Carboplatin-induced severe hypersensitivity reaction: Role of IgE-dependent basophil activation and FcεRI

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Basophil activation was observed in patients with a history of carboplatin-induced severe hypersensitivity reaction (HR). However, the precise mechanism by which carboplatin induces basophil activation and the associated surrogate markers remains to be elucidated. To investigate whether IgE-dependent mechanisms, including the overexpression of FcεRI, participate in carboplatin-induced basophil activation, 13 ovarian cancer patients were enrolled: 5 with a history of carboplatin-induced severe hypersensitivity reaction within the past 2 years, and 8 with no such history. The expression levels of FcεRI, IgE, and CD203c on basophils were measured using a flow cytometer. Immunoglobulin E-dependent basophil activation was evaluated by testing for IgE passive sensitization using lactic acid, and by testing for phosphatidylinositol 3-kinase inhibition, using wortmannin. In three patients positive for carboplatin hypersensitivity, pretreatment with wortmannin almost completely inhibited carboplatin-induced basophil activation (P < 0.05). In a healthy control subject, whose own IgE showed no response to carboplatin, acquired reactivity to carboplatin when exposed to plasma from patients positive for carboplatin hypersensitivity. This did not occur when the same experiment was carried out using plasma from the patients negative for carboplatin hypersensitivity. Moreover, pretreatment with omalizumab, a monoclonal anti-IgE antibody, almost completely blocked carboplatin-induced basophil activation in the plasma of patients positive for carboplatin hypersensitivity. On further investigation, the HR-positive group had significantly higher levels of FcεRI compared with the negative group (P < 0.05). In conclusion, an IgE-dependent mechanism incorporating FcεRI overexpression participates in carboplatin-induced severe HR. These results establish the relevance of monitoring the pharmacodynamic changes of basophils to prevent carboplatin-induced severe HR.

C arboplatin (CBDCA)-induced hypersensitivity reaction (HR) is a potentially lethal adverse event that frequently requires discontinuation of the drug. The rate of HR has been reported to be as high as 26.7% in patients receiving more than seven cycles of CBDCA.1 As CBDCA has been widely used for many types of cancer as a first or second line treatment, clarifying the mechanism of CBDCA-induced HR is extremely important for prevention of severe HR in such cases. The risk of CBDCA-induced severe HR is thought to be associated with a previous history of drug allergy, a prolonged platinum-free interval, or high dosages of CBDCA.2,3 We previously confirmed this by showing that patients with a CBDCA-free interval >13 months had a 22-fold higher risk of HR and that patients with a maximum dose/body of CBDCA >650 mg had a 9.5-fold higher risk of HR.4,5 Despite these findings, these factors have been insufficient for predicting HR in clinical practice. Alternatively, intradermal injections of CBDCA have also been used to predict CBDCA-induced severe HR.4,5 However, there are some risks associated with the clinical application of skin testing for anticaner drugs. For example, in patients with a history of life-threatening anaphylaxis, skin testing may be contraindicated. It is also not recommended due to several other factors including potential skin damage, concerns about environmental contamination, and risks to medical and nursing professionals resulting from excessive exposure to anticancer drugs.6

An improved understanding of basophils is useful for understanding and diagnosing allergic diseases, due to their role in the release of various inflammatory mediators, such as histamine.7,8 CD203c has been identified as a specific surface marker for basophils and mast cells of the hematopoietic lineage.9 CD203c is expressed on resting cells at low levels and its expression is rapidly upregulated following activation.9 In addition, it has been reported that CD203c is a useful marker for allergies induced by amoxicillin and (L)-asparaginase.10,11 We recently confirmed that patients with a history of CBDCA-induced severe HR showed an increase in CD203c-positive basophils compared with patients without HR.12 In addition, all five patients with grade 2 or higher HR showed increased CD203c on basophils.
during the months following the onset of HR, whereas no such increase was not observed in the previous courses of CBDCA. Therefore, it is reasonably certain that the mechanism of CBDCA-induced severe HR somehow involves basophil activation. However, the expression of CD203c on the day prior to anaphylaxis was found to be insufficient for predicting HR in half of the patients. Recently, Caiado et al. reported that 59% of patients have CBDCA-specific IgE; this was identified using the ImmunoCAP assay (Phadia, MI, USA). These findings strongly suggest that IgE-mediated basophil activation plays a key role in the development of CBDCA-induced HR.

