an antitumor effect on various experimental tumor models(2,3). In earlier studies, camptothecin was demonstrated to be an inhibitor of DNA topoisomerase I(4,5). Recent studies revealed that camptothecin stabilizes the DNA-topoisomerase I cleavable complex resulting in lethal impediment of cells(6-9) through the strong inhibition of DNA synthesis(10-13). SN-38 is a metabolite of CPT-11, a semisynthesized camptothecin derivative which has also been shown

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The abbreviations used are: CPT-11, 7-ethyl-10-[4-(1-pipiridino)-1-piperidino] carbonyloxy-camptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; CPT, camptothecin.

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to have antitumor activity and reduced toxicity compared with camptothecin(14-16). CPT-11 is converted into SN-38 to affect the activity of topoisomerase I, that is, CPT-11 itself possesses only a marginal antiproliferative effect, but it is SN-38 that directly does interact with the DNA-topoisomerase I cleavable complex(17,18).

In the studies for mechanism of the antitumor activity of these drugs, the cross-sensitivity(-resistance) between CPT-11 and SN-38 has been tested in some cell lines(19-21). These two compounds showed good correlation in their inhibitory effect on cell growth. In the course of our study, however, we found a human pancreatic tumor cell line showing inconsistent sensitivity to CPT-11 and SN-38. The present study is the first demonstration that CPT-11 itself can affect the cell growth.

MATERIALS AND METHODS

Cells and Drug sensitivity test

QGP-1N is a human pancreatic tumor cell line established previously(22). SUIT-2 is also a human pancreatic tumor cell line, kindly provided by Dr. Iwamura et al.(23). Cells were maintained in a humidified atmosphere of 5% CO_2 in air. Daigo's T medium supplemented with 7% fetal bovine serum(FBS) was used as a growth medium. For the drug sensitivity test, cells(1×10^3) were seeded into 96-well plates. After 2 days of incubation, the cells were exposed to CPT-11 or SN-38 for 48 hr. Washed with PBS twice, the cells were cultured for 5 days. Fixed and stained, the number of viable cells was determined by the absorbance at 620nm on a Micro Plate Reader(MPR A4,TOSOH). The IC_{50} of each test compound for cell growth was estimated from the dose-response curve.

Intracellular content of CPT-11 and SN-38

Intracellular levels of CPT-11 and SN-38 were determined as described by Kawato et al.(18).

Inhibition of DNA synthesis

Inhibition of DNA synthesis was determined by incorporation of thymidine into the acid-insoluble fraction of the cells as described by Kawato et al.(18). Briefly, 2×10^4 cells were seeded into 24-well plates. After 2 days, the cells were incubated with medium containing CPT-11 or SN-38 for 60 min or 120 min, respectively. For the last 15 min, [^{3}H]thymidine(18.5KBq/ml) was added into medium. The radioactivity incorporated into the acid-insoluble fraction was measured by a liquid scintillation counter. The IC_{50} of each compound for DNA synthesis was evaluated from the dose-response curve.

Topoisomerase I activity assay

The topoisomerase I activity was determined by the relaxation of pBR322 DNA(200 ng) in the presence or absence of CPT-11 or SN-38(24), under the same conditions as reported before(25).

RESULTS

Cross-sensitivity test between CPT-11 and SN-38

SUIT-2 and QGP-1N gave almost the same dose-response curves toward CPT-11. Their IC_{50}s of CPT-11 were 1.525 ± 0.025 μg/ml and 1.850 ± 0.141 μg/ml, respectively. While IC_{50} of SN-38 was 152 times greater for QGP-1N than for SUIT-

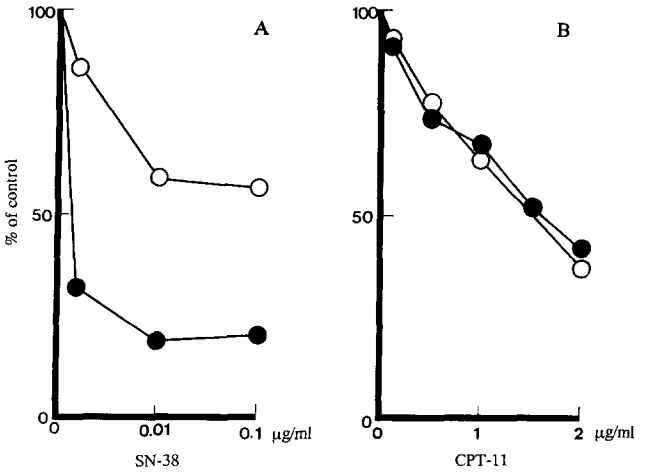

Fig.1. Inhibitory effect of CPT-11 and SN-38 on cell growth of QGP-1N and SUIT-2. Cells were seeded into a 96-well plate ($1x10^3$ /well). After attachment, cells were treated with indicated concentrations of SN-38(A) or CPT-11(B) for 48h., washed with PBS twice and cultured for 5 days. ●; SUIT-2. ○; QGP-1N.

