Early prediction of triple negative breast cancer response to cisplatin treatment using diffusion-weighted MRI and 18F-FDG-PET

Huong Nguyen-Thu1 · Hirofumi Hanaoka2 · Takahito Nakajima1 · Aiko Yamaguchi2 · Tien Nguyen-Cong1 · A. Adhipatria P. Kartamihardja1 · Yoshito Tsushima1,3

Received: 23 July 2017 / Accepted: 15 January 2018 / Published online: 22 January 2018
© The Japanese Breast Cancer Society 2018

Abstract

Background We evaluated the potential of diffusion-weighted MRI (DW-MRI) and 18F-FDG-PET for the early prediction of a triple negative breast cancer (TNBC) response to cisplatin.

Methods Cisplatin-treated TNBC tumor-bearing mice were categorized as responders or non-responders based on the tumor growth rate. DW-MRI and 18F-FDG-PET were performed before and after treatment (day 0 and days 3 and 7, respectively). The average apparent diffusion coefficient value (ADCmean), the highest standardized uptake value (SUVmax), and the metabolic tumor volume (MTV) were measured. The ratios of each parameter relative to day 0 were calculated [ΔADCmean (day 3) and (day 7), ΔSUVmax (day 3) and (day 7), and ΔMTV (day 3) and (day 7), respectively]. Overall survival rates were compared based on the thresholds determined by these parameters.

Results Both the day 3 and day 7 ratios of ADCmean and MTV showed significant differences between the responder and non-responder groups, whereas the ratios of SUVmax did not. Mice with ΔADCmean (day 3) exceeding the threshold showed a longer overall survival rate. Mice with ΔSUVmax (day 7), ΔMTV (day 3), and ΔMTV (day 7) below the respective thresholds showed a longer overall survival rate.

Conclusions The ratios of ADCmean, SUVmax, and MTV have the potential to predict the therapeutic response and to screen non-responders in the ultra-early phase following cisplatin treatment in patients with TNBC.

Keywords Triple negative breast cancer · Early response prediction · Cisplatin · Diffusion-weighted MRI · 18F-FDG-PET

Introduction

Triple negative breast cancer (TNBC) is a refractory subtype of breast cancer due to the lack of therapeutic targets, such as estrogen receptors, progesterone receptors, and human epidermal growth factor receptor 2 [1, 2]. Among the few therapeutic options, the effectiveness of neoadjuvant chemotherapy (NAC) using platinum-based agents including cisplatin has been frequently reported [3, 4]. However, the cases responsive to cisplatin treatment are limited. Therefore, there arose a need to screen cisplatin non-responders immediately after the onset of each course of the treatment to refine the patient care.

Diffusion-weighted magnetic resonance imaging (DW-MRI) and 18F-fluorodeoxyglucose positron emission tomography (18F-FDG-PET) are the advanced imaging modalities which can evaluate the tumor response to NAC in the early phase [5–8]. The apparent diffusion coefficient (ADC) calculated by DW-MRI represents microstructural water diffusion,
which reflects tissue cellularity [9]. The previous studies in breast cancer patients showed that, in successful cases, the tumor showed an increased average ADC (ADC_{mean}) after one or two courses of NAC, while the tumor size remains unchanged [10, 11]. Utilizing the maximum standardized uptake values (SUV_{max}) or metabolic tumor volume (MTV), both of which reflect glucose metabolism, ^{18}F-FDG-PET can also detect viable tumor tissue before anatomical change become apparent [12, 13]. Although currently available reports set the early prediction point to ‘after several cycles of NAC’, it would be more beneficial if physicians could select the patients suitable for cisplatin therapy much earlier than that by providing patients with opportunities to try alternative therapeutic options.

To establish a clinically applicable early prediction method, a study should be implemented in a highly controlled setting, because various factors would affect the imaging results. However, it is challenging to apply these methods in patient cohorts simultaneously during cisplatin treatment, primarily because of the limited time frame of imaging, the therapeutic cost, and differences in the individual patients’ conditions. Molecular imaging in living animals allows us to organize studies of DW-MRI and ^{18}F-FDG-PET in controlled conditions such as the time, treatment protocol, and size or position of the tumor. Here, we carried out a longitudinal molecular imaging study using DW-MRI and ^{18}F-FDG-PET for the early prediction of tumor response to cisplatin by assessing the changes of ADC_{mean}, SUV_{max}, and MTV in a TNBC tumor-bearing mouse xenograft model.

