Research Article

Pattern of F-18 FDG Uptake in Colon Cancer after Bacterial Cancer Therapy Using Engineered Salmonella Typhimurium: A Preliminary In Vivo Study

Ari Chong,1,2 Dinh-Huy Nguyen,2,3 Hyeon Sik Kim,4 June-Key Chung,2,5 and Jung-Joon Min2,3

1Department of Nuclear Medicine, Chosun University Medical School, Gwangju, Republic of Korea
2Department of Nuclear Medicine, Chonnam National University Medical School, Jeonnam 519-763, Republic of Korea
3Department of Nuclear Medicine, Chonnam National University Hwasun Hospital, Jeonnam 519-763, Republic of Korea
4Medical & Bio Photonics Research Center, Korea Photonics Technology Institute, Gwangju, Republic of Korea
5Department of Nuclear Medicine, Seoul National University College of Medicine, Republic of Korea

Correspondence should be addressed to Jung-Joon Min; jjmin@jnu.ac.kr

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Purpose. Bacterial cancer therapy (BCT) research using engineered Salmonella typhimurium has increased in recent years. 2-Deoxy-2[18F]fluoro-D-glucose positron emission tomography (FDG PET) is widely used in cancer patients to detect cancer, monitor treatment responses, and predict prognoses. The aim of this pilot study was to investigate FDG uptake patterns in a mouse tumor model after BCT.

Procedures. BCT was performed via the intravenous injection of attenuated S. typhimurium (SLΔppGpp/lux) into female mice bearing a tumor (derived from CT26 murine colon cancer cells) in the right thigh. 18F-FDG PET images acquired before BCT and at different time points after BCT. In vivo bioluminescence imaging confirmed bacterial presence in the tumor. The tumor volume, standardized uptake value (SUV) of FDG (SUVmax and SUVmean), early SUV reduction%, and normalized tumor volume change were analyzed.

Results. Early after BCT (1 or 2 days post-injection (dpi)), FDG tumor uptake decreased in 10 out of 11 mice and then increased at later stages. FDG uptake before BCT was correlated with normalized tumor volume change after BCT. Early FDG reduction% after BCT was correlated with normalized volume change after BCT.

Conclusions. Early after BCT, FDG tumor uptake decreased and then increased at later stages. The higher the FDG tumor uptake before BCT, the better the BCT response. FDG uptake patterns were related to tumor volume change after BCT. Therefore, FDG uptake was a good candidate for evaluating BCT.

1. Introduction

Despite recent and considerable advances in cancer therapy, several major treatment limitations still persist, including drug resistance, limited effective treatment of metastatic tumors, and toxicity toward normal tissue [1]. As a result, alternative treatments have been suggested. Bacterial cancer therapy (BCT) is one such alternative; while its origins date back approximately 150 years, contemporary therapies involve the use of Streptococcus pyogenes to treat inoperable sarcoma [2]. Several other bacterial strains are also used for BCT [2–9], including Escherichia coli [5], Clostridium [10], Salmonella [3], Listeria [11], and Bifidobacterium [12], where the bacteria tend to accumulate in a broad range of tumors [7]. Several studies have reported tumor suppression after BCT [5, 6, 13]. Importantly, BCT studies are no longer limited to animal models; several clinical phase I–III trials have been conducted in cancer patients [9, 14–19].

A common bacteria strain for BCT is attenuated Salmonella typhimurium which is defective in ppGpp synthesis (AppGpp S. typhimurium) [20]. The bacteria displays a 100,000–1,000,000-fold increased median lethal dose [21]. Zheng et al. reported
that ΔppGpp S. typhimurium was more than 10,000-fold higher in tumor tissue when compared with other organs [8]. Critically, bacteria injected during BCT were rapidly cleared from internal organs [5], while after tumor targeting, BCT bacteria were not released from tumors and did not re-enter the blood circulation [22].

