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Direct Detection and Quantification of Abasic Sites for In Vivo Studies of DNA Damage and Repair

Yanming Wang¹, Lili Liu², Chunying Wu¹, Alina Bulgar², Eduardo Somoza¹, Wenxia Zhu¹, and Stanton L. Gerson²

¹Department of Radiology, Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44122

²Department of Hematology and Oncology, Case Comprehensive Cancer Center, Case Western Reserve University, Cleveland, OH 44122

Abstract

Use of chemotherapeutic agents to induce cytotoxic DNA damage and programmed cell death is a key strategy in cancer treatments. However, the efficacy of DNA-targeted agents such as temozolomide is often compromised by intrinsic cellular responses such as DNA base excision repair (BER). Previous studies have shown that BER pathway resulted in formation of abasic or apurinic/apyrimidinic (AP) sites, and blockage of AP sites led to a significant enhancement of drug sensitivity due to reduction of DNA base excision repair. Since a number of chemotherapeutic agents also induce formation of AP sites, monitoring of these sites as a clinical correlate of drug effect will provide a useful tool in the development of DNA-targeted chemotherapies aimed at blocking abasic sites from repair. Here we report an imaging technique based on positron emission tomography (PET) that allows for direct quantification of AP sites *in vivo*. For this purpose, positron-emitting carbon-11 has been incorporated into methoxyamine ([¹¹C]MX) that binds covalently to AP sites with high specificity. The binding specificity of [¹¹C]MX for AP sites was demonstrated by *in vivo* blocking experiments. Using [¹¹C]MX as a radiotracer, animal PET studies have been conducted in melanoma and glioma xenografts for quantification of AP sites. Following induction of AP sites by temozolomide, both tumor models showed significant increase of [¹¹C]MX uptake in tumor regions in terms of radioactivity concentration as a function of time, which correlates well with conventional MRP-based bioassays for AP sites.

Keywords

abasic sites; Apurinic/apyrimidinic (AP) sites; TMZ; DNA; Tumor; PET Imaging

Requests for reprints: Yanming Wang, 11100 Euclid Ave, Department of radiology, University Hospital of Cleveland, Cleveland, Ohio, 44106. Phone: 216 844 3288; Yanming.wang@case.edu.

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Introduction

Current cancer therapies rely heavily on radiation and DNA damaging agents to induce both cytotoxic DNA changes and programmed cell death (1). Cytotoxic DNA damages include bulky lesions, inter-strand crosslinks, double-strand breaks, interruption of transcription, replication, and chromosome segregation (2). These lesions interfere with DNA metabolic processes and inhibit normal cell and tumor growth.

Among the DNA-targeted chemotherapeutic agents is temozolomide (TMZ, 3, 4-dihydro-3-methyl-4-oxoimidazo [5, 1-d]tetrazine-8-carboxamide), which has been widely utilized in cancer therapies (3). The drug easily penetrates the blood-brain barrier making it particularly useful in treating malignant brain tumors (4). It has shown promising anti-tumor activity in recent clinical trials (5–8). However, drug resistance remains a critical consequence, often causing treatment failure in clinical use (9). A major resistance factor is the presence of elaborate mechanisms of DNA repair (10). This resistance is based on the fact that TMZ reacts with DNA forming O⁶-methylguanine (O⁶mG), 7-methylguanine (N7mG), and 3-methyladenine (N3mA) DNA adducts that are repaired by three major mechanisms.

The O⁶mG DNA adduct is a cytotoxic and genotoxic lesion mainly repaired by O⁶-methylguanine DNA- methyltransferase (MGMT) (11). Cell death from O⁶mG adducts is also promoted by mismatch repair (MMR)(12). Deficiency in MMR is associated with pronounced resistance to TMZ (13). Meanwhile, N7mG, the dominant lesion formed by TMZ, and N3mA DNA adducts are removed by the base excision repair (BER) pathway (14–16). Efficient BER minimizes the impact of these lesions in normal and tumor cells.

Among the DNA repair pathways, BER is the predominant pathway for repair of damaged single bases with small modifications (17, 18). BER plays an important role in drug resistance observed in TMZ treatment (19). This is due to the fact that a significant proportion of DNA damages induced by TMZ is repaired by the BER pathway. Only those methylated DNA adducts that fail to be repaired will exhibit toxicity. Thus, intrinsic cellular resistance to TMZ results from robust repair by the BER pathway.

