Effect of Tritoqualine on the Proliferation of Interleukin-3 Dependent Cell Line and Sensitive Cells

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Abstract—In this paper, the effect of tritoqualine (TRQ) on the proliferation of interleukin (IL) sensitive cells was investigated. TRQ inhibited the proliferation of FDCp-2 (IL-3 dependent cell line), CTLL-2 (IL-2 dependent cell line) and bone marrow cells (BMC) stimulated by IL, giving an ID50 of about 3 μM equally in the three systems. However, a ten times higher concentration of TRQ was required to inhibit the tumor cell proliferation. TRQ did not affect the unstimulated bone marrow cells. Accordingly, it is suggested that TRQ may show its anti-allergic effect, at least partially, by interfering with the proliferation/differentiation to mast cell and basophils of multi-functional hemato-poietic cells stimulated by IL-3.

Tritoqualine (TRQ, Fig. 1) has been used clinically in Europe for the treatment of various allergic diseases (1–3). Recently, it was shown to decrease the serum transaminase level and suppress the progression of fibrosis in the liver of patients with chronic hepatitis (4).

In an animal model, Yuasa et al. (5–7) demonstrated the therapeutic and prophylactic effect of TRQ on the chronic liver injury in rats treated with CCl4.

Since the first observation of Parrot et al. (8, 9) and Capri and Maggi (10) demonstrating the inhibitory effect of TRQ on histidine decarboxylase, extensive studies have been conducted to clarify the pharmacological properties of TRQ, and some proposals for its mode of action have been pointed out. One proposal by Umezu et al. (11–13) suggested that the main pharmacological activity of TRQ can be ascribed to the membrane stabilization of various inflammatory cells, resulting in the inhibition of a) collagen types of secretion from fibroblast, b) histamine release from mast cells and c) lysosomal enzyme release from polymorphonuclear leukocytes.

It is of interest to study the effect of TRQ on the proliferation (or differentiation) of interleukin-3 (IL-3) sensitive cells, since Ihe et al. (14) recently reported that IL-3 exhibited functional activity as mast cell growth factor, P-cell growth factor and histamine producing cell stimulation factor, and involvement of those cells in the allergic state has been well documented. IL-3 might be, in part, responsible for the spontaneous auto-immune disease appearing in MRL/ lpr/lpr mice, where IL-3 production increases accompanying aging as shown by Palacios. (15)

In this paper, we reported the effect of TRQ on interleukin (IL) induced proliferation of IL sensitive cell line, FDCp-2 cells and CTLL-2 cells and also murine bone marrow

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Fig. 1. Chemical structure of tritoqualine.
cells (BMC), as well as the effect on IL-3 production.

Materials and Methods

Animals: BALB/c, C3H/He and C57BL/6 were purchased from Japan Charles River, Inc. (Kanagawa, Japan). Male mice were used at the age of 6 weeks.

Medium: Unless mentioned, the medium used throughout these experiments was RPMI-1640 supplemented with 2 mM l-glutamine, 50 \( \mu \)M 2-mercaptoethanol, 1 \( \mu \)g/ml of streptomycin, 100 U/ml of penicillin G, and 10% heat inactivated fetal calf serum (Armour Inc., U.S.A.).

Reagents: The following reagents were used in these experiments: TRQ (Medichenie AG, Switzerland), disodium chromoglycate (DSCG: Fujisawa Inc., Japan), dexamethasone (DEX: Sigma, U.S.A.), mitomycin-C (MMC: Kyowa Inc., Japan) and concanavalin A (Con A: Sigma, U.S.A.). IL-3 containing supernatant (IL-3 SUP) was prepared by harvesting the culture supernatant of WEHI-3 cells, IL-3 producing cell line, after 3 days at 37°C in a 7% CO2 atmosphere. IL-2 containing supernatant (IL-2 SUP) was prepared by harvesting the culture supernatant of rat spleen cells with 10 \( \mu \)g/ml Con A after 24 hr at 37°C in a 7% CO2 atmosphere.