Materials and methods

Preparation of tumor-bearing mice

All experimental protocols were approved by the local Laboratory Animal Care and Use Committee of Gunma University. A human TNBC cell line, MDA-MB-231, was purchased from the American-Type Culture Collection (ATCC, Manassas, VA, USA). Prior to injection, mice (BALB/c nu/nu, 5 weeks, Japan SLC, Hamamatsu, Japan) were anesthetized by isoflurane (Wako Pure Chemical, Osaka, Japan). The cell suspension (5 × 10^5 cells/mice) was implanted subcutaneously into the mid-lateral right flank of the mouse. Tumor volume was measured twice per week using a digital caliper; the volume (V) was calculated with this formula:

\[ V[\text{mm}^3] = 0.52 \times (\text{length[mm]}) \times (\text{width[mm]})^2. \]

Cisplatin therapy

After the tumor volume reached about 90 mm^3, mice were randomly divided into a no-treatment control group (n = 8) and a cisplatin treatment group (n = 14). A single maximum tolerated dose of cisplatin (10 mg/kg) was injected intraperitoneally [14]. Image-based observation of the tumor response using DW-MRI and ^{18}F-FDG-PET was performed 3 and 7 days after the treatment (days 3 and 7, respectively). Baseline images were acquired on day 0, before the cisplatin injection.

DW-MRI

DW-MRI was performed on a 1 Tesla animal MRI scanner (ICON, Bruker, Billerica, MA, USA). Mice were anesthetized during the whole process and placed in a prone position on the body coil. Following the localization scan, five continuous axial T2-weighted images (TR = 2000, TE = 85, slice thickness = 1 mm, matrix = 96 × 96) were obtained at the tumors. Subsequently, 5 slices of transverse DW-MRI (matrix = 96 × 96, NEX = 2, FOV = 30 × 30 mm, slice thickness = 1.5 mm) were obtained in the same positions with T2-weighted images using echo planar imaging, and diffusion maps were created with two b factors of 100 s/mm^2 (b0) and 500 s/mm^2 (b1). Geographic regions of interest (ROI) were drawn as large as possible to include the whole tumor using the EV insite software ver. 3.1 (PSP, Tokyo, Japan). The tumor margins were carefully excluded from the ROIs, and necrotic areas that exceeded one-quarter of the tumor in size were also excluded. ROIs were drawn twice for each tumor by two specialists individually. ADC_{mean} was manually calculated as follows: ADC_{mean} = –ln [(S1_{mean}–S0_{mean})/(b1–b0)] (S1_{mean} and S0_{mean}; the average of all voxels of DW-MRI intensity in b1 and b0, respectively) [15].

^{18}F-FDG-PET

The mice fasted for more than 8 h prior to ^{18}F-FDG injection. The mice were kept warm on a heat pad and were anesthetized during the whole process. 60 min after ^{18}F-FDG injection (5 MBq/mouse), images with matrix size = 128 × 128 × 159 without attenuation correction were obtained in 10 min acquisition time using a small animal PET scanner (Inveon; Siemens, Knoxville, TN, USA). Tumor uptake of ^{18}F-FDG (SUV_{max}) was quantified using an Inveon Research Workplace workstation (Siemens). Volumes of interest (VOI) were drawn manually to trace the contours of the tumor without correction for partial volume effects. VOIs were drawn twice for each tumor by two specialists individually. The maximum intensity projection (MIP) images were displayed using AMIDE 1.0.4 (Stanford University, Stanford, CA, USA).

MTV was automatically calculated using the PET VCAR software (GE Healthcare, Chicago, IL, USA). The same
three ROIs were selected to cover the surrounding area of the tumor, and then, the average SUV of the three areas (background $\text{SUV}_{\text{mean}}$) and its standard deviation (SD) were determined. MTV was defined as the total tumor volume of the region enclosed by the voxels with the threshold SUV: background $\text{SUV}_{\text{mean}}$ plus 2 SD [16]. MTV was calculated twice for each tumor by two specialists individually.