A major goal of our study is to construct an appropriate way for predicting CBDCA-induced severe HR. To accomplish this, we attempted to confirm whether an IgE-dependent mechanism participates in CBDCA-induced basophil activation.

Materials and Methods

Study subjects. This study included patients who were admitted to Mie University Hospital (Tsu, Japan) between December 2011 and July 2013. Thirteen female patients with ovarian cancer were enrolled in this study. Five patients had a history of CBDCA-induced grade 3 or higher anaphylaxis, whereas 8 had no such history (Table 1). Upon study entry, blood samples were obtained from the patients on the day before CBDCA treatment. Five healthy control subjects with no history of treatment with CBDCA and no history of allergic diseases were also enrolled in this study. In addition, for further control, we enrolled a healthy basophil donor, whose IgE can only be recognized by polyclonal anti-IgE antibodies (Vector Laboratories, Burlingame, CA, USA), but not the positive control, we enrolled a healthy basophil donor, whose IgE can only be recognized by polyclonal anti-IgE antibodies (Vector Laboratories, Burlingame, CA, USA), but not the positive control.

To confirm the dissociation of IgE from FcεRI by acid treatment and binding of IgE to FcεRI after passive sensitization, pre- and post-passive-sensitized basophils were stained with an FITC-conjugated anti-IgE (Dako, Tokyo, Japan) and R-phycocerythrin (PE)-conjugated anti-FcεRI antibody. Mouse IgG2bk was used as an isotype control of anti-FcεRI antibody. The expression levels of FcεRI on each sample were then analyzed using a flow cytometer.

Reverse transcription-PCR analysis. Total RNA was prepared from whole blood samples using NucleoSpin RNA Blood (Takara Bio, Shiga, Japan). Messenger RNA was detected by RT-PCR using ReverTra Ace qPCR RT Master Mix (Toyobo, Osaka, Japan) with 50 ng total RNA, EagleTaq Master Mix (Roche Applied Science, Tokyo, Japan), and corresponding primer sets. Real-time PCR analysis was carried out using StepOnePlus (Applied Biosystems, Tokyo, Japan). The expression levels of the target molecules relative to GAPDH were measured using a fluorescence detection system.

Table 1. Characteristics of ovarian cancer patients who participated in this study (n = 13)

|                        | HR(−) group | HR(+) group | P-value |
|------------------------|-------------|-------------|---------|
| No. of patients        | 8           | 5           |         |
| Age, years†            | 57.5 (50–73)| 61 (54–69)  | 0.30    |
| No. of CBDCA treatments prior to study† | 7 (7–8) | 14 (11–22) | <0.01  |
| History of drug/food allergy‡ | 4 | 3 | 1.00 |
| Dose of CBDCA during study, mg† | 575 (400–690) | 500 (150–700) | 0.66 |

†Data are expressed as median values (minimum–maximum), and statistical analyses between the two groups were carried out using the Mann-Whitney U-test. § Statistical analyses between the two groups were carried out using Fisher’s exact test.

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evaluated with StepOne software version 2.2.2 (Applied Biosystems).

Statistical analysis. The non-parametric Mann–Whitney U-test and Fisher’s exact test were used to assess differences between patients with and without CBDCA-induced HR. The non-parametric Dunnett’s multiple comparison test was used to compare results among the three groups. We also carried out parametric one-way repeated measures ANOVA to test for differences among several related samples. All statistical analyses were carried out using GraphPad Prism 5 (version 5.01; GraphPad Software, San Diego, CA, USA). The \( P \)-values were two-sided and \( P < 0.05 \) was considered statistically significant.

