J. Pharm. Pharmacol. 1999, 51: 543–548
Received October 13, 1998
Accepted December 15, 1998

© 1999 J. Pharm. Pharmacol.

Synthesis and Cytotoxicity Evaluation of Some 8-Hydroxyquinoline Derivatives

AI-YU SHEN, SHENG-NAN WU* AND CHIH-TSAO CHIU

*Department of Pharmaceutical Science, Foo Yin Institute of Technology, Kaohsiung 831 and *Department of Medical Education and Research, Veterans General Hospital-Kaohsiung, Kaohsiung, Taiwan, Republic of China*

Abstract

Interest in Mannich bases of 8-hydroxyquinoline stems from reports of their high potency against human cancer cells. In the search for potential anticancer drug candidates, Mannich bases of 8-hydroxyquinoline (7-pyrrolidinomethyl-8-hydroxyquinoline, 7-morpholinomethyl-8-hydroxyquinoline, 7-piperidinomethyl-8-hydroxyquinoline and 7-diethylaminomethyl-8-hydroxyquinoline) were synthesised by reaction with various secondary amines and formaldehyde. They were prepared as hydrochlorides.

The cytotoxic activity of 7-pyrrolidinomethyl-8-hydroxyquinoline, 7-morpholinomethyl-8-hydroxyquinoline and 7-diethylaminomethyl-8-hydroxyquinoline compounds in the National Cancer Institute in-vitro cancer cell line panel was determined. It was found that they exhibited substantial cytotoxic activity against leukaemia. The log concentration of 7-pyrrolidinomethyl-8-hydroxyquinoline, 7-morpholinomethyl-8-hydroxyquinoline and 7-diethylaminomethyl-8-hydroxyquinoline that inhibited 50% of 60 cell lines' growth were $-4{\cdot}81$ M, $-5{\cdot}09$ M and $-5{\cdot}35$ M, respectively. Compound 7-pyrrolidinomethyl-8-hydroxyquinoline was selected for further in-vivo testing. The electrophysiological effect of 7-pyrrolidinomethyl-8-hydroxyquinoline also was tested in human myeloma cells (RPMI 8226). The outward current was voltage dependent, activating at -40 mV and believed to be the voltage-activated K^+ current $I_{K(V)}$. 7-Pyrrolidinomethyl-8-hydroxyquinoline (1–30 μM) caused the inhibition of $I_{K(V)}$ in a concentration-dependent manner. The IC50 value of 7-pyrrolidinomethyl-8-hydroxyquinoline-induced inhibition of $I_{K(V)}$ is 23 μM. The GI50 value of 7-pyrrolidinomethyl-8-hydroxyquinoline-induced inhibition of cell growth is 14 μM.

The results suggest that at least part of the cytotoxicity effect of 7-pyrrolidinomethyl-8-hydroxyquinoline on myeloma cells could be related to blockade of voltage-activated K^+ channels.

Mannich bases exhibit several pharmacological properties. Many substituted aminomethyl derivatives of naphthol or unsaturated ketones are known to possess antitumour or antifungal activities (Dimmock et al 1990; Shen et al 1995). Mannich bases of 8-hydroxyquinoline with these biological activities have seldom been described. As shown in previous studies, 8-hydroxyquinoline inhibited the growth of B16 cells as well as human melanoma cell lines (Nordenberg et al 1990). 7-Morpholinomethyl-8-hydroxyquinoline was prepared previously and found to have higher potency than 8-hydroxyquinoline for inhibition of DNA and RNA synthesis in AS-30D cells at micromolar concentrations in our preliminary trial. As an extension of our synthesis of cytotoxic antitumour analogues related to 8-hydroxyquinoline derivatives and to continue the search for agents that are active against tumours, we planned to synthesize the Mannich bases of 8-hydroxyquinoline through the introduction of pyrrolidino, piperidino, and diethylamino groupings. Interest in the introduction of these particular groupings is based upon reports of its high potency against p-388 leukaemia and EMT 6 tumour (Dimmock et al 1990).

It has been reported that voltage-dependent K^+ channels are implicated in the initiation of mitosis in T lymphocytes (De Coursey et al 1984; Sidell & Schlichter 1986), neuroblastoma cells (Rouzaire-

Correspondence: A.-Y. Shen, Department of Pharmaceutical Science, Foo Yin Institute of Technology, Kaohsiung 831, Taiwan, Republic of China.