**Image analysis based on tumor growth rate**

Modelling of tumor growth was performed with an exponential fitting curve model to estimate responders and non-responders to the cisplatin treatment (Fig. 1). Mice that took longer to reach a 2.5-fold increase in tumor volume ($T_{2.5}$) than the longest period in the control group (9 days) were categorized as responders ($n = 7$), and mice that took 9 days or less to reach $T_{2.5}$ were categorized as non-responders ($n = 7$). The $\text{ADC}_{\text{mean}}$, $\text{SUV}_{\text{max}}$, and MTV values in each group on days 0, 3, and 7 were compared. The ratios of $\Delta\text{ADC}_{\text{mean}}$, $\Delta\text{SUV}_{\text{max}}$, and $\Delta\text{MTV}$ (day 3), and $\Delta\text{MTV}$ (day 7), respectively. The ratios of these parameters on day 7 to those on day 0 were calculated in the same manner [$\Delta\text{ADC}_{\text{mean}}$ (day 7), $\Delta\text{SUV}_{\text{max}}$ (day 7), and $\Delta\text{MTV}$ (day 7), respectively].

**Survival analysis based on the ratio of $\text{ADC}_{\text{mean}}$, $\text{SUV}_{\text{max}}$, and MTV**

The survival time was determined as the time from treatment to death or when the tumor volume reached a volume of 2000 mm$^3$. The Kaplan–Meier method was used to estimate survival as a function of time. Cisplatin-treated mice were divided into two groups based on whether the ratio of $\text{ADC}_{\text{mean}}$, $\text{SUV}_{\text{max}}$, or MTV was less than or greater than each threshold, which was defined as the average of each value in the control group minus 2 SD ($\text{SUV}_{\text{max}}$ and MTV) or plus 2 SD ($\text{ADC}_{\text{mean}}$), and we compared the survival time between these groups.

To provide an overview of the relationship between image-based analysis and the therapeutic response to the cisplatin treatment, we compared the $\text{ADC}_{\text{mean}}$, $\text{SUV}_{\text{max}}$, and MTV ratios, the time-to-tumor progression, which was defined as the time when the tumor reached 2.5 times its size measured on day 0 in accordance with a previous report [17], and the overall survival in cisplatin-treated mice.

**Statistical analysis**

Data were expressed as mean ± SD. GraphPad Prism software version 6.0 (GraphPad Software, La Jolla, CA, USA) or IBM SPSS software version 24 (IBM, Armonk, NY, USA) was used for statistical analysis. Differences in multiple groups were evaluated by one-way analysis of variance followed by Tukey’s multiple comparison. Survival differences were analyzed by the log-rank test, while the Mantel–Cox test was used to compare survival distributions across groups. Inter- or intra-observer variability was evaluated by intraclass correlation (ICC) analysis and Bland–Altman analysis. $p$ values less than 0.05 were considered significant.

**Results**

**Quality control**

There was no significant difference between average tumor volume of control, responder, and non-responder group at day 0 ($97.2 ± 31.7$, $83.4 ± 13.4$, and $97.8 ± 18.0$ mm$^3$, respectively). The tumor volume change of each mouse
was summarized in Supplemental Table S1. The relative tumor volume of responder group was significantly lower than those of non-responder group and control group at day 3 (1.08 ± 0.11, 1.44 ± 0.33, and 1.52 ± 0.25, respectively, \( p < 0.05 \)) and day 7 (1.38 ± 0.34, 2.12 ± 0.25, and 2.43 ± 0.34, respectively, \( p < 0.05 \)) after treatment. Images that passed our standard were used for further analysis. Individual ADC_{mean}, SUV_{max}, and MTV values are summarized in Supplemental Table S1. There was no intra- or interobserver variability for the measurement of ADC_{mean}, SUV_{max}, and MTV (ICC range 0.81–0.99). Bland–Altman plots for intra- or interobserver variability were summarized in Supplemental Fig. S1. The Bland–Altman plot showed strongly agreeing intra- or interobserver measurements.