Our previous S. typhimurium in vivo research showed that tumor growth after BCT exhibited two phases: tumor growth suppression for 1–10 days, but regrowth thereafter [23]. The trajectory of individual tumor growth after BCT often shows significant variations in treatment responses [24]. However, no modalities are currently available to predict the tumor suppression effects of BCT; therefore, in vivo imaging techniques are required to monitor and predict BCT effects.

The glucose analog, 2-deoxy-2\(^{[18F]}\)fluoro-D-glucose (FDG) is widely used as a radiotracer for positron emission tomography (PET) and typically shows high FDG uptake in malignant tumors due to increased anaerobic glycolysis [25]. In patients with various cancers, FDG PET/computed tomography (PET/CT) strategies are used for tumor detection, monitoring treatment responses, and prognosis predictions. FDG also accumulates in activated immune cells [26], whereas BCT mechanisms trigger immune responses [7, 23, 27]. Therefore, based on these uptake modalities, FDG appears to be a good candidate for evaluating BCT. To our knowledge, no previous reports have analyzed FDG uptake changes in tumors after BCT.

We therefore investigated FDG uptake patterns in tumors in a murine tumor model using attenuated S. typhimurium during and after BCT.

2. Materials and Methods

2.1. Study Overview. This study was performed using two separate trials: experiment A (\(n = 6\) mice) and B (\(n = 5\) mice). We used this approach to avoid overburdening individual animals with different preparation procedures, such as fasting and general anesthesia (Figure 1). After generating colon cancer animal model (murine CT26 colon adenocarcinoma cells, BALB/c female mice, 6-weeks-old), BCT was initiated using a single intravenous injection of bacterial cells via the tail vein (bacterial dose = \(4 \times 10^7\) CFU/mouse in 100μL PBS). Two trials were designed to avoid animal preparation issues such as fasting and general anesthesia. In trial A (\(n = 6\)), FDG PET/CT images were acquired at 0 dpi, 2 dpi, 10 dpi, and 16 dpi (a). In trial B (\(n = 5\)), FDG PET/CT images were acquired at 0 dpi, 1 dpi, 4 dpi, 7 dpi, and 15 dpi (b). In vivo BLI confirming the presence of tumor-targeting bacteria was performed at 2 dpi, immediately after PET/CT in trial A, and immediately after each PET/CT in trial B. Abbreviations: FDG, 2-deoxy-2\(^{[18F]}\)fluoro-D-glucose; dpi, days post-injection; SUV\(_{\text{max}}\), maximum standardized uptake value; SUV\(_{\text{mean}}\), mean standardized uptake value; PBS, phosphate-buffered saline; CFU, colony-forming units; BLI, bioluminescence imaging.
Type Culture Collection, CRL-2638) was xenografted into animals. Cells were cultured in Dulbecco’s Modified Eagle Medium at 37°C in 5% CO₂ and used under specific pathogen-free conditions. A tumor was generated via the subcutaneous injection of 1 × 10⁶ CT26 cells in phosphate buffered saline (PBS) into the right thigh of each mouse. BCT was then initiated when the tumor volume reached approximately 180 mm³. Tumor length (L), width (W), and depth (D) dimensions were measured using a single caliper (mm³) by one researcher. Tumor volume (V) is calculated using the formula: 

\[ V = \frac{L \times W \times W}{2} \]

2.3. The Bacterial Strain and BCT. Attenuated S. typhimurium defective in ppGpp synthesis (RelA::cat, spoT::kan) and expressing the bacterial luciferase (lux) operon (SH2037) (SLppGpp/lux) [28, 29] were used for BCT, which was performed using a one-time intravenous injection via the tail vein (bacterial dose = 4.5 × 10⁷ colony-forming units [CFU]/mouse in 100 μL PBS [29]).