Dubois & Dubois 1990) and myeloma cells (Wu et al 1998). It has been shown that the classical K^+-channel blockers tetraethylammonium, 4-aminopyridine and the anticancer drug tamoxifen block both voltage-dependent K^+ channels and mitogenesis. Therefore, we sought to examine the effect of 7-pyrrolidinomethyl-8-hydroxyquinoline on voltage-activated K^+ currents in human myeloma cells (RPMI-8226). Patients whose myeloma clones produce only light chains, particularly of the λ-type, have poorer responses to treatment with early relapse and short survival (Handelsman 1996; Wu et al 1998). Therefore, we discuss the cytotoxicity of these compounds as well as the ionic interaction with the myeloma cell.

Materials and Methods

The melting point of compounds was determined with a Yanagimoto MP-3 micromelting apparatus and was uncorrected. Infrared spectra were obtained from a Shimadzu IR-408 spectrophotometer. Nuclear magnetic resonance spectra were recorded from a Varian Gemini T-300 spectrometer at the National Sun Yat-Sen University, Kaohsiung, and were expressed in parts per million (δ) with tetramethylsilane used as an internal standard. Mass spectra obtained for the purposes of structure confirmation were obtained from a Jeol JMS-HX 110 mass spectrometer at the National Sun Yat-Sen University, Kaohsiung. Elemental analysis was performed on a CHN-O-Rapid Heraeus Elemental Analyzer at the National Cheng-Kung University, Tainan. Thin-layer chromatography was carried out on precoated silica gel F254 chromatographic plates (20 × 20 cm; 0·2 mm).

Preparation of 7-dialkylaminomethyl-8-hydroxyquinoline (general procedure)

Mannich bases 7-pyrrolidinomethyl-8-hydroxyquinoline, 7-piperidinomethyl-8-hydroxyquinoline, 7-morpholinomethyl-8-hydroxyquinoline and 7-diethylaminomethyl-8-hydroxyquinoline were prepared by reaction of 0·013 mol of 8-hydroxyquinoline with 0·026 mol of secondary amine (morpholine, pyrrolidine, piperidine and diethylamine) and 1·5 g of 37% formaldehyde in ethanol, as shown in Figure 1. The mixture was heated to reflux for 2 h and then evaporated to give a brown oil as a crude product which was purified by silica-gel chromatography using a mixed solvent of ethylacetate and methanol (9 : 1) as eluent. The pure oily free base was dissolved in ethanol. Concentrated hydrochloric acid was added and the resulting crystals were collected. The solid hydrochloride salt was recrystallized from ethanol and ether. The structural assignment was based on its NOE spectra, in which irradiation of H_3 enhances H_4 (14·62%), in agreement with the structure. It indicated substitution of the dialkylaminomethyl group at position 7 of 8-hydroxyquinoline.

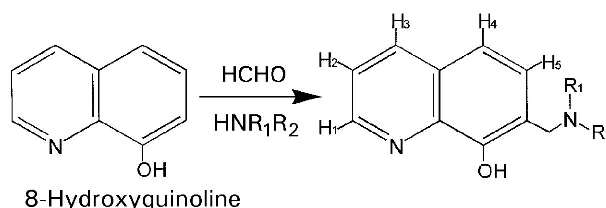

Figure 1. Synthesis of Mannich bases of 8-hydroxyquinoline.

7-Pyrrolidinomethyl-8-hydroxyquinoline hydrochloride. 7-Pyrrolidinomethyl-8-hydroxyquinoline hydrochloride was recrystallised from ethanol and ether (55% yield). IR (KBr): 3430, 2995, 2894, 2445 cm^{-1}, ^{1}H-NMR 300 MHz, (D_2O) δ: 9·09 (d, J = 4·2 Hz, 1H), 9·06 (d, J = 8·7 Hz, 1H), 8·07 (dd, J = 4·2, 8·7 Hz, 1H), 7·90 (d, J = 8·7 Hz, 1H), 7·85 (d, J = 8·7 Hz, 1H), 4·72 (s, 2H), 3·60 (m, 2H), 3·29 (m, 2H), 2·20 (m, 2H), 2·01 (m, 2H). MS, m/z (%) 159 (100: M^+), 158 (53), 70 (9·88). Anal. calcd for $C_{14}H_{16}N_2O.2HCl.2H_2O$ (337): C 49·85, H 6·53, N 8·30, O 14·24. Found: C 49·47, H 6·59, N 8·25, O 14·13.