Results

Inhibitory effect of wortmannin, a PI3-K inhibitor, on basophil activation. In the three patients with a history of CBDCA-induced severe anaphylaxis, CBDCA-induced CD203c expression on basophils was almost completely inhibited by pretreatment with wortmannin in a way similar to positive control (anti-IgE antibody) exposure (Fig. 1a) (\( P < 0.05 \) and \( P < 0.01 \), for 0.1 and 10 \( \mu \)M wortmannin, respectively) (Fig. 1b).

Confirmation of passive sensitization by flow cytometry analysis. We tested the involvement of CBDCA-specific IgE on CBDCA-induced severe HR using in vitro sensitization. Dissociation of IgE from the basophils from the healthy basophil donor as well as binding of IgE to the basophils in CBDCA patients’ plasma were confirmed either by staining with anti-FceRI antibodies, CRA1 or CRA2, or with an anti-IgE antibody. As has already been shown, CRA1 reacts with a region other than the IgE binding site, whereas CRA2 reacts directly with the IgE binding site. (18) We were thus able to confirm the successful dissociation of IgE by confirming that acid treatment increased CRA2 staining levels (Fig. 2a). After passive sensitization, CRA2 staining levels decreased, thereby suggesting that IgE-dissociated FceRI on the acid-treated basophils was occupied by IgE in patient plasma (Fig. 2a). Consistent with the results of the CRA2 staining, the level of IgE on negative control

| Wortmannin (μM) | Negative control | Positive control (anti-IgE mAb) | CBDCA (50 μg/mL) |
|-----------------|-----------------|-----------------|-----------------|
| 0 μM  | 1.5% | 66.8% | 69.3% |
| 0.1 μM | 2.1% | 4.3% | 2.9% |
| 10 μM | 1.9% | 3.3% | 1.4% |

(a)

(b)

\( \text{Fig. 1. Expression levels of CD203c-positive basophils after in vitro exposure to carboplatin (CBDCA) and wortmannin, a phosphatidylinositol 3-kinase inhibitor (measured by flow cytometric analysis). Whole blood with or without wortmannin was stained for CD3, prostaglandin D2 receptor (CRTH2), and CD203c. Flow cytometer charts for CD3− and CRTH2+ cells (basophils) are shown. Uregulation of CD203c on basophils (shown as a percentage in (a)) was determined using a threshold that was defined as the expression level above which 2% of basophils in the negative control column fluoresce, on average. (a) Data are from the patient whose response to CBDCA was highest among the hypersensitivity reaction-positive patients. This patient’s basophils were pretreated with the indicated concentrations of wortmannin, and subsequently exposed to the negative control, positive control, and 50 μg/mL CBDCA. Percentages shown indicate the upregulation rate of CD203c. Mean fluorescence intensities (MFIs) indicated for binding levels of CD203c on basophils. (b) Difference between the respective mean upregulation rates (\( n = 2 \)) of three patients were analyzed using one-way repeated measures ANOVA.} \)

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The expression levels of FcεRI (Fig. 4). Figure 4(a,b) shows results typical of HR(+) patients. (a) Immunostained healthy subject’s basophils with anti-FcεRI antibodies; PBMCs with or without acid treatment were stained for CD3, prostaglandin D2 receptor (CRTH2), and FcεRI. (b) Immunostained healthy subject’s basophils with anti-IgE antibody; PBMCs with or without acid treatment were stained for CD3, CRTH2, and IgE. Flow cytometer charts for CD3+ PBMCs are shown. Basophil fraction (CD3+ and CRTH2+) PBMC was gated. MFI is indicated for binding levels of anti-CRA1, anti-CRA2, and anti-IgE antibodies on basophils. MFI, mean fluorescence intensity.

basophils decreased after acid treatment, and increased after passive sensitization (Fig. 2b).