2(Fig.1). The ratios of $IC_{50.CPT-11}$ to $IC_{50.SN-38}$ were 2.89 for QGP-1N and 363 for SUIT-2.

Drug uptake and intracellular formation of SN-38

Intracellular uptake of CPT-11 after 240 min of incubation in medium containing CPT-11, was about 3 times greater for SUIT-2 than for QGP-1N(Fig.2A).

The intracellular content of SN-38 converted from CPT-11 was similar for SUIT-2 and QGP-1N(Fig.2B). The amount of SN-38 incorporated from the medium was also the same(Fig.2C).

Inhibition of DNA synthesis by CPT-11 or SN-38

The IC_{50} of CPT-11 for DNA synthesis of QGP-1N was 3.5 μg/ml, while that of SUIT-2 was not inhibited by CPT-11 up to 10 μg/ml. Conversely, the IC_{50} of SN-38 for DNA synthesis of SUIT-2 was 7.2 ng/ml, and IC_{50} for QGP-1N was greater than 1.0 μg/ml(Fig.3).

Inhibitory effect of CPT-11 and SN-38 on topoisomerase I activity

Activities of topoisomerase I in crude nuclear extracts from SUIT-2 and QGP-1N were almost proportional to their intracellular contents of the enzyme as reported before(25) as shown in Fig.4A. For the inhibition test, the amount of nuclear extract giving similar relaxation activity of the topoisomerase I enzyme were used.

SN-38 dose-dependently inhibited the relaxation of pBR322 DNA by topoisomerase Is extracted from QGP-1N and SUIT-2 cells. A slight but not significant difference in inhibition rates was observed between QGP-1N and SUIT-2. CPT-11

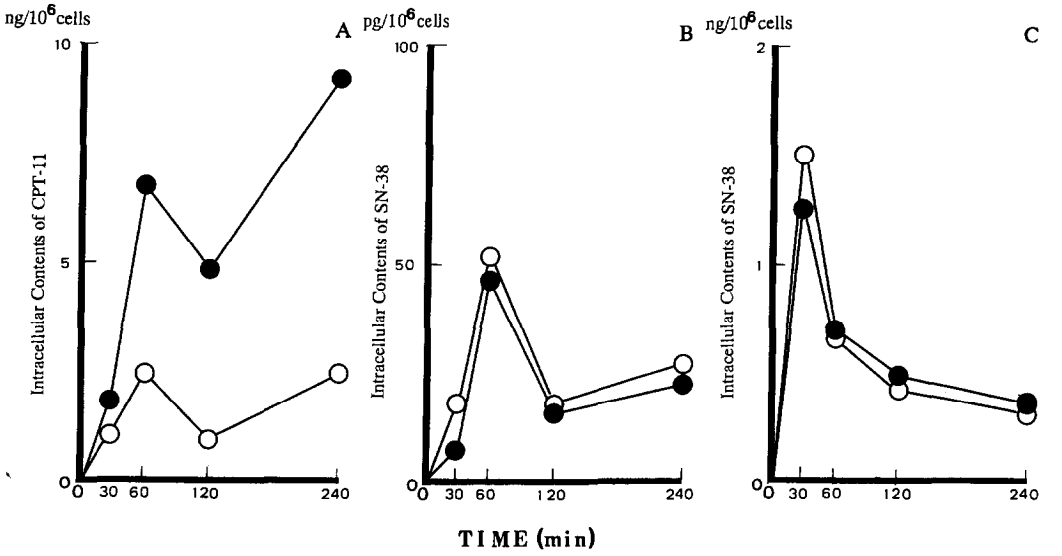

Fig.2. Time course study of intracellular uptake of CPT-11(A) or SN-38(C) and intracellular formation of SN-38 from CPT-11(B) ; Cells were exposed to 100μM CPT-11 (A, B) or 5μM SN-38(C). ●; SUIT-2. ○; QGP-1N.

also affected the catalytic activity of topoisomerase I to shift the position of relaxed DNA as reported before(18), and also no significant difference was detected(Fig.4B).