7-Piperidinomethyl-8-hydroxyquinoline hydrochloride. 7-Piperidinomethyl-8-hydroxyquinoline hydrochloride was recrystallized from ethanol and ether (50% yield). IR (KBr): 3507, 2995, 2918, cm^{-1}, ^{1}H-NMR 300 MHz, (D_2O) δ: 9·07 (d, J = 5·7 Hz, 1H), 9·06 (d, J = 8·4 Hz, 1H), 8·07 (dd, J = 5·7, 8·4 Hz, 1H), 7·88 (d, J = 8·4 Hz, 1H), 7·83 (d, J = 8·4 Hz, 1H), 4·60 (s, 2H), 3·53 (t, J = 12·60 Hz, 2H), 3·09 (t, J = 12·60 Hz, 2H), 1·95-1·66 (m, 4H). MS, m/z (%) 159 (100: M^+), 158 (55), 84 (53). Anal. calcd for $C_{15}H_{18}N_2O.2HCl.\frac{1}{2}H_2O$ (323): C 55·70, H 6·51, N 8·67, O 7·43. Found: C 55·52, H 6·38, N 8·61, O 7·79.

7-Morpholinomethyl-8-hydroxyquinoline hydrochloride. 7-Morpholinomethyl-8-hydroxy-quinoline hydrochloride was recrystallized from ethanol and ether (55% yield). IR (KBr): 3400, 3000, 2800, 2400 cm^{-1}, ^{1}H-NMR 300 MHz (DMSO-d_6) δ: 8·98 (d, J = 4·20 Hz, 1H), 8·56 (d, J = 7·80 Hz, 1H), 7·86 (d, J = 8·70 Hz, 1H), 7·76 (dd, J = 4·2, 7·8 Hz, 1H), 7·57 (d, J = 8·70 Hz, 1H), 4·57 (s, 2H), 4·05 (m, 4H), 3·91 (m, 4H), 3·27 (br. S, 1H). MS, m/z 158 (88: M^+), 159 (100). Anal. calcd for $C_{14}H_{16}N_2O_2.2HCl.H_2O$ (334): C 50·30, H 5·98, N

8·38, O 14·37. Found: C 50·22, H 6·01, N 8·44, O 14·58.

7-Diethylaminomethyl - 8-hydroxyquinoline hydrochloride. 7-Diethylaminomethyl-8-hydroxyquinoline hydrochloride was recrystallized from ethanol and ether (55% yield). IR (KBr): 3500, 2917 cm^{-1}. ^{1}H-NMR 300 MHz (D_2O) δ: 9·08 (d, J = 5·4 Hz, 1H), 9·06 (d, J = 8·4 Hz, 1H), 8·09 (dd, J = 5·4, 8·4 Hz, 1H), 7·92 (d, J = 8·7 Hz, 1H), 7·86 (d, J = 8·7 Hz, 1H), 4·66 (s, 2H), 3·64 (s, 1H), 3·29 (q, J = 7·20 Hz, 4H), 1·36 (t, J = 7·20 Hz, 6H). MS, m/z (%) 159 (92: M^+), 158 (100), 72 (25). Anal. calcd for $C_{14}H_{18}N_2O.2HCl.½H_2O$ (312): C 53·81, H 6·73, N 8·96, O 7·69. Found: C 53·49, H 6·78, N 8·52, O 8·05.

Nucleic acid synthesis activity

[^{3}H]-Thymidine and [^{3}H]-uridine (50 Ci $mmol^{-1}$) were purchased from ICN Biomedicals Inc., Costa Mesa, CA. AS-30D rat hepatoma cell line (Smith et al 1970) was generously provided by Dr J. P. Chang, Institute of Zoology, Academia Sinica, R.O.C. AS-30D cells were maintained in RPMI 1640 medium (Gibco BRL, Grand Island, NY) supplemented with 5% heat-inactivated foetal bovine serum, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin.

