Blockers of \(K^+/{\text{Cl}}^-\) transporter/channels diminish proliferation of osteoblastic cells

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(Received 9 January 2009; and accepted 14 January 2009)

ABSTRACT

In the present report, we studied if blockers of the \(K^+/{\text{Cl}}^-\) cotransporter, and \(\text{Cl}^-\) and \(K^+\) channels modify the proliferation of MC3T3-E1 osteoblastic cells. A blocker of the \(K^+/{\text{Cl}}^-\) cotransporter, DIOA (100 and 500 μM), diminished the cell proliferation. NPPB (100 and 300 μM, a blocker of \(\text{Cl}^-\) channel) and quinine (100 and 500 μM, a blocker of \(K^+\) channel) also suppressed the proliferation of MC3T3-E1 cells. These blockers of the \(K^+/{\text{Cl}}^-\) cotransporter/channels (DIOA, NPPB and quinine) would increase the intracellular \(\text{Cl}^-\) concentration ([\(\text{Cl}^-\)]). On the other hand, our previous study (Biochem. Biophys. Res. Commun. 361, 1038–1043, 2007) reported that a decrease in [\(\text{Cl}^-\)] diminished the proliferation of MC3T3-E1 osteoblastic cells. Taken together, the observations indicate that both increases and decreases in [\(\text{Cl}^-\)] diminish the proliferation of MC3T3-E1 osteoblastic cells.

Some studies (3, 4, 6, 8) have reported multiple factors regulating migration, proliferation, and differentiation of the osteoblast. Ion channels/transporters such as the \(K^+/{\text{Cl}}^-\) cotransporter, \(\text{Cl}^-\) and \(K^+\) channels, and the \(\text{Na}^+/K^+/{\text{Cl}}^-\) cotransporter (NKCC) would have some action on various cell function including cell proliferation (7, 14, 21), but the actions of the \(K^+/{\text{Cl}}^-\) cotransporter, and \(\text{Cl}^-\) and \(K^+\) channels on cell proliferation remain controversial. Our previous report has indicated that reduction of the intracellular \(\text{Cl}^-\) concentration ([\(\text{Cl}^-\)]) diminishes the proliferation of osteoblasts (9), in which ion transports including \(\text{Cl}^-\) actively occur. The \(K^+/{\text{Cl}}^-\) cotransporter, and \(\text{Cl}^-\) and \(K^+\) channels would control [\(\text{Cl}^-\)], and blockade of the \(K^+/{\text{Cl}}^-\) cotransporter (KCC), and the \(\text{Cl}^-\) and \(K^+\) channels would elevate the [\(\text{Cl}^-\)]. Therefore, the aim of the present study is to clarify if blockade of the \(K^+/{\text{Cl}}^-\) cotransporter, and \(\text{Cl}^-\) and \(K^+\) channels, which would elevate the [\(\text{Cl}^-\)], affects the proliferation of osteoblasts.

The culture medium containing 10% fetal bovine serum (FBS), 100 IU/mL penicillin and 100 μg/mL streptomycin was prepared using αMEM (Cell Science and Technology Institute, Sendai, Japan) with 2 mM L-glutamine (Sigma, St. Louis, MO, USA). We obtained mouse calvaria-derived osteoblast-like cell, MC3T3-E1, from the RIKEN CELL BANK (Tsukuba, Japan), and cultured the cells in the culture medium at 37°C in a humidified atmosphere with 5% CO\(_2\) in air. Dihydroindolyl-oxy alkanoic acid (DIOA), a blocker of the \(K^+/{\text{Cl}}^-\) cotransporter (KCC), was prepared as 50 and 100 mM stock solutions in dimethyl sulfoxide (DMSO). 5-N-2-(3-phenyl-propylamino)benzoic acid (NPPB), a Cl\(^-\) channel blocker, was prepared as 100 and 300 mM stock solutions in DMSO. Quinine, a \(K^+\) channel blocker, was prepared as 100 and 500 mM stock solutions in DMSO. Each drug was added to the culture medium by diluting 1000-fold; e.g., 1 μL of the

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NPPB, on the proliferation of MC3T3-E1 cells. The treatment with NPPB, 100 or 300 μM, abolished the cell proliferation. We further studied the effect of quinine, a K$^+$ channel blocker, on the proliferation of MC3T3-E1 cells. Quinine (100 and 500 μM) diminished the cell proliferation (Fig. 3).
K⁺ channels suppress the proliferation of MC3T3-E1 osteoblastic cells, suggesting the existence of a special proliferation mechanism dependent on K⁺/Cl⁻ transport (movement).

The change in [Cl⁻]i modifies the cell growth (11), the gene expression of COX2 in renal cells (23), the Na⁺/K⁺/2Cl⁻ cotransporter (NKCC) activity in dog tracheal cells (5) and in human trabecular meshwork cells (20), gene expression of epithelial Na⁺ channel (ENaC) in renal epithelium (15), and src kinase activity in renal cells (17). KCC extrudes KCl from the cytosolic space, keeping [Cl⁻]i low, suggesting that the blockade of KCC would elevate [Cl⁻]i, by diminishing the KCl efflux via KCC (10, 12). We indicate that a blocker of KCC, DIOA, diminished the cell proliferation (Fig. 1), suggesting that an increase in [Cl⁻]i would diminish the cell proliferation. Both NPPB (a Cl⁻ channel blocker) and quinine (a K⁺ channel blocker) diminished the cell proliferation (Figs. 2 and 3). Both compounds, NPPB and quinine, are expected to elevate [Cl⁻]i by blocking K⁺/Cl⁻ effluxes (10, 12). These observations suggest that an increase in [Cl⁻]i would diminish the cell proliferation. On the other hand, the reduction of [Cl⁻]o, which would decrease [Cl⁻]i, diminished the proliferation of gastric cancer cells (13, 22). Further, MC3T3-E1 cells used in the present study also showed the same response to the reduction of [Cl⁻]o; i.e., the proliferation of MC3T3-E1 cells was diminished in a low Cl⁻ culture medium (9). These observations seem to be inconsistent; i.e., both increases and decreases in [Cl⁻]i diminish the cell proliferation. To explain these apparently inconsistent observations, we propose an idea that the cell proliferation of MC3T3-E1 cells depends on the conductance (movement) of Cl⁻ but not on the change in [Cl⁻]i. This idea is supported by our previous report that the activity of the Na⁺, K⁺-ATPase depends on the Cl⁻ conductance (movement) (16). An alternative idea is that [Cl⁻]i of MC3T3-E1 cells cultured in the normal medium (normal [Cl⁻]i) is the most suitable concentration for cell proliferation, and deviations from normal [Cl⁻]i (both higher and lower than normal [Cl⁻]i) diminish the proliferation of MC3T3 cells, although more experiments are required to confirm this idea. The observations shown in the present study indicate here a new idea that the K⁺/ Cl⁻ movement would play an important role in the proliferation of osteoblastic cells.

Acknowledgements
This work was supported by Grants-in-Aid from Japanese Society for The Promotion of Science (1739057, 17590191, 17790154, 18659056, 19590212, 19790168, 20390060), Fuji Foundation for Protein Research, and The Salt Science Research Foundation (0837).

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