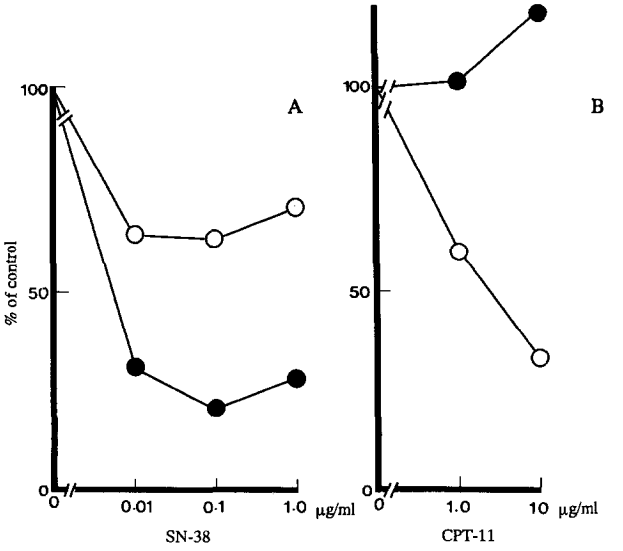

Fig.3. Inhibitory Effect of CPT-11 and SN-38 on DNA synthesis of QGP-1N and SUIT-2. Rate of DNA synthesis was determined by counting the amount of [^{3}H]-thymidine incorporated into acid-insoluble fractions. Cells were treated at 37℃ with SN-38 for 120min.(A), or with CPT-11 for 60min.(B).
●; SUIT-2. ○ ; QGP-1N.

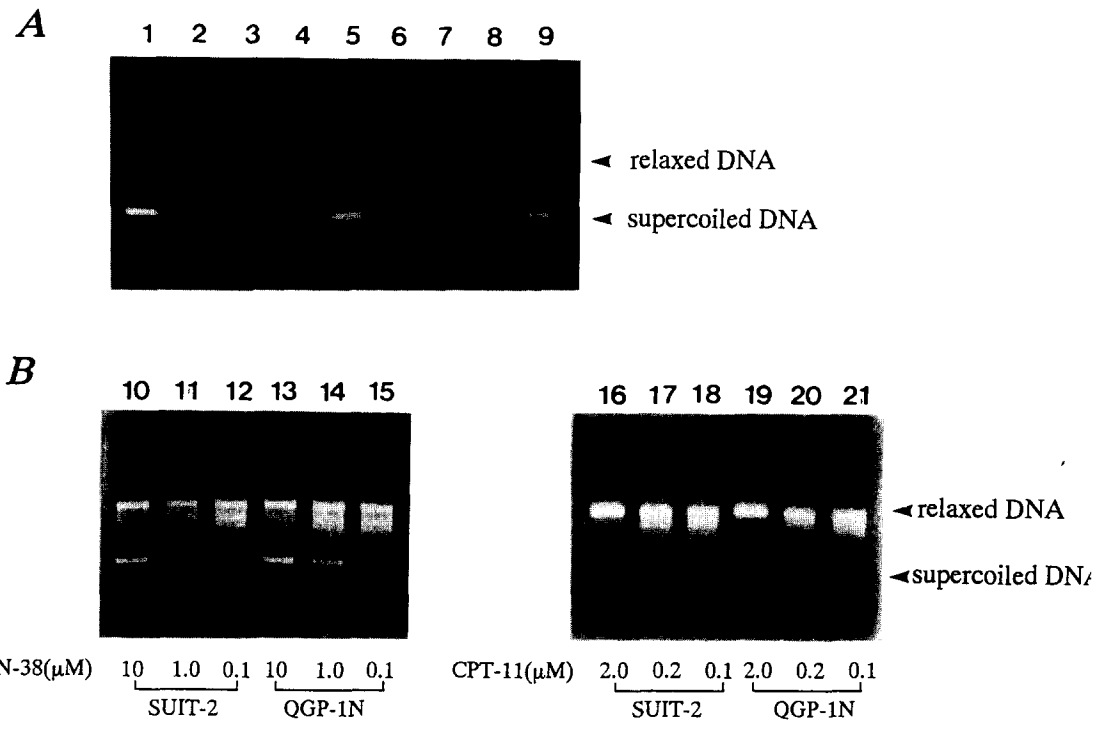

Fig. 4. *A.* Catalytic activity of DNA topoisomerase I from SUIT-2 and QGP-1N. Supercoiled pBR322 DNA was treated at 37℃ for 10min. with no drug, no protein (*lane* 1), with 15, 10, 5, and 1ng protein of nuclear extract from SUIT-2 (*lane* 2-5, respectively), with 300, 200, 100, and 50ng protein of nuclear extract from QGP-1N (*lane* 6-9, respectively).
B. Inhibition of catalytic activity of topoisomerase I by CPT-11 and SN-38. Supercoiled pBR322 DNA was treated with 15ng protein from SUIT-2 (*lane* 10-12, 16-18), with 300ng protein from QGP-1N (*lane* 13-15, 19-21) under the indicated conditions.