DNA and RNA synthesis of cell cultures were determined as described previously (Roffler et al 1992). Briefly, AS-30D cells were plated overnight in 96-well microtitre plates at 2×10^4 cells per well. Serial dilutions of compound in medium containing 5% foetal calf serum were added to cells for 24–48 h at 37°C. Cells were subsequently washed once with sterile PBS and incubated for 2 h with [^{3}H]-thymidine or [^{3}H]-uridine (1 mCi per well) for DNA and RNA synthesis determinations, respectively. Radioactivity of trichloroacetic acid precipitated and washed RNA or DNA were measured in a Beckman LS 6000 series liquid scintillation counter.

Electrophysiological measurement

For the preparation of cells, RPMI-8226, a human myeloma cell line, originally derived from the peripheral blood of multiple myeloma, was obtained from the American Type Culture Collection ([CLL 155], Rockville, MD). Myeloma cells were grown in suspension in RPMI 1640 culture medium supplemented with 2 mM L-glutamine (Gibco), containing 10% heat-inactivated foetal bovine serum (Gibco), 100 U mL^{-1} penicillin, and 100 mg mL^{-1} streptomycin at 37°C in 5% CO_2 in a humidified atmosphere. Cells were always studied between four and seven days of incubation and the diameter of the cells ranged between 15 and 20 μM.

Cells were transferred to a recording chamber that was mounted on the stage of an inverted phase-contrast microscope (Diaphot-200, Nikon, Tokyo, Japan). To monitor the change in cell size, the microscope was also combined with a video camera system with magnification up to ×1500. Cells were bathed at room temperature (20–25°C) in normal Tyrode's solution. The patch pipettes were made from Kimax capillary tubes (Kimble Products, Vineland, NJ) utilising a vertical two-step electrode puller (PB-7, Narishige, Tokyo, Japan) and the tips were fire-polished with a microforge (MF-83, Narishige). The resistance of the patch pipette was 3–5 MΩ when it was bathed in normal Tyrode's solution. A three-dimensional micromanipulator (WR-6, Narishige), which was mounted on the fixed stage of an inverted microscope, was used to place the pipette near the cell. Voltage-clamp command potentials of either step or ramp depolarization were digitally generated using a programmable stimulator (SMP-311, Biologic, Claix, France) which was directed by a personal computer through an RS-232 port. Ionic currents or membrane potentials were recorded in voltage-clamp or current-clamp configuration with the use of a patch clamp amplifier (RK-400, Biologic) (Shen & Wu 1998).

Data recording and analysis

The signals consisting of voltage and current tracings were monitored on a digital storage oscilloscope (Model 1602, Gould Instrument Systems, Inc., Valley View, OH) and recorded on-line with a digital audio tape recorder (Model 1204, Biologic). After the experiments, the collected data were digitized at the sampling frequency of 5–10 KHz with a Digidata 1200 analogue-to-digital device and pClamp 6.03 software package (Axon Instruments, Foster City, CA) (Liu et al 1998).

To calculate the inhibition percentage of 7-pyrrolidinomethyl-8-hydroxyquinoline, the cells were depolarized from a holding potential of −80 mV to +30 mV, and the amplitude of $I_{K(V)}$ during the application of 7-pyrrolidinomethyl-8-hydroxyquinoline was compared with the control value. The half maximal concentration (EC50) was represented as the 7-pyrrolidinomethyl-8-hydroxyquinoline concentration required for inhibition of the amplitude of $I_{K(V)}$ by 50%. All values were reported as the mean ± standard error of the mean (s.e.m.). Student's paired or unpaired *t*-test was used for statistical analyses. One-way analysis of variance (ANOVA) was used for comparisons of more than two treatment group means and the Newman–

Keuls test was then used for multiple post-hoc comparisons. Probability values of $P < 0.05$ were considered significant.

Drugs and solutions

Ethylene glycol-bis (β-aminoethylether) *N*, *N*, *N′*, N′-tetraacetic acid (EGTA) and 8-hydroxyquinoline were purchased from Sigma Chemical (St Louis, MO). All chemicals were obtained from regular commercial chemicals suppliers and were of reagent grade. The composition of normal Tyrode's solution was as follows (in mM): NaCl 136·5, KCl 5·4, $CaCl_2$ 1·8, $MgCl_2$ 0·53, glucose 5·5, HEPES–NaCl buffer 5 (pH 7·4). To measure the voltage-activated K^+ current, the pipettes were filled with solution (mM): K-aspartate 130, KH_2PO_4 1, $MgCl_2$ 1, EGTA–KOH 10, Na_2ATP 3, Na_2GTP 0·1 and HEPES–KOH buffer 5 (pH 7·3).

