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Comparison of the Antihistaminic Activity Between Cetirizine Enantiomers

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Abstract – The antiallergic drug, cetirizine, inhibits the histamine release from a rat basophilic leukemia (RBL-2H3) cell line, which is frequently used as a mast cell model. By investigating inhibitory activities of (+)- and (−)-cetirizine in RBL-2H3 cells on the histamine release, we aimed to evaluate the effect of their structual characteristics on the antihistamine activity. The study on RBL-2H3 cell has clearly demonstrated that the (−)-cetirizine is significantly more potent than the (+)- or the racemic cetirizine, although there was no difference in pharmacokinetics between (+)- and (−)-cetirizine in rats.

Keywords □ cetirizine; RBL 2H3 cell; antihistaminic activity

INTRODUCTION

Most drugs are still used as racemates; i.e., the mixtures of the enantiomers. However, it is gradually becoming clear that the enantiomers could frequently possess different pharmacological action and/or pharmacokinetic property. For example, not only the *d*-isomer of chlorpheniramine has been shown to be more effective pharmacologically in its clinical use than the *l*-isomer but also more stereoselective in pharmacokinetic profiles (Nishikata *et al.*, 1992).

Cetirizine is a potent second generation antihistamine that is used in seasonal and perennial allergic rhinitis. Although cetirizine is still marketed as a racemate, it is well known that the (+)-cetirizine is more effective for treating urticaria and the (−)-cetirizine is more effective for treating seasonal and perennial allergic rhinitis in humans (Gray[1)], 1997; Gray[2)], 1997). Since allergic responses are related to mediator released from mast cells and basophils (Gentile & Skoner, 1996; Fisher *et al.*, 1995; Jeannette *et al.*, 1998; Berthon *et al.*, 1993), we strongly felt that it was necessary to compare the antihistamine activity of (+)- and (−)-cetirizine enantiomers using RBL 2H3 cell.

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MATRIALS AND METHODS

Cell culture conditions

RBL-2H3 cells, kindly provided by Dr. Kyung-Rim Lee (Ewha Womans University, Seoul, Korea), were maintained as monolayer cultures in Eagles Minimum Essential Medium (EMEM) supplemented with 10% fetal calf serum and 1% glutamine (all from Biofluids, Rockville, MD, U.S.A.). Cells were routinely cultured for 48 h at 37°C in a humidified incubator with 5% CO_2/95% air and harvested by treatment with trypsin/versene (Biofluids). Following the asessment of viability by trypan blue exclusion, cells were either subcultured or used in the following experiments.

Anti IgE-induced histamine release

For the determination of histamine release, cells were grown until 2×10^7 cells confluently in 100 mm dish 2×10^6 cells were placed to 24 well plate and incubated 18 h at 37°C in a humidified incubator with 5% CO_2/95% air. After the media was removed, IgE (0.2 μg/ml; Serotec, Oxford, U.K.)

was added in EMEM. Cells were incubated at 37°C for 45~60 min, then each well was washed twice with 0.5 ml of the release buffer (25 mmol/l HEPES, pH 7.1, 100 mmol/l NaCl, 5 mmol/l KCl, 0.4 mmol/l $MgCl_2$, 9.0 mmol/l $CaCl_2$, 5.0 mmol/l glucose and 0.1% BSA (all from Sigma, St Louis, Missouri, USA)). Release buffer (0.2 ml) containing AntiIgE (1.25 μg/ml; Setotec, Oxford, U. K.), (+)-, (−)-, or racemic cetirizine was then added to each well and cells were incubated at 37°C for 10 min. Racemic cetirizine was a gift from UCB pharmaceutical company (Seoul, Korea) and (+)- and (−)-cetirizine were prepared from racemic cetirizine (Choi *et al.*, 2000).

Radioimmunoassay of released histamine

Released histamine was determined using a commercially available radioimmunoassay kit (Immunotech, Marseille, France). Bound radio activity was measured in a Kobra 4002 γ-counter (Packard, USA). Results were expressed as percentages of histamine release as follow; (1-bound cpm/RBL 2H3 cell cpm)× 100.

RESULTS

The antihistamine activities of (+)- and (−)-cetirizine enantiomers as well as racemic cetirizine were evaluated by determining their inhibitory potencies of histamine release in RBL 2H3 cell. Fig. 1 illustrates the percentage of the histamine release in RBL 2H3 cell when treated with (+)-, (−)- or racemic cetirizine.

Time course of the reactions were monitored at 5 min, 10 min and 15 min by treating (+)-, (−)- or racemic cetirizine in RBL 2H3 cell. The reaction time of 10 min was selected as the optimized choice.

The five controls employed in this study were as follows; RBL 2H3 cell was used as a negative control, RBL 2H3 cell treated AntiIgE after treating IgE was used as positive control. RBL 2H3 cell treated (+)-, (−)- or racemic cetirizine, (+)-, (−)- or racemic cetirizine after treating IgE and IgE was used as controls, also.

Inhibitory effect of (+)-, (−)-, and racemic cetirizine in RBL 2H3 cell was increased as the concentration of the drugs increased.

Fig. 2 shows drug concentration-histamine release curves. The antihistamine activity of (−)-cetirizine, expressed as the percentages of histamine release, was found to be more potent than racemic and (+)-cetirizine within the range of the experiment.