Analysis of passive sensitization by basophil activation test. Furthermore, to verify the replacement of IgE on basophils, we examined the basophils from a healthy control subject whose own IgE was not recognized by the positive control of the Aller-genicity Kit (Fig. 3a). Prior to this, all HR(+) and HR(−) patients’ IgE could be recognized by the positive control (Fig. 3a). After passive sensitization, basophils from the control subject also acquired reactivity to the positive control (Fig. 3b). Subsequently, control subject basophils sensitized with plasma from HR(+) patients also acquired reactivity to CBDCA, whereas control subject basophils sensitized with plasma from HR(−) patients did not respond to CBDCA (Fig. 3b). Furthermore, we were able to confirm that omalizumab specifically blocked the binding between IgE and FcεRI (Figs 2b, 3b). Additionally, we observed a high correlation between the respective response levels to CBDCA of patients’ sensitized basophils and patients’ original basophils (n = 5, r = 0.99) (Fig. 3c), which suggests that a CBDCA-specific IgE is required for the activation of basophils by CBDCA in HR(+) patients.

Expression levels of FcεRI on basophils in the HR(−) and HR(+) patients. The expression levels of FcεRI on basophils in HR(−) and HR(+) patients were measured by flow cytometry (Fig. 4). Figure 4(a,b) shows results typical of HR(−) and HR(+) patients, representing ΔMFI of 2385 and 10 193, respectively. ΔMFI is calculated as the difference in mean fluorescence intensity (MFI) between the FITC labeled anti-human FcεRI a chain antibody and its isotype control. The HR(+) group had significantly higher levels of FcεRI than both the HR(−) group (P < 0.05) and control subjects (P < 0.01) (Fig. 4c). In contrast, the expression levels of the reference protein, CRTH2, on basophils were comparable among the three groups (Fig. 4d).

Expression levels of FcεRI mRNA in the HR(−) and HR(+) patients. The overall expression levels of FcεRI mRNA in whole blood were measured by real-time RT-PCR in HR(−) and HR(+) patients (Fig. 5). Significant differences in all chains of FcεRI mRNA were found between the two groups. The expression levels of α, β, and γ chains of FcεRI mRNA in the HR(+) group were found to be 3.2-fold (P < 0.05), 6.8-fold (P < 0.01), and 1.9-fold (P < 0.05) higher than those in the HR(−) group.

Discussion

In this study, we confirmed for the first time that an IgE-dependent mechanism directly participates in CBDCA-induced basophil activation and that either CBDCA itself or a CBDCA-serum albumin conjugate can act as a complete antigen. In addition, IgE-dependent basophil activation by CBDCA was revealed to incorporate FcεRI overexpression, along all chains of FcεRI mRNA.

First, we showed that basophil activation was almost completely inhibited by pretreatment with wortmannin, a PI3-K-specific inhibitor. Previous studies also reported that wortmannin inhibited basophil activation when stimulated with an anti-IgE antibody, but not when N-formyl-L-methionyl-L-leucyl-phenylalanine was used as the basophil stimulator. Furthermore, other studies have shown that, in addition to high-affinity FcεRI, human basophils also express both activating (FcγRIIA) and inhibiting (FcγRIIB) low-affinity IgG receptors, as well as minute amounts of the low-affinity IgG receptor, FcγRIIB, while expressing neither FcγRIIA nor high-affinity IgG receptor (FcγRI). Moreover, PI3-K activation has also been reported to play distinct roles in FcγRIIA-mediated degranulation and the production of cytokines. However, FcγRIIA-dependent passive systemic allergy has generally been thought to be dependent on monocytes/macrophages and neutrophils, but not on mast cells and basophils. These reports are consistent with the idea that an IgE-FcεRI-mediated mechanism is primarily responsible for basophil activation by CBDCA.
**Original Article**