**Efficacy of ADC_{mean} for predicting tumor response to cisplatin treatment**

Figure 2a, b shows typical MRI images of tumor in responder and non-responder mice. The responder group showed a significantly lower ADC_{mean} than the non-responder group before treatment (\( p < 0.05 \); Fig. 2c). No significant differences were observed in ADC_{mean} after treatment (days 3 and 7) between these two groups, although the ADC_{mean} value in the responder group had a tendency to increase over time. In contrast, the \( \Delta \text{ADC}_{\text{mean}} \) (day 3) and (day 7) showed significantly higher values in the responder group than in the non-responder group (\( p < 0.05 \) and \( p < 0.01 \), respectively; Fig. 2d), as these values reflected the change in ADC_{mean} from days 0 to 3 and from days 0 to 7, respectively.

**Efficacy of SUV_{max} for predicting tumor response to cisplatin treatment**

As shown in Fig. 3a, b, visual assessment of PET images was unable to distinguish the change of \(^{18}\text{F-FDG} \) uptake over time in responder and non-responder tumors. As illustrated in Fig. 3c, SUV_{max} the representative semi-quantitative parameter for \(^{18}\text{F-FDG-PET} \), clarified the difference in \(^{18}\text{F-FDG} \) uptake between the control, responder, and non-responder groups after treatment. On days 3 and 7, the responder group showed a significantly lower SUV_{max} than did the non-responder group (\( p < 0.05 \) and \( p < 0.01 \), respectively). However, \( \Delta \text{SUV}_{\text{max}} \) (day 3) and (day 7) showed no
significant differences in the responder and non-responder groups (Fig. 3d).

**Efficacy of MTV for predicting tumor response to cisplatin treatment**

Unlike SUV\textsubscript{max}, MTV clearly uncovered the difference in the metabolic tumor response to cisplatin treatment in each group. Both the control and non-responder groups showed prominent increases in MTV value over time, whereas the MTV slightly decreased in the responder group (Fig. 4a), even though tumor volume was increased. On days 3 and 7, the responder group showed a significantly lower MTV value than the non-responder group (\(p<0.05\) and \(p<0.01\), respectively). The ratio of MTV showed an even more notable difference between the groups, particularly \(\Delta\text{MTV}\).
(day 7), which showed no overlap between the responder and non-responder groups (0.76 ± 0.19 and 1.95 ± 0.54, respectively; *p* < 0.01; Fig. 4b).

**Efficacy of ADCmean, SUVmax, and MTV ratios for predicting overall survival of cisplatin-treated mice**

To determine the usefulness of DW-MRI and 18F-FDG-PET for the prediction of prognosis after cisplatin treatment, mice were divided into two groups based on the thresholds determined by the ratios of ADCmean, SUVmax, and MTV. As illustrated in Fig. 5, it turned out that all ratios except for ΔADCmean (day 7) could be prognostic factors for the overall survival of cisplatin-treated mice. Mice with higher ΔADCmean (day 3) ratios showed a significantly longer median overall survival than mice with lower ΔADCmean (day 3) ratios (*p* < 0.05, Fig. 5a). Mice with ΔSUVmax (day 7), ΔMTV (day 3), and (day 7) ratios below each threshold showed an extended mean overall survival (*p* < 0.05; Fig. 5c–e, respectively). Table 1 summarizes the ADCmean, SUVmax, and MTV ratios, relative tumor volume (ΔTumor volume), the time to progression, and the overall survival of each cisplatin-treated mouse. Overall, mice with ADCmean, SUVmax, and MTV ratios comparable to the control group exhibited no therapeutic response, while mice with better imaging results in multiple analysis (higher ΔADCmean and lower ΔSUVmax or ΔMTV than each threshold), such as mice Nos. 8, 9, 10, and 13, showed a higher therapeutic response.

![Fig. 5](image-url)
Discussion

We found that as early as days 3 and 7 post-treatment, DW-MRI with ADC\textsubscript{mean} and 18\textsuperscript{F}-FDG-PET with MTV successfully predicted the TNBC xenograft tumor response to cisplatin therapy. This is the first basic study that evaluated these parameters at this ultra-early phase after a single treatment with cisplatin, and it clearly demonstrated the possibility of early prediction.