DISCUSSION

Recent studies revealed that CPT-11 is converted into SN-38 to stabilize the DNA-topoisomerase I cleavable complex(17,18), resulting in lethal impediment of cells. Accordingly, it is natural that some cell lines should show cross-sensitivity(-resistance) between these two compounds(19-21).

Contradictory to this prediction, we found a human pancreatic tumor cell line, QGP-1N, showing inconsistent sensitivity to CPT-11 and SN-38. If CPT-11 played no other role but as a prodrug of SN-38, a good correlation in sensitivities should be found excepting the case that intracellular uptake were fairly different between CPT-11 and SN-38. To ascertain whether a difference in membrane transportation contributes to the inconsistency or not, we determined the intracellular contents of these two compounds. As a control, we used SUIT-2 cells , which is almost as sensitive to CPT-11 as QGP-1N and shows cross-sensitivity between these two compounds. Decreased conversion of CPT-11 to SN-38 in resistant cells has been reported (20,21), and we have previously

suggested that decreased uptake of CPT-11 is one of the reasons for acquired resistance to CPT-11(25). Intracellular uptake of CPT-11 and/or SN-38 does not correlate with their inconsistent sensitivities, although the time course studies for intracellular uptake suggest the presence of something which discharges the compounds out of the cells, such as the efflux pump.

For further study, we also examined the inhibitory effect of CPT-11 on DNA synthesis. In the recent studies, the effect of CPT-11 itself has been detected only when inhibition of DNA synthesis was consequent on blocking intracellular uptake of the precursors of DNA macromolecule(18). CPT-11, however, inhibited DNA synthesis of QGP-1N cells(Fig.2) without affecting the membrane transportation of thymidine(data not shown). Moreover, at the same concentration, CPT-11 had no inhibitory effect on the DNA synthesis of SUIT-2 cells. If this distinct difference is attributable to the activity of SN-38, the same tendency should be observed in the case of SN-38. DNA synthesis of QGP-1N, however, was not affected by a 60 min of incubation(the same period as for CPT-11) with SN-38 up to 1.0μg/ml(data not shown), while that of SUIT-2 was reduced about 30-35% under the same test condition. Furthermore, the $IC_{50.SN-38}$ for DNA synthesis of QGP-1N was more than 140 times greater than that of SUIT-2 under the condition of 120 min-incubation time. These are consistent with the sensitivities of the cells to SN-38. These findings indicate that CPT-11 should surely have its own inhibitory effect at least in QGP-1N cells, but that the intrinsic effect of CPT-11 does not appear in SUIT-2 cells.

Several cell lines resistant to CPT-11 have been demonstrated to show decreased level of topoisomerase I enzyme as a mechanism of resistance(19,20, 26-28). We have previously reported that QGP-1N contains an extremely lower level of topoisomerase I enzyme(about one-thirtieth), compared with SUIT-2(25), which might be one of the reasons QGP-1N is about 152-fold resistant to SN-38 than SUIT-2, although it is still insufficient.

Qualitatively changed topoisomerase Is showing biochemical resistance to CPT(CPT-11) have been reported(19,20,26). We studied the inhibitory effect of SN-38 on topoisomerase I activity in a cell-free system. SN-38 dose-dependently inhibited topoisomerase I activity similarly in both cell lines. The resistance to SN-38 shown in QGP-1N was not due to the biochemical resistance of topoisomerase I enzyme. No difference was also detected in the inhibition of topoisomerase I activity by CPT-11 between the two cell lines. In QGP-1N cells, CPT-11 might affect something resulting in growth inhibition of the cell other than the topoisomerase I enzyme or at least by a manner different from SN-38. There is little possibility of their having point mutation site(s) in topoisomerase I causing hypersensitivity to CPT-11 and/or resistance to SN-38, such as CPT-K5(19,29). The mechanism of resistance of QGP-1N

cells to SN-38 is not clear at present and plausible mechanisms of acquired resistance would not apply to this native resistant cells, QGP-1N. Other possibilities of different mechanisms, such as correlation with intracellular glutathione(17, 21), is now under investigation.

The present findings indicate that CPT-11 should have its own growth inhibitory effect on QGP-1N cells. It is probable that some wild type cells should show sensitivity to CPT-11 itself. Further study for the mechanisms of cytotoxic activity of CPT-11 is required, which might lead to exploitation of a new antitumor agent. QGP-1N is a good model for this study.

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