Results and Discussion

Mannich bases of 8-hydroxyquinoline were prepared by reaction of 8-hydroxyquinoline with various secondary amines and formaldehyde in ethanol at room temperature, and the products were isolated as their hydrochlorides. 7-Pyrrolidinomethyl-8-hydroxyquinoline, 7-morpholinomethyl-8-hydroxyquinoline and 7-diethylaminomethyl-8-hydroxyquinoline were submitted to the National Cancer Institute (NCI) for cytotoxicity testing. The biological results obtained in-vitro showed that these compounds were cytotoxic. The mean log GI50 values (GI50 is the concentration of compounds which inhibits cell growth up to 50%) were obtained by screening compounds against up to 60 human cancer cell lines including leukaemia, non-small-cell lung cancer, colon cancer, central nervous system cancer, melanoma, ovarian cancer, renal cancer, prostate cancer and breast cancer. The activity of the compounds were lower than the mean GI50 against all cell lines in the melanoma panel and prostate cancer, but substantial activity was demonstrated against the cell lines in the leukaemia panel. Other panels usually show mixed sensitivity to these compounds. The log GI50 of 7-pyrrolidinomethyl-8-hydroxyquinoline in the NCI's 60 cell line screen was −4·81 M whereas that of 7-morpholinomethyl-8-hydroxyquinoline and 7-diethylaminomethyl-8-hydroxyquinoline were −5·09 M and −5·35 M, respectively. The substitution on position 7 of the 8-hydroxyquinoline derivatives containing a diethylaminomethyl group had considerably lower log GI50 than 7-pyrrolidinomethyl-8-hydroxyquinoline and 7-morpholinomethyl-8-hydroxyquinoline. 7-Diethylaminomethyl-8-hydroxyquinoline also showed higher potency with log GI50 values generally in the range of −6·35 M to −5·51 M in leukaemia cells. However, 7-pyrrolidinomethyl-8-hydroxyquinoline was selected by NCI for further in-vivo evaluation using the human xenograft assay (Hollow Fiber Assay), which was performed by the Developmental Therapeutics Program, Division of Cancer Treatment, Diagnosis and Centers. The activity seen was against MDA-MB-435, MDA-MB-231, LOX and NCI-H23. According to the NCI's protocol, compounds with a combined intraperitoneal + subcutaneous score $\geq$20, a subcutaneous score $\geq$8 or a net cell kill of one or more cell lines, are referred for xenograft testing. The results of the hollow-fibre assay for 7-pyrrolidinomethyl-8-hydroxyquinoline in-vivo were reported as an intraperitoneal score = 4, subcutaneous score = 14, and a total score = 18. This compound also produced a reduction in the viable cell mass below that present at the time of implantation. Thus, 7-pyrrolidinomethyl-8-hydroxyquinoline actually killed the target cells rather than just suppressing their growth. In our preliminary trial, we tested the cytotoxic mechanism of 7-morpholinomethyl-8-hydroxyquinoline on AS-30D cells by nucleic acid synthesis determination. Treatment of AS-30D cells with 7-morpholinomethyl-8-hydroxyquinoline resulted in inhibition of DNA and RNA synthesis. DNA synthesis in AS-30D cells was inhibited by 7-morpholinomethyl-8-hydroxyquinoline within 24 h. 8-Hydroxyquinoline, in contrast, inhibited DNA synthesis of AS-30D cells only after 48 h of contact. Both 8-hydroxyquinoline and 7-morpholinomethyl-8-hydroxyquinoline were less effective at inhibiting RNA synthesis, higher concentrations of compounds and longer incubation times were required to inhibit RNA synthesis. 7-Morpholinomethyl-8-hydroxyquinoline showed higher potency than 8-hydroxyquinoline (Table 1). On the basis of this promising activity, further study of the ionic mechanism of action of derivatives of 8-hydroxyquinoline were undertaken.