The absolute value analysis revealed that pre-treatment ADC\textsubscript{mean}, and post-treatment SUV\textsubscript{max} and MTV were able to predict response to cisplatin treatment. The pre-treatment ADC\textsubscript{mean} in the non-responder group, which is consistent with the previous clinical studies [5, 18], indicates possible association between cellularity of tumor [19] and response to treatment. Despite these promising results, it is impractical to adopt absolute values as the early predictor in clinical practice, because they are variably affected by diverse factors.

By contrast, the post-treatment/pre-treatment value ratio, which includes the change over time relative to the baseline, should be more reliable than the value itself. Indeed, ADC\textsubscript{mean} ratios as well as MTV ratios showed significant differences between the responder and non-responder groups, as previously reported [5, 6, 13, 20]. ADC\textsubscript{mean} and MTV would have sharply reflected the decrease in the density and the number of viable tumor cells, respectively, that occurred shortly after cisplatin therapy. Not surprisingly, SUV\textsubscript{max} ratios did not show any differences within any of the groups, probably because SUV\textsubscript{max} is unable to reflect the whole tumor condition as it is a single voxel value [20, 21].

Despite the short observation period, the treatment efficacy influenced the relative tumor volumes. We consider that this difference would not affect the imaging parameter analysis, because not only ΔADC\textsubscript{mean} and ΔSUV\textsubscript{max} but also ΔMTV are tumor volume independent. Indeed, 5 in the 7 responder mice showed more than 30% decrease in MTV at day 7, despite approximately 50% increase in the tumor volume. This result confirmed the benefit of the use of ΔMTV, and also suggested that our MTV values reflected the tumor metabolism rather than the mere tumor volume.

We then analyzed potential predictive ability of these parameters using overall survival as an indicator of therapeutic response. Consistent with the abovementioned results, when ΔMTV (day 3), ΔMTV (day 7), ΔSUV\textsubscript{max} (day 7), or ΔADC\textsubscript{mean} (day 3) was employed as the thresholds, the overall survival rate showed significant differences between the two groups. ΔSUV\textsubscript{max} (day 3) in all treated mice exceeded the threshold perhaps, because 3 days were too short for the every part of the tumor to metabolically subside. If any highly active part remained, SUV\textsubscript{max} represents that part instead of the other less

<table>
<thead>
<tr>
<th>Mouse No.</th>
<th>ΔADC\textsubscript{mean}</th>
<th>ΔSUV\textsubscript{max}</th>
<th>ΔMTV</th>
<th>ΔTumor volume</th>
<th>Time to progression\textsuperscript{a}</th>
<th>Overall survival \textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (responder)</td>
<td>1.95</td>
<td>2.22</td>
<td>1.23</td>
<td>1.02</td>
<td>0.87</td>
<td>1.04</td>
</tr>
<tr>
<td>2 (non-responder)</td>
<td>0.61</td>
<td>0.69</td>
<td>1.10</td>
<td>1.21</td>
<td>1.60</td>
<td>2.36</td>
</tr>
<tr>
<td>3 (non-responder)</td>
<td>1.33</td>
<td>0.73</td>
<td>1.44</td>
<td>1.70</td>
<td>1.21</td>
<td>1.93</td>
</tr>
<tr>
<td>4 (non-responder)</td>
<td>0.60</td>
<td>0.36</td>
<td>1.04</td>
<td>1.02</td>
<td>1.31</td>
<td>1.56</td>
</tr>
<tr>
<td>5 (non-responder)</td>
<td>0.79</td>
<td>0.88</td>
<td>0.94</td>
<td>0.88</td>
<td>1.45</td>
<td>1.97</td>
</tr>
<tr>
<td>6 (non-responder)</td>
<td>0.76</td>
<td>1.03</td>
<td>0.80</td>
<td>0.62</td>
<td>1.03</td>
<td>1.22</td>
</tr>
<tr>
<td>7 (non-responder)</td>
<td>0.39</td>
<td>0.92</td>
<td>0.69</td>
<td>0.96</td>
<td>1.18</td>
<td>1.61</td>
</tr>
<tr>
<td>8 (responder)</td>
<td>1.72</td>
<td>1.34</td>
<td>0.72</td>
<td>0.64</td>
<td>0.58</td>
<td>0.64</td>
</tr>
<tr>
<td>9 (responder)</td>
<td>1.77</td>
<td>1.85</td>
<td>1.09</td>
<td>0.90</td>
<td>0.74</td>
<td>0.68</td>
</tr>
<tr>
<td>10 (responder)</td>
<td>2.47</td>
<td>2.51</td>
<td>0.86</td>
<td>0.64</td>
<td>0.77</td>
<td>0.54</td>
</tr>
<tr>
<td>11 (non-responder)</td>
<td>1.38</td>
<td>1.45</td>
<td>1.35</td>
<td>1.26</td>
<td>2.45</td>
<td>3.00</td>
</tr>
<tr>
<td>12 (responder)</td>
<td>1.55</td>
<td>0.96</td>
<td>1.08</td>
<td>1.02</td>
<td>0.94</td>
<td>1.07</td>
</tr>
<tr>
<td>13 (responder)</td>
<td>1.47</td>
<td>2.15</td>
<td>0.74</td>
<td>0.53</td>
<td>0.68</td>
<td>0.66</td>
</tr>
<tr>
<td>14 (responder)</td>
<td>0.89</td>
<td>1.10</td>
<td>0.82</td>
<td>1.07</td>
<td>0.98</td>
<td>0.67</td>
</tr>
<tr>
<td>Control mice average</td>
<td>0.91</td>
<td>0.74</td>
<td>1.29</td>
<td>1.16</td>
<td>1.71</td>
<td>2.58</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Time-to-tumor progression was defined as the time when the tumor reached a size 2.5 times greater than its size on day 0