2.4. PET/CT Scanning. Six hours before the FDG injection, the mice were caged separately and all food and sawdust removed. Mice were masked and anesthetized using 2.5% isoflurane in air. Before the FDG injection, serum glucose levels were measured using a glucometer (Accu-Chek® Performa, Roche Diagnostics, Indianapolis, IN, USA) to ensure a fasting status. Then, ¹⁸F-FDG (7.4 MBq) was injected via the tail vein. After 1 h, PET/CT scanning was performed except head area. We used a microPET scanner (Inveon, Siemens Medical Solutions, Knoxville, TN, USA). Acquired images were reconstructed using a three-dimensional ordered-subset expectation maximization algorithm, with four iterations. The reconstructed pixel size was 0.78 mm in axial and transverse directions (128 × 128 pixels in each of 159 transverse slices). Data normalization, decay corrections, and dead time corrections were also performed. PET/CT images were analyzed using PMOD v.3.310 (PMOD Technologies, Ltd., Zurich, Switzerland). FDG uptake in tumors was measured as the standardized uptake value (SUV) [30, 31]. After manually drawing a region-of-interest (ROI) in the tumor at the highest uptake point, maximum standardized uptake (SUVmax) and mean standardized uptake values (SUVmean) were obtained using PMOD.

2.5. In Vivo Bioluminescence (BLi) Imaging. In vivo BLi confirmed successful injection and SLppGpp/lux accumulation in tumors. Images were acquired after PET/CT scanning on the same day. To capture images, an IVIS-100 imaging system equipped with a charged coupled device camera (Caliper Life Sciences, Waltham, MA, USA) was used. After anesthetization with 2.5% isoflurane in air, mice were placed in the light-tight chamber of the IVIS-100 system. Living image software v.2.25 (Caliper Life Sciences) was used for image acquisition and processing. In trial A, in vivo BLi was acquired at 2 dpi. In trial B, in vivo BLi was performed on the same day as PET scanning and in vivo BLi counts were also acquired.

2.6. Analysis of Variables. SUVmax, SUVmean, and tumor volume values at each time point were used for analyses. The following formula is used:

\[ SUV = \frac{\text{decay corrected activity} [\text{kBq}] / \text{mL of tissue volume}}{\text{injected } F - 18 \text{ FDG activity} [\text{kBq}] / \text{g of body mass}} \]

A circular ROI was drawn over the FDG uptake area in the tumor, after which SUVmax and SUVmean values were obtained.

In addition, early SUVmax reduction%, early SUVmean reduction%, normalized tumor volume change, and growth ratio are calculated as follows:

\[ \text{Early SUVmax reduction\%} = 100 \times \frac{\text{SUVmax}(0 \text{ dpi}) - \text{SUVmax}(1 \text{ or } 2 \text{ dpi})}{\text{SUVmax}(0 \text{ dpi})} \]

\[ \text{Early SUVmean reduction\%} = 100 \times \frac{\text{SUVmean}(0 \text{ dpi}) - \text{SUVmean}(1 \text{ or } 2 \text{ dpi})}{\text{SUVmean}(0 \text{ dpi})} \]

\[ \text{Normalized volume change}(6 \text{ dpi}) = \frac{\text{tumor volume}(0 \text{ dpi}) - \text{tumor volume}(6 \text{ dpi})}{\text{tumor volume}(0 \text{ dpi})} \]

\[ \text{Normalized volume change}(16, 17 \text{ dpi}) = \frac{\text{tumor volume}(0 \text{ dpi}) - \text{tumor volume}(16 \text{ or } 17 \text{ dpi})}{\text{tumor volume}(0 \text{ dpi})} \]

\[ \text{Growth ratio}(x \text{ dpi}) = \frac{\text{tumor volume at } x \text{ dpi}}{\text{tumor volume at } 0 \text{ dpi}} \]