**Carboplatin-induced basophil activation**

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**Negative control**

HR (+)

| CD203c PE | MFI 107.9 |
| CD203c PE | MFI 100.5 |

HR (-)

| CD203c PE | MFI 99.8 |
| CD203c PE | MFI 100.5 |

Healthy (control) subject

| CD203c PE | MFI 24.8 |
| CD203c PE | MFI 24.9 |

**Positive control (anti-IgE mAb)**

HR (+)

| CD203c PE | MFI 435.9 |
| CD203c PE | MFI 384.0 |

HR (-)

| CD203c PE | MFI 100.7 |
| CD203c PE | MFI 107.0 |

**CBDCA (50 μg/mL)**

HR (+)

| CD203c PE | MFI 284.7 |
| CD203c PE | MFI 107.0 |

HR (-)

| CD203c PE | MFI 100.4 |
| CD203c PE | MFI 100.4 |

**Anti-IgE pAb**

HR (+)

| CD203c PE | MFI 160.8 |
| CD203c PE | MFI 326.0 |

HR (-)

| CD203c PE | MFI 70.4 |
| CD203c PE | MFI 58.7 |

**CRTH2 FITC**

HR (+)

| CD203c PE | MFI 26.7 |
| CD203c PE | MFI 26.7 |

HR (-)

| CD203c PE | MFI 24.2 |
| CD203c PE | MFI 24.2 |

**ΔMFI of CD203c**

R = 0.99

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study subjects with FITC-conjugated anti-human FcγRI 
basophils was performed by incubation of whole blood sample from
patients (HR(−)) and HR(+) patients' plasma with or without omalizumab treatment, or with HR(−) patients' plasma. In (a) and (b), flow cytometer charts for CD3 PBMCs are shown. Upregulation of CD203c on basophils was determined using a threshold that was defined as the expression level above which 2% of basophils in the negative control column fluoresce, on average. Mean fluorescence intensities (MFIs) indicated for binding levels of CD203c on basophils. (c) Correlation of response levels to CBDCA of sensitized basophils and of corresponding IgE donor patients' original basophils (n = 5). AMFI was calculated as the difference between the negative control and CBDCA-stimulated sample, with respect to the MFI of PE-labeled CD203c.

Fig. 4. Expression levels of FcγRI on basophils in hypersensitivity reaction (HR(−) and HR(+) patients. Flow cytometric analysis of FcγRI on basophils was performed by incubation of whole blood sample from study subjects with FITC-conjugated anti-human FcγRI α chain antibody. Histograms from a typical HR(−) (a) and HR(+) patient (b) are shown (unshaded histograms). An isotype control antibody was also used as a negative control (shaded histograms). ΔMFI is calculated as the difference in mean fluorescence intensity (MFI) between the FITC-labeled anti-human FcγRI α chain antibody and its isotype control. The ΔMFI is compared between HR(+) (n = 5) and HR(−) (n = 8) patients, as well as healthy subjects (n = 5). (d) The AMFI of PE-conjugated anti-CRTH2 (prostaglandin D2 receptor) antibody used as a reference protein is compared between the three groups. The horizontal line inside the graph represents the median value within each group. The expressions of FcγRI and CRTH was analyzed using two or three different samples from each patient. Differences between the three groups were analyzed using Dunnett's multiple comparison tests.

Fig. 5. Expression levels of α, β, and γ chains of FcγRI mRNA in the peripheral blood of hypersensitivity reaction (HR(−) and HR(+) patients. Total RNA was isolated from blood samples, and analyzed by real-time RT-PCR. Relative mRNA levels of FcγRIα, β, and γ chains were quantified relative to the expression levels of GAPDH. Data are presented as the mean ± SD. Statistical analyses between the two groups were carried out using the Mann-Whitney U-test.