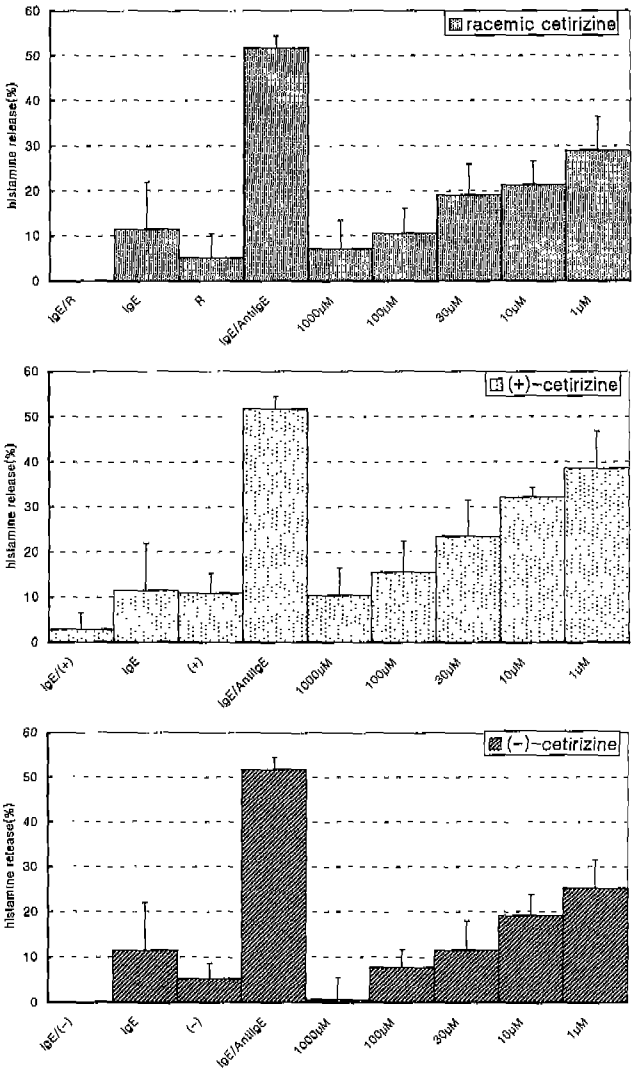

Fig. 1. Histamine release response of RBL 2H3 cell after treating (+)-, (−)- and racemic cetirizine (n=3). IgE/R, IgE/(+) or IgE/(−), Freshly isolated cells were sensitized with IgE, washed with release buffer and release buffer containing anti IgE and 30 μM of racemic, (+)- or (−)-cetirizine was added.; IgE, Cells were sensitized only IgE.; R, (+) or (−) , Cells were treated only racemic, (+)- or (−)-cetirizine.; IgE/AntiIgE, Cells were sensitized with IgE, washed with release buffer and release buffer containing antiIgE was added.; 1000 μM, 100 μM, 30 μM, 10 μM, 1 μM, Concentration of racemic, (+)- or (-)-citirizine which was contained in the release buffer.

Typically at 10 μM percent inhibition of histamine release by (−)-cetirizine was 63.0 and that of (+)-cetirizine was 37.9. This significant trend is also shown at all concentration of cetirizine (n=3).

Discussion

Cetirizine is presently sold as a racemic mixture and is used

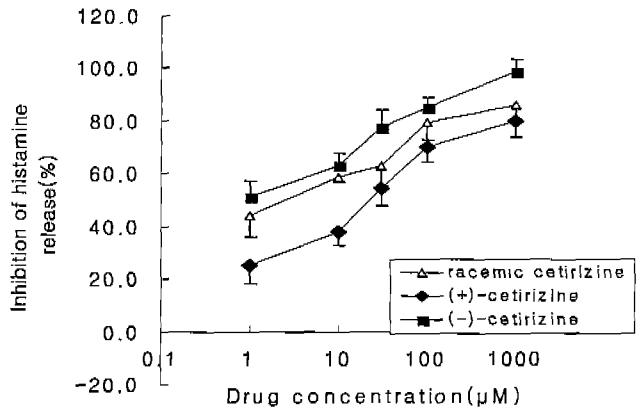

Fig. 2. Inhibition of histamine release percentage of racemic, (+)- and (−)-cetirizine in RBL 2H3 cell (n=3).

primarily in seasonal and allergic rhinitis. The optically pure (+)- or (−)-cetirizine be utilized, it should result in an enhanced efficacy and thus an improved therapeutic index. Therefore, cetirizine could well be the candidate drug of a racemic switch. In a previous study, we looked into the differences between (+)- and (−)-cetirizine in rat plasma after the racemic cetirizine was administered to the rat. We found the results showed that the pharmacokinetics of (+)- and (−)-cetirizine in rat plasma not to be significantly different (Choi *et al.*, 2000).

In this study, we have compared the antihistamine activity of (+)-, (−)- and racemic cetirizine in RBL 2H3 cell. The results of this study show that (−)-cetirizine is more potent than (+)- and racemic cetirizine and especially, (−)-cetirizine is significantly more potent than (+)-cetirizine ($p<0.05$). And the allergic effect of (+)-cetirizne in RBL 2H3 cell was larger than (−)- and racemic cetirizine.

Therefore, this study shows that there is the possibility of the racemic switch of the racemic cetirizine to the (−)-cetirizine.

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