\textsuperscript{b}The threshold was defined as the average of each value in the control group plus 2 SDs (ΔADC\textsubscript{mean}) or minus 2 SDs (ΔSUV\textsubscript{max} and ΔMTV)

Underlined values are higher than the threshold for ΔADC\textsubscript{mean} or lower than the threshold for ΔSUV\textsubscript{max} or ΔMTV

\[ \Delta S_{\text{MTV}} \text{ (day 3)} \]
active parts of the tumor. The lack of significance in the ΔADC_{mean} (day 7)-based analysis may be attributable to the experimental error in manual ROI delineation in small animal tumor. However, the ADC_{mean} ratio is expected to be a valuable parameter in clinical practice [5, 22], considering the better qualities of the clinical MRI and analytical software.

Table 1 highlights the complementary roles of ADC_{mean}, SUV_{max}, and MTV to increase the diagnostic accuracy, since none of them could completely distinguish the responder and non-responder mice. Our results suggest that physicians may potentially be able to predict therapeutic response even after only one cycle of chemotherapy, which is much shorter than those of the currently reported methods [5, 12]. Although imaging conditions, the thresholds, and observation interval should be optimized, it is worth investigating the predictive ability of these parameters in breast cancer patients undergoing NAC therapy.

There were some limitations to this study. We used only one TNBC cell line, whereas clinical TNBC is a heterogeneous disease, and thus would respond differently to the chemotherapy. However, there is a great potential that the ratios of ADC_{mean}, SUV_{max}, and MTV could predict early response even for the heterogeneous disease like TNBC, since these values reflect the changes in the cellular activity and/or metabolism occurs as a result of chemotherapy, which is independent of genetic factors. We evaluated cisplatin mono-therapy to simplify the analysis by avoiding overlap effects from other drugs. The applicability of our results to combination chemotherapy in clinical practice should be the subject of future research. Although the usefulness of ADC_{mean} and MTV was validated, the sample size was small. Increasing the number of mice can be expected to yield more precise results. Further optimization is necessary to apply our method in clinical practice, because it is impractical to perform imaging multiple times in a short term after treatment, and imaging condition for animals is distinct from that for patients.

In conclusion, the ratios of ADC_{mean} and MTV successfully differentiated the responder and non-responder groups, and the ratios of ADC_{mean}, SUV_{max}, and MTV enabled early prediction of the overall survival rate after cisplatin therapy. Thus, these parameters have the potential to predict early therapeutic response and to screen non-responders in the ultra-early phase following cisplatin treatment in patients with TNBC.

Compliance with ethical standards

Conflict of interest There is no conflict of interest to declare.

References