The outward voltage-activated K^+ currents of human myeloma cells which were shown here could be observed following depolarizing pulses to −40 mV, and were increased in amplitude with greater depolarization. This voltage-activated K^+ channel is similar to the λ-type channel described in T lymphocyte K^+ channels (De Coursey et al 1984; Wu et al 1998). Our results showed that the outward current we recorded in myeloma cells essentially consisted of the voltage-activated K^+ current. K^+ channels, with an open probability increased by mitogens, are assumed to play a role in the progression through the cell cycle at a critical period in the G1 phase, before entry into the S

Table 1. The effect of 7-morpholinomethyl-8-hydroxyquinoline and 8-hydroxyquinoline on DNA and RNA synthesis in AS-30D cells after 24 h and 48 h incubation.

Compound	IC50 (μM)			
	DNA[a]	DNA[b]	RNA[a]	RNA[b]
7-Morpholinomethyl-8-hydroxyquinoline	10·76	6·04	12·43	25·78
8-Hydroxyquinoline	20·88	9·69	109·21	94·55

[a]24 h incubation, [b]48 h incubation. Each experiment was carried out in triplicate and repeated once.

phase (Amigorena et al 1990). Their possible role may result from a maintenance of a hyperpolarized resting potential directly involved in DNA synthesis and cell proliferation (Rouzaire-Dubois et al 1993). Based on our preliminary study, the finding that 7-morpholinomethyl-8-hydroxyquinoline effect-ively suppressed DNA and RNA synthesis could be related to the biological activity of the K^+ channel. Thus, the ionic mechanism of action of 7-pyrrolidinomethyl-8-hydroxyquinoline on myeloma cells was investigated. The presence of 7-pyrrolidinomethyl-8-hydroxyquinoline in the bath effectively inhibited the amplitude of $I_{K(V)}$ in the myeloma cell line. As shown in Figure 2, the cell was held at the level of −40 mV, and ramp voltage pulses from −90 to +50 mV (1 s in duration) were applied. 7-Pyrrolidinomethyl-8-hydroxyquinoline (5 μM) inhib-ited the amplitude of $I_{K(V)}$ to 2560 ± 105 pA from control values of 2950 ± 118 pA (n = 8 cells). After pyrrolidinomethyl-8-hydroxyquinoline was removed, $I_{K(V)}$ returned almost to the control level. The present study showed that 7-pyrrolidinomethyl-8-hydroxyquinoline blocked currents through voltage-activated K^+ channels in a dose-dependent manner (Table 2). The IC50 value for the inhibitory effects of the voltage-activated K^+ channel and cell growth were 23 and 14 μM, respectively. Deutsch et al (1986) reported that K^+-current blockers could simply modulate cell proliferation by depolarizing the membrane. The sensitivity of cell proliferation to 7-pyrrolidinomethyl-8-hydroxyquinoline (Table 3) was comparable with that of voltage-activated K^+ currents, and the relation between the reduction in current and proliferation was observed. It is thus suggested that 7-pyrrolidinomethyl-8-hydroxyquinoline - mediated inhibition of the K^+ channels may partially, if not entirely, contribute to its antineoplastic action. Because of the similarities in the molecular structures of these compounds, we suggest a common action.

Antitumour therapy based on targeted drug delivery to tumour cells has the potential of overcoming limitations that are inherent in conventional cancer chemotherapy. Many animal and human

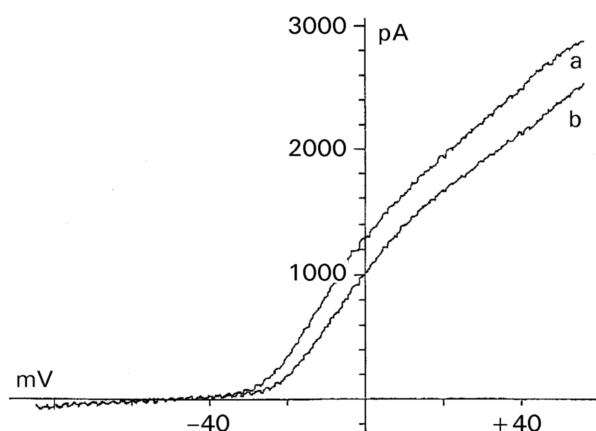

Figure 2. Effect of 7-pyrrolidinomethyl-8-hydroxyquinoline on the current–voltage relations of $I_{K(V)}$ in human myeloma cells. The inhibitory effect of 7-pyrrolidinomethyl-8-hydroxyquinoline on the voltage-activated K^+ current is illustrated. a. Control current trace of current voltage relation. b. Recording after the application of 5 μM 7-pyrrolidinomethyl-8-hydroxyquinoline.