The early SUVmax reduction% calculation was the earliest measurement after BCT; thus, SUVmax at 2 dpi was used for trial A and SUVmax at 1 dpi for trial B. The same acquisition times were used for early SUVmean reduction%. To analyze the effects of BCT, early (6 dpi) and late (16 or 17 dpi) time points were used, and from growth graphs, the
Figure 2: Three representative FDG PET/CT images after bacterial cancer therapy (BCT) using engineered Salmonella typhimurium. A tumor was generated in the right thigh of each mouse (BALB/c female mice, 6-weeks-old) using murine CT26 colon adenocarcinoma cells. After the tumor volume reached at least 180 mm³, BCT was performed by the intravenous injection of bacterial cells via the tail vein (bacterial dose = 4.5 × 10⁷ CFU/mouse in 100 μL PBS). FDG PET/CT images were acquired before and after BCT. Coronal PET/CT fusion images are shown. FDG uptake was measured as SUVmax. White arrows indicate FDG uptake in tumors before BCT (a–c). Yellow arrows (a & b) indicate decreased FDG uptake 1 day after BCT. The red arrow (c) indicates a tumor showing no decrease in FDG uptake after BCT. Abbreviations: FDG, 2-deoxy-2-[¹⁸F]fluoro-D-glucose; dpi, days post-injection; SUVmax, maximum standardized uptake value; SUVmean, mean standardized uptake value; PBS, phosphate-buffered saline. CFU, colony-forming units.
Figure 3: Continued.
3.1. General Features before BCT. Between trials A and B, we observed no significant differences in tumor volume (0 dpi). Among the 11 mice, two died during the study: A5 at 2 dpi and A3 at 16 dpi (size measurements were performed until 12 dpi). Tumor volume at 0 dpi was not correlated with SUVmax \( (p > 0.5) \) or SUVmean \( (p > 0.5) \) at 0 dpi.

3.2. Tumor Growth and BLi after BCT. In vivo BLi confirmed successful SLΔppGpp/lux accumulation in all mice tumors. After BCT, tumor growth was suppressed until 6–9 dpi in most mice, with growth then recommencing (Figures 2 and 3). However, in a1 and a3 mice, tumors were not suppressed and continued to grow (Figure 2(a)).

In vivo BLi detected SLΔppGpp/lux accumulation signal in tumors until 10 dpi, whereas no signals were detected at 15 or 16 dpi. In trial B, counting by optical signal was performed. Signals from in vivo BLi (1 dpi) correlated with FDG uptake in the tumor before BCT (SUVmax 0 dpi, rho = 0.95 and \( p = 0.0001 \); with SUVmean 0 dpi, rho = 0.67 and \( p = 0.0002 \)). However, optical signals from in vivo BLi were not correlated with tumor volume (0 dpi), volume change, tumor growth ratio, early SUVmax reduction%, or early SUVmean reduction% \( (p > 0.1) \).

In a separate experiment, we enumerated bacterial accumulation in tumor after intravenous injection of SLΔppGpp/lux \( (4.5 \times 10^7 \text{ CFU/mouse}) \). More than \( 10^8 \text{ CFU/gram} \) of SLΔppGpp/lux were accumulated in the tumor at 1, 3, and 5 dpi (Figure S1, electronic supplementary material (ESM)).

3.3. FDG Uptake Patterns in Tumors after BCT. FDG uptake in tumors decreased in the early days after BCT (1 or 2 dpi) in 10 of 11 mice, but it increased in later days. In only one mouse (a3), FDG uptake did not decrease in the early days after BCT (Figure 3(c)).
Between trials A and B, we observed no significant differences in hepatic FDG uptake and lung FDG uptake (Figure S2, ESM).