Cell lines: WEHI-3 cell line was maintained in the medium which was exchanged every 3 days. FDCp-2, IL-3 dependent cell line, was maintained in the medium supplemented with 20% IL-3 SUP, and CTLL-2, IL-2 dependent cell line, maintained in the medium supplemented with 20% IL-2 SUP. The tumor cell lines used in the experiment were J-774 cells (murine macrophage-like cell line), FM-3A cells (murine mammary), P-815 cells (murine leukemia), PLC cells (human hepatoma) and KN cells (human stomach cancer).

Preparation of spleen cell suspension: Spleens of mice were teased on a stainless mesh, and the cell suspension was washed three times with RPMI-1640. The lymphocyte fraction was obtained by using the Ficoll-Isopaque density gradient centrifugation method.

Preparation of bone marrow cell sus-
Results

The effect of TRQ on interleukin stimulated cell proliferation: As shown in Table 1, TRQ strongly inhibited the proliferation of FDCp-2 cells induced by IL-3 SUP in a dose-dependent manner; and even the lowest dose, 0.33 $\mu$M, caused almost complete inhibition. It was also noted that TRQ did not affect the incorporation of $^3$H-TdR without IL-3 SUP in the dose range tested in this experiment. This inhibitory effect of TRQ was compared with those of DEX and DSCG (Fig. 2). The approximate ID50, the dose to give 50% inhibition of proliferation, was 2.2 $\mu$M of TRQ and 460 $\mu$M of DEX, respectively; DSCG did not inhibit even at 33 $\mu$M. BMC proliferation induced by IL-3 SUP was also inhibited by TRQ, and the ID50 was approximately 3 $\mu$M (Table 2). However,

Table 1. Effect of TRQ on IL-3 stimulated proliferation of FDCp-2 cell line

| IL-3 SUP conc. (%) | TRQ conc. ($\mu$M) | Uptake of $^3$H-TdR (cpm×100) | % Inhibition |
|-------------------|-------------------|-------------------------------|--------------|
| 0                 | 0                 | 37±1.7                        | —            |
| 0                 | 0.33              | 38±3.6                        | 0            |
| 0                 | 3.3               | 34±0.7                        | 1            |
| 0                 | 33                | 30±0.9                        | 2            |
| 10                | 0.33              | 246±16                        | —            |
| 10                | 3.3               | 75±11 (<0.01)                 | 90           |
| 10                | 33                | 41±7.0 (<0.01)                | 99           |
| 10                | 33                | 27±2.0 (<0.01)                | 100          |

FDCp-2 cells were incubated with TRQ and IL-3 SUP for 24 hr at 37°C and then pulsed with $^3$H-TdR for 17 hr to measure cell proliferation. Results represent the mean±S.E.M. (triplicate). Numbers in parenthesis represent P values (Student’s t-test).

Fig. 2. Effect of tritoqualine, dexamethasone and disodium chromoglycate on IL-3 stimulated proliferation of FDCp-2 cell line. FDCp-2 cells were incubated with 10% IL-3 SUP and reagents. Other experimental details are the same as described in Table 1. Data represent the mean±S.E.M. (triplicate). *P<0.05, **P<0.01 (Student’s t-test).
pretreatment of BMC with TRQ before IL-3 stimulation did not affect the proliferation as shown in Fig. 3, suggesting that TRQ had no effect on the unstimulated BMC. Table 3 shows that TRQ inhibited not only IL-3 stimulation, but also IL-2 stimulated pro-

Table 2. Effect of TRQ on IL-3 stimulated proliferation of bone marrow cells

| IL-3 SUP conc. (%) | TRQ conc. (µM) | Uptake of $^3$H-TdR (cpm ×100) | % Inhibition |
|-------------------|---------------|-------------------------------|--------------|
| 0                 | 0             | 10±2.7                        |              |
| 0                 | 0.33          | 13±3.1                        | 0            |
| 3.3               | 8±0.8         | 10±1.1                        | 0            |
| 33                | 220±5.3       |                               |              |
| 10                | 0.33          | 222±14                        | 0            |
| 3.3               | 120±3.0 (<0.01) | 72±2.9 (<0.01) | 48           |
| 33                | 72±2.9 (<0.01) |                               | 70           |

Bone marrow cells were incubated with TRQ and IL-3 SUP for 48 hr at 37°C and then pulsed with $^3$H-TdR for 17 hr to measure cell proliferation. Results represent the mean±S.E.M. (triplicate). Numbers in parenthesis represent the P value (Student's t-test).