Table 2. Concentration-dependent inhibition of the voltage-activated K^+ current in human myeloma cells by 7-pyrrolidinomethyl-8-hydroxyquinoline.

Concn	Relative amplitude
0	1·00 ± 0·20
1	0·93 ± 0·20
5	0·84 ± 0·19
10	0·69 ± 0·11
30	0·19 ± 0·02

All data represent the mean ± s.e.m. of 8 cells.

tumours exhibit high levels of β-glucuronidase activity relative to most normal tissues (Henle et al 1988). β-Glucuronidase is an enzyme that deconjugates glucuronides and may cause secondary accumulation of active drug in tissues with high β-glucuronidase activity (Connors et al 1973). It has been reported that the glucuronide of 8-hydroxyquinoline can be converted to 8-hydroxyquinoline in tumour cells with elevated β-glucuronidase activity (Monson et al 1991). The relative accumulation of 8-hydroxyquinoline in tumours which exhibited high activities of β-glucuronidase after treatment with the glucuronide of 8-hydroxyquinoline increased the therapeutic index of the chemotherapy. A glucuronide of 7-pyrrolidinomethyl-8-hydroxyquinoline, 7-morpholinomethyl-8-hydroxyquinoline, 7-diethylaminomethyl-8-hydroxyquinoline or 7-piperidinomethyl-8-hydroxyquinoline, the derivatives of 8-hydroxyquinoline, are also expected to be selectively toxic to tumour cells expressing β-glucuronidase. The β-glucuronidase activity of tumour cells can be elevated by targeting this enzyme to tumours as an

Table 3. Cytotoxicity of 8-hydroxyquinoline derivatives against human myeloma cells.

Compound	Inhibition (%)				GI50 (μM)
	0·1 μM	1 μM	10 μM	100 μM	
7-Pyrrolidinomethyl-8-hydroxyquinoline	6	6	35	126	14
7-Diethylaminomethyl-8-hydroxyquinoline	0	21	80	134	3·1
7-Morpholinomethyl-8-hydroxyquinoline	0	0	21	NT	>10

Data obtained from the National Cancer Institute's in-vitro tumour cells screen. GI50 = concentration inhibiting cell growth by 5%. NT = not tested.

antibody–enzyme conjugate (Roffler et al 1992; Wang et al 1992). It also has been reported that direct radio-iodination of metabolic 8-hydroxy-quinoline-glucuronide can selectively increase anticancer activity (Unak & Unak 1996). The chemotherapy plays an important role in treating cancer. The basic limitation of chemotherapy is the physiological similarity between normal cells and tumour cells. Consequently, K^+-channel blockers should act on both normal and malignant cells. However, it was found that the membrane lipid composition of malignant cells differs from that of normal cells (Shinitzky 1984) and conversely, the pharmacological properties of K^+ channels depend on their lipid environment (Rouzaire-Dubois et al 1991). In light of the above observations, 7-pyrrolidinomethyl-8-hydroxyquinoline may be useful in cancer chemotherapy.

Acknowledgements

This work was supported in part by a grant from the National Science Council (NSC 86-2314-B-242-001). The authors thank Dr Steve Roffler (Institute of Biomedical Science, Academia Sinica) and Dr Ji Wang Chern (Department of Pharmacy, National Taiwan University) for their helpful suggestions.

References

Amigorena, S., Choquet, D., Teillaud, J. L., Korn, H., Fridman, W. H. (1990) Ion channel blockers inhibit B cell activation at a precise stage of the G1 phase of the cell cycle. J. Immunol. 144: 2038–2045

Connors, T. A., Farmer, P. B., Foster, A. B., Gilsenan, A. E., Jarman, M., Tisdale, M. J. (1973) Metabolism of aniline mustard [N,N-di-(2-chloro-ethyl) aniline]. Biochem. Pharmacol. 22: 1971–1980

De Coursey, T. E., Chandy, K. G., Gupta, S., Cahalan, M. D. (1984) Voltage-gated K^+ channels in human T lymphocytes: a role in mitogenesis? Nature 307: 465–468