3.4. Correlations between FDG Uptake before BCT and Treatment Responses. During treatment responses, we analyzed normalized tumor volume change (Figure 4). At 6 dpi, both pre-BCT SUVmax and SUVmean indices in tumors correlated with normalized volume change after BCT (rho = 0.697 and p = 0.0251 and rho = 0.818 and p = 0.0038, respectively, Figures 4(a) and 4(b)). This meant that the higher the FDG uptake before BCT, the greater the tumor suppression. However, at 16 or 17 dpi, only the SUVmean value (rho = 0.8, p = 0.0096) was correlated with a normalized tumor volume change (Figures 4(c) and 4(d)).

Tumor volume at 0 dpi did not correlate with normalized tumor volume change at 6 dpi (p > 0.4) or at 16 or 17 dpi (p > 0.5, Figure S3, ESM). Neither SUVmax nor SUVmean at 0 dpi was correlated with tumor volume at 6 dpi (p > 0.4) or at 16 or 17 dpi (p > 0.05, Figure S4, ESM).

3.5. Correlations between FDG Uptake after BCT and Treatment Responses. After BCT, FDG uptake in tumors decreased in the early days (1 or 2 dpi). We calculated this using early SUVmax reduction% and early SUVmean reduction%. We also observed a significant correlation between normalized tumor volume change and early SUV% (Figure 5). At the early response time point (6 dpi), both early SUVmax reduction% and early SUVmean reduction% were correlated with normalized tumor volume change (rho = 0.685 and p = 0.0289 for SUVmax reduction% and rho = 0.891 and p = 0.0005 for SUVmean reduction%; Figures 5(a) and 5(b)). At the late response time point (16 or 17 dpi), both early SUVmax reduction% and early SUVmean reduction% were correlated with normalized tumor volume change (rho = 0.733 and p = 0.0246 for SUVmax reduction% and rho = 0.917 and p = 0.0005 for SUVmean reduction% and p = 0.0038, respectively, Figures 4(a) and 4(b)). This meant that the higher the FDG uptake before BCT, the greater the tumor suppression. However, at 16 or 17 dpi, only the SUVmean value (rho = 0.8, p = 0.0096) was correlated with a normalized tumor volume change (Figures 4(c) and 4(d)).

Tumor volume at 0 dpi did not correlate with normalized tumor volume change at 6 dpi (p > 0.4) or at 16 or 17 dpi (p > 0.5, Figure S3, ESM). Neither SUVmax nor SUVmean at 0 dpi was correlated with tumor volume at 6 dpi (p > 0.4) or at 16 or 17 dpi (p > 0.05, Figure S4, ESM).

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Tumor volume at 0 dpi did not correlate with normalized tumor volume change at 6 dpi (p > 0.4) or at 16 or 17 dpi (p > 0.05, Figure S4, ESM).
**Figure 5**: Correlations between treatment responses and early FDG uptake changes after bacterial cancer therapy (BCT) using engineered *Salmonella typhimurium* in a CT26 colon cancer cell line mouse model. Treatment responses were determined at two time points: early (6 dpi) and late (16 or 17 dpi). Normalized tumor volume change is calculated using tumor volume (0 dpi) – tumor volume (x dpi)/tumor volume (0 dpi). Early SUV reduction% was defined as 100 × (SUV at 0 dpi – SUV at 1 or 2 dpi)/SUV at 0 dpi. A higher early SUV reduction% meant a larger decrease in FDG uptake after BCT (1 or 2 dpi). (a) Early SUVmax reduction% and normalized volume change at 6 dpi. (b) Early SUVmean reduction% and normalized volume change at 6 dpi. (c) Early SUVmax reduction% and normalized volume change at 16 or 17 dpi. Abbreviations: FDG, 2-deoxy-2-[18F]fluoro-D-glucose; dpi, days post-injection; SUVmax, maximum standardized uptake value; SUVmean, mean standardized uptake value; early SUVmax reduction%, 100 × (SUV max at 0 dpi – SUV max at 1 or 2 dpi)/SUVmax at 0 dpi; early SUVmean reduction%, 100 × (SUV mean at 0 dpi – SUV max at 1 or 2 dpi)/SUVmean at 0 dpi.

reduction%; Figures 5(c) and 5(d)). Taken together, these data indicated that a reduction in FDG uptake in the early days after BCT predicted BCT outcomes.