Fig. 3. Effect of TRQ pretreatment on IL-3 stimulated proliferation of bone marrow cells. Bone marrow cells were preincubated with TRQ in the absence of IL-3 SUP for 16 hr at 37°C and then washed. Cells were incubated with IL-3 SUP as described in Table 2.
liferation of the CTLL-2 cell line, giving an ID50 of about 3 μM, similar to that against IL-3 stimulation.

Effect of TRQ on the tumor cell proliferation: To see whether the inhibitory effect of TRQ might be specific for interleukin

Table 3. Effect of TRQ on IL-2 stimulated proliferation of CTLL-2 cell line

| IL-2 SUP (%) | TRQ conc. (μM) | Uptake of ³H-TdR (cpm (S.E.M.)) | % Inhibition |
|--------------|----------------|---------------------------------|--------------|
| 0            | 0              | 690 (94)                        | -            |
| 0.33         | 0              | 654 (115)                       | 5            |
| 3.3          | 0              | 447 (12)                        | 35           |
| 33           | 0              | 202 (49)                        | 71           |
| 0.1          | 0              | 8680 (325)                      | -            |
| 0.33         | 0              | 7933 (239)                      | 8            |
| 3.3          | 0              | 4137 (78)                       | 52           |
| 33           | 0              | 241 (21)                        | 97           |
| 1.0          | 0              | 54502 (3058)                    | -            |
| 0.33         | 0              | 49186 (1582)                    | 10           |
| 3.3          | 0              | 28085 (1317)                    | 48           |
| 33           | 0              | 447 (156)                       | 99           |

CTLL-2 cells were incubated with TRQ and IL-2 SUP for 24 hr at 37°C and then pulsed with ³H-TdR for 17 hr to measure cell proliferation. Results represent the mean±S.E.M. (triplicate).

Table 4. Effect of TRQ on proliferation of various tumor cell lines

| Cell line | TRQ conc. (μM) | cpm     | % Control |
|-----------|----------------|---------|-----------|
| J774      | 0              | 44692   | 100       |
|           | 0.33           | 31706   | 71        |
|           | 3.3            | 35116   | 79        |
|           | 33             | 26128   | 59        |
| FM3A      | 0              | 44820   | 100       |
|           | 0.33           | 44765   | 100       |
|           | 3.3            | 39251   | 88        |
|           | 33             | 28470   | 64        |
| P-388     | 0              | 23774   | 100       |
|           | 0.33           | 18321   | 77        |
|           | 3.3            | 15241   | 64        |
|           | 33             | 7236    | 30        |
| PLC       | 0              | 3971    | 100       |
|           | 0.33           | 3250    | 82        |
|           | 3.3            | 3638    | 92        |
|           | 33             | 4155    | 105       |
| KN        | 0              | 13769   | 100       |
|           | 0.33           | 11984   | 87        |
|           | 3.3            | 8461    | 61        |
|           | 33             | 6641    | 48        |

Tumor cells were incubated with TRQ for 24 hr at 37°C and then pulsed with ³H-TdR for 17 hr to measure cell proliferation. Results represent the mean of triplicate cultures with S.E.M. of less than 15%.
induced proliferation, or simply a non-specific cytostatic effect, the effects of TRQ on various tumor cell lines which proliferate without interleukin was investigated. As shown in Table 4, TRQ inhibited tumor cell proliferation; however, only at a higher concentration. The ID50 was more than 30 μM in 4 out of 5 tumor cell lines, suggesting that TRQ was rather specific for interleukin stimulated proliferation.

Effect of TRQ on production of IL-3 by allogeneic mixed lymphocytes reaction: As shown in Fig. 4, TRQ did not significantly suppress nor augment IL-3 production appearing in the culture supernatant of MLR.