It is commonly believed that IgE is largely responsible for the development of type I allergy, and the activation of basophils and mast cells is prevented by the dissociation of IgE from FcγRI.(22,23) We examined the involvement of CBDCA-specific IgE at the onset of CBDCA-induced severe HR using in vitro sensitization. Flow cytometric analyses using CRA1 and CRA2 showed that pretreatment with lactic acid almost completely dissociated IgE from FcγRI on basophils, without decreasing the number of FcγRI receptors. The decrease of IgE on basophils was reversed following IgE passive sensitization from HR(+) or HR(−) patients, and the specific binding of IgE and FcγRI was confirmed by the inhibitory effect of omalizumab. Interestingly, when IgE passive sensitization was applied to plasma from HR(+) patients, it induced the acquisition of reactivity to CBDCA in the basophils of a control subject, whose own IgE showed no previous response to CBDCA. However, this acquisition of reactivity did not occur in the plasma of HR(−) patients. Furthermore, we found that the pretreatment with omalizumab almost completely blocked the basophil activation by CBDCA in the previously mentioned sample sensitized by HR(+) patient plasma. Incidentally, this was the same result produced by the monoclonal anti-IgE antibody treatment. These results are the first conclusive evidence showing that a CBDCA-specific IgE participates in basophil activation by CBDCA and that an IgE-mediated pathway plays a key role in CBDCA-induced severe HR. It is currently thought that platinum drug-specific IgE directly recognizes the primary amine groups in CBDDCA and cisplatin.(13) Furthermore, in vitro basophil activation has been reported in a patient with cisplatin allergy.(24) Therefore, not only CBDCA but also cisplatin may induce severe HR, which requires an IgE-mediated activation of...
basophils. On the other hand, the molecular structure of oxalipatin does not contain a primary amine. We previously confirmed that specific basophil activation did not occur by the in vitro exposure to oxalipatin in five patients with a history of oxalipatin-induced HR (data not shown).

In this study, we showed that patients with CBDCA-induced severe HR had significantly higher levels of FcRI than the HR(−) group and healthy control subjects. In addition, we found that the expression levels of all chains of FcεRI mRNA were significantly elevated in the HR(+) patients compared with those in the HR(−) patients. We also noted that, among these chains, the β chain is expressed exclusively on basophils, while the α and γ chains are also expressed on monocytes and eosinophils to form trimers of FcεRI. Incidentally, it has been found that the γ chain, FeγRγ, is required for the expression and function of FcεRI as well as FcγRI, FcγRII, and FcεR (25,26). Therefore, it is well within reason that the level of β chain specifically expressed in basophils would be significantly higher in the HR(+) patients than in the HR(−) patients. In addition, the β chain has an important role in amplifying the expression and function of FcεRI (27,28). These results would seem to imply that a significant increase in FcεRI β-chain expression contributes substantially to the overexpression of FcεRI on basophils in HR(+) patients. It should be noted that, despite the fact that patients’ respective expression levels of FcεRI were found to be associated with the number of CBDCA treatments (Table 1), the actual pharmacokinetic association is thought to be relatively insignificant. This is because the expression levels of FcεRI in the patients without CBDCA-induced HR were comparable to that of the healthy subjects, who had no history of CBDCA treatment (Fig. 4c). Indeed, these results are consistent with previous reports of an overexpression of FcεRI in type I allergy due to ragweed and peanuts (29,30). Furthermore, in addition to circulating total IgE, FcεRI has also attracted much attention as a therapeutic target for IgE-dependent allergies (31).

Recently, a desensitization protocol for CBDCA treatment has been developed for patients with a history of CBDCA-induced HR, who are at a high risk of developing severe HR (5,32). As previously noted, a CBDCA-specific IgE was recently observed in patients with CBDCA-induced HR (13). Therefore, monitoring the pharmacodynamic changes of basophils including CBDCA-specific IgE, CD203c, and FcεRI expressions in these patients must be considered to be an essential part of the process. We believe that CBDCA-induced severe HR, which has been difficult to predict up to now, will come to be a controllable side-effect in the near future. Moreover, the reactivity of basophils against CBDCA usually declines after several months from the onset of severe HR, as previously indicated (12). Therefore, the basophil activation status following severe HR may also help identify patients for whom CBDCA is safe to administer, which is of extreme clinical significance for a large number of patients.

In conclusion, these results strongly imply that an IgE-dependent mechanism incorporating FcεRI overexpression participates in CBDCA-induced severe HR. Our results establish the relevance of monitoring the pharmacodynamic changes of basophils following repeated exposure to CBDCA and may aid in the development of a highly sensitive in vitro test for the detection of severe CBDCA-induced HR. Ultimately, we feel this work should be of considerable interest to healthcare workers concerned with the safe management of cancer chemotherapy.

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