From tumor volume analysis at 6 dpi, both early SUVmax reduction% (rho = −0.738 and p = 0.0149) and early SUVmean reduction% (rho = −0.817 and p = 0.0039) were negatively correlated with tumor volume. From analyses at 16 or 17 dpi, both early SUVmax reduction% (rho = −0.733 and p = 0.0246) and early SUVmean reduction% (rho = −0.833 and p = 0.0053) were negatively correlated with tumor volume (Figure S5, ESM).

**4. Discussion**

To our knowledge, this is the first pilot study to analyze FDG uptake changes in tumors after BCT. We observed several unique FDG uptake features: (1) Tumor FDG uptake decreased in the first 2 days after BCT and then increased, before tumor graft re-growth; (2) FDG uptake before BCT was correlated with normalized tumor volume change after BCT; and (3) early FDG reduction% after BCT was correlated with normalized tumor volume change after BCT.

The mechanisms underlying tumor-targeting bacteria may involve the chemotactic system [32–34], inflammatory cytokine-mediated dilation of tumor blood vessels [35, 36], and immune-privileged tumor environments [7, 37, 38]. However, the mechanism underpinning the therapeutic effects of BCT using *Salmonella* species is unclear. *Salmonella* species are believed to kill tumor cells by apoptosis and/or autophagy via alterations in host antitumor immune responses or nutrient deprivation [1]. *Salmonella* also activates the inflammasome pathway via damage signal release from cancer cells and macrophages [39]. These effects may contribute to the BCT therapeutic effects of *Salmonella*.

FDG is not a specific radiotracer for apoptosis. However, BCT mechanisms are not simply limited to apoptosis. A recent
This pilot study had some limitations. The number of study animals was small. Further imaging studies are underway to compare the characteristics of different radiotracers such as FDG and fluorodeoxyglucose in BCT. Also, we did not perform in vitro histological and genomic analyses before and after BCT. However, our study was exclusively focused on the analysis of FDG uptake patterns in BCT. Further research investigating in vitro molecular genomic changes following BCT is warranted.

5. Conclusions

This was the first study investigating FDG uptake patterns in BCT using attenuated S. typhimurium. Early after BCT, FDG tumor uptake decreased and then increased at later stages. The higher the FDG uptake before BCT, the better the BCT response, and FDG uptake patterns were related to tumor volume change after BCT. Therefore, FDG uptake appears to be a good candidate for BCT evaluating BCT.

Data Availability

The data is available on request.

Ethical Approval

All applicable institutional and/or national guidelines for the care and use of animals were followed. All animal experiments were conformed to the Chonnam National University Animal Research Committee protocols.

Conflicts of Interest

All authors declare no conflict of interest associated with the work in this study.

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Supplementary Materials

Figure S1: accumulation of engineered Salmonella typhimurium in a CT26 colon cancer model. Tumor targeting of engineered S. typhimurium (SLppGpp-lux) in a CT26 colon cancer model was confirmed by in vivo BLI from 1 dpi after BCT. Data are showing bacterial load colonisation efficiency at the tumor after BCT in a CT26 colon cancer model. Figure S2: comparison of tumor volume and liver and lung FDG uptake before BCT in a CT26 colon cancer model between trial A and B. Figure S3: correlation between pretreatment tumor volume and tumor FDG uptake before treatment and treatment results in a CT26 colon cancer model. Figure S4: correlation between pretreatment FDG uptake and tumor volume after BCT in a CT26 colon cancer model. Figure S5: correlation between early SUV reduction% and tumor volume after BCT in a CT26 colon cancer model.
Figure S6: changes in FDG uptake in liver and lung before and after BCT in a CT26 colon cancer model. (Supplementary Materials)

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