Deutsch, C., Krause, D., Lee, S. C. (1986) Voltage-gated potassium conductance in human T lymphocytes stimulated with phorbol ester. J. Physiol. 372: 405–423

Dimmock, J. R., Erciyas, E., Raghavan, S. K., Kirkpatrick, D. L. (1990) Evaluation of the cytotoxicity of some Mannich bases acetophenone against the EMT6 tumour. Pharmazie 45: 755–757

Handelsman, H. (1996) Haematopoietic stem-cell transplantation in multiple myeloma. Cancer Treat. Rev. 22: 119–125

Henle, K. J., Monson, T. P., Nagle, W. A., Moss, A. J. (1988) Tumor-targeted cell killing with 8-hydroxyquinolyl-glucuonide. Radiat. Res. 115: 373–386

Liu, S. I., Chi, C. W., Lui, W. Y., Mok, K. T., Wu, C. W., Wu, S. N. (1998) Correlation of hepatocyte growth factor-induced proliferation and calcium-activated potassium current in human gastric cancer cells. Biochim. Biophys. Acta 1368: 256–266

Monson, T. P., Henle, K. J., Nagle, W. A., Mansouri, A. (1991) Tumor-targeted delivery of 8-hydroxyquinoline. I. J. Radiat. Oncol. Biol. Phys. 20: 1263–1271

Nordenberg, J., Novogrodsky, A., Beery, E., Patia, M., Wasserman, L., Warshawsky, A. (1990) Anti-proliferative effects and phenotypic alterations induced by 8-hydroxyquinoline in melanoma cell lines. Eur. J. Cancer 26: 905–907

Roffler, S .R., Yu, M. H., Chen, B, M., Tung, E., Yeh, M. Y. (1992) Therapy of human cervical carcinoma with monoclonal antibody-Pseudomonas exotoxin conjugates. Cancer Res. 51: 4001–4008

Rouzaire-Dubois, B., Dubois, J. M. (1990) Tamoxifen blocks both proliferation and voltage-dependent K^+ channels of neuroblastoma cells. Cell Signal 2: 387–393

Rouzaire-Dubois, B., Gerard, V., Dubois, J. M. (1991) Modification of K^+ channel properties induced by fatty acids in neuroblastoma cells. Pflugers Arch. 419: 467–471

Rouzaire-Dubois, B. R., Gerard, V., Dubois, J. M. (1993) Involvement of K^+ channels in the quercetin-induced inhibition of neuroblastoma cell growth. Eur. J. Physiol. 423: 202–205

Shen, A. Y., Wu, S. N. (1998) Effects of 1-pyrrolidinylmethyl-2-naphthol on contractile force and ionic current in cardiac and vascular smooth myocytes. Drug Dev. Res. 44: 87–96

Shen, A. Y., Hwang, M. H., Roffler, S., Chen, C. F. (1995) Cytotoxicity and antimicrobial activity of some naphthol derivatives. Arch. Pharm. 328: 197–201

Shinitzky, M. (1984) Membrane fluidity in malignancy adversative and recuperative. Biochim. Biophys. Acta 738: 251–261

Sidell, N., Schlichter, L. (1986) Retinoic acid blocks potassium channels in human lymphocytes. Biochem. Biophys. Res. Commun. 138: 560–567

Smith, D. F., Walborg, E. F., Chang, J. P. (1970) Establishment of a transplantable ascites varient of a rat hepatoma induced by 3-methyl-4-dimethylaminoazo benzene. Cancer Res. 30: 2306–2309

Unak, T., Unak, P. (1996) Direct radioiodination of metabolic 8-hydroxyquinolyl-glucuronide, as a potential anti-cancer drug. Appl. Radiat. Isot. 47: 645–647

Wang, S. M., Chern, J. W., Yeh, M. Y., Ng, J. C., Tung, E., Roffler, S. R. (1992) Specific activation of glucuronide prodrugs by antibody-targeted enzyme conjugates for cancer therapy. Cancer Res. 52: 4484–4491

Wu, S. N., Yu, H. S., Jan, C. R., Li, H. F., Yu, C. L. (1998) Inhibitory effects of berberine on voltage- and calcium-activated potassium currents in human myeloma cells. Life Sci. 62: 2283–2294