Discussion

The mast cell and histamine released by mast cells are very closely related to various types of allergic diseases through the release of histamine or other chemical mediators, and IL-3 is considered to be one of the growth or differentiation factors for mast cells. Considering the clinical efficacy in allergic diseases of TRQ and the recent finding of cell membrane stabilizing activity, we investigated the effect of TRQ on interleukin production and interleukin induced cell proliferation in vitro systems.

The concentration range of TRQ used in this study was selected because a blood level of 1 μM TRQ was sufficient to produce a therapeutic effect on liver injury in rats treated by CCl4 (17).

TRQ strongly inhibited FDCp-2 cells proliferation induced by IL-3 as shown in Tables 1 and 2. A difference in the ID50 values was observed between two experiments. However, the ID50 of 2 μM seemed to be more reliable, since the third experiment in the same system gave an ID50 of 3 μM for TRQ. In the experiment using BMC, TRQ inhibited the BMC proliferation induced by IL-3 the same dose. IL-2 induced proliferation was also inhibited by TRQ, and TRQ inhibited the proliferation of CTLL-2 with 0% of IL-2 SUP. However, when CTLL-2 cells were incubated in the absence of IL-2 SUP for 24 hr with or without TRQ, the viability of CTLL-2 cells was less than 5%. Therefore, this inhibitory effect might be caused by a synergetic effect of decreasing cell viability.
and TRQ. In the three systems of cell proliferation, TRQ had almost the same ID50 value.

Accordingly, one may ask whether TRQ is a cytocidal or a non-specific cytostatic reagent. However, this could be excluded since 1) pretreatment of BMC with TRQ at the conc. of 30 μM had no effect on the subsequent proliferation induced by IL-3 as shown in Fig. 3, 2) presence of TRQ in MLR culture did not affect IL-3 production (Fig. 4), 3) the growth of mastocytoma P-815 cells was not affected by 10 μM of TRQ; however, histamine release from cells was inhibited (12).

Tumor cell proliferation was also inhibited by TRQ; however, its ID50 value was about 30 μM, ten times weaker than that of interleukin stimulated cells.

We reported in our previous paper (18) that TRQ inhibited spleen cell proliferation stimulated by Con A at an ID50 of about 30 μM, and this effect was very similar to that against tumor cell proliferation. So the effect of TRQ on the cell proliferation could be stated as: 1) no effect on the unstimulated cell, 2) ten times more specific inhibition against interleukin stimulated cell proliferation, and 3) non-specific cytostatic effect at more than 30 μM.

It is known that each interleukin dependent cell line possesses the receptor for its own interleukin on the cell membrane, and one way that TRQ can inhibit proliferation may be an antagonistic action against IL-3. However this is not the case, since pretreatment of BMC with TRQ did not have any effect on the subsequent proliferation. Although the details of the sequential response that induced the cell proliferation after the IL stimulation have not been elucidated sufficiently, the activation of protein kinase C is thought to be a general process involved in the cell proliferation.

Umezu et al. (19) reported that TRQ inhibited calcium influx and calmodulin activation of mast cells stimulated by several stimuli, and the proliferation inhibitory effect of TRQ might be mediated at least partially through the same mechanism. It was also noted in this study that DEX or DSCG did not inhibit the cell proliferation stimulated by IL-3, and as far as we know, TRQ is the only compound which inhibits it at the physiological concentration and without cytocidal activity. As shown in Table 1, 0.33 μM of TRQ caused almost complete inhibition, but in Fig. 2, the same dose of TRQ gave about 50% inhibition. The two culture supernatants of WEHI-3 cells used in these experiments might contain different IL-3 activity.

It is reported that mast cell/basophils can be differentiated from multi-functional hemato-poietic cells by IL-3 stimulation after several passages (20). This phenomenon was confirmed in our experiment. However, the presence of TRQ in the culture did not generate the mast cells and/or basophils (data not shown).

It is conceivable that TRQ might play a role in the allergic diseases associated with IL-3 and mast cells and basophils.

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