In the BER pathway, apurinic/apyrimidinic (AP) sites are generated through loss of damaged bases (purine or pyrimidine) in the DNA strands. AP sites are the most common damages induced by TMZ and related alkylating agents. However, these chemotherapies often exert minimal toxicity in the presence of efficient BER. Recent evidence indicates that AP sites can act as Topo II poisons that significantly increase the physiological concentration of Topo II-DNA cleavage complexes (20, 21). When AP sites are located within Topo II cleavage sites, they remarkably stimulate Topo II-mediated DNA fragmentation (22, 23), leading to lethal toxicity. Under normal conditions, the AP site is recognized by AP-endonuclease in the BER pathway and repaired with reinsertion of the proper base. Because persistent AP sites bound by Topo II would be highly toxic, AP sites are potentially an important target for cancer therapy. We have recently identified that a number of other agents also induce AP sites and activate the BER pathway. These include fludarabine, which is directly incorporated into DNA and is repaired by a DNA glycosylase, and other agents such as

premetrexate, that alter nucleotide pools, resulting in insertion of uracil which is recognized by the uracil-D-glycosidase.

Since AP sites are key intermediates in the BER pathway, their quantitative and dynamic measurement in cellular DNA is crucial for efficacy evaluation of therapeutic treatments. Currently, assessment of AP sites can only be achieved *in vitro* using extracted DNA from tumor tissues. However, this is a relative measure based on chemiluminescence using aldehyde reactive probe (ARP). It is also an invasive assay and cannot be used to directly monitor AP-site content in tumors. Direct imaging and quantitative assessment of AP sites *in vivo* will provide a platform technology for efficacy evaluation of a variety of DNA-targeted chemotherapies – all those that produce AP sites and invoke BER. Understanding the dynamic of AP site formation and repair will allow physicians and researchers to determine optimal dose strategies of single and combination treatment schedules. Furthermore, with the advent of agents to block BER, direct imaging of AP sites will help to determine the optimal dose schedule to potentiate drug administration based on persistence of AP sites. For instance, if one agent induces AP sites, and another blocks BER repair, while a third induces Topo II, understanding the relationship between these events may considerably impact therapeutic efficacy. In addition, direct imaging of AP sites will also play an important role in drug discovery. It will facilitate screening of new agents that are designed to either induce AP sites in tumor cells or block AP sites from DNA repair.

Studies from our group and others have shown that methoxyamine (MX) potently binds to AP sites (24, 25). The resulting blockage of AP sites overcomes the intrinsic resistance of tumor cells to chemotherapeutic agents (26, 27). MX was first introduced as a tool to study the BER pathway in 1985 by Liuzzi and Talpaert-Borle (28). Since then, MX has been reported and studied as an inhibitor of BER by several laboratories. The specific action of MX in disruption of BER has been clearly demonstrated. MX reacts with the tautomeric open-ring form of deoxyribose generated from the removal of an abnormal base by any one of the DNA glycosylases (28). The reaction of MX with AP sites is fast, even faster than with AP endonuclease (APE) (24). Considering that MX binds potently to AP sites and possesses a unique structure that permits for radiolabeling with C-11 without any structural alternation, we hypothesized that MX can be used as a radiotracer for PET imaging of AP sites *in vivo*. To test this hypothesis, we developed a synthetic approach to [¹¹C]MX. We then used small animal PET (microPET) to evaluate *in vivo* pharmacokinetic profiles of [¹¹C]MX in xenograft tumor mouse models. Because the same imaging modality can be used in clinical setting, the results obtained from microPET can be directly translated into clinical trials. Here we report the radiosynthesis of [¹¹C]MX and *in vivo* microPET studies of AP sites in animal tumor models.

Materials and Methods

1. Radiosynthesis of [¹¹C]MX

The radiosynthesis of [¹¹C]MX was achieved in two steps as shown in Figure 1. Using an on-site cyclotron, [¹¹C]CO₂ was first generated, which was reduced to [¹¹C]methyl iodide by lithium aluminum hydride. The *N*-Boc-protected hydroxylamine was then methylated with [¹¹C]methyl iodide to yield *N*-Boc-protected [¹¹C]-labeled MX. Following

radiomethylation, the Boc-group was then cleaved by hydrochloric acid. The reaction mixture was then neutralized with sodium hydroxide and the product was purified by solid phase extraction using Sep-Pak. HPLC analysis on a cation exchange column indicated that the retention time of the radiolabeled [^{11}C]MX as determined by radiodetector was identical to the retention time of the non-labeled MX as determined by UV detection under the same condition. The resulting compound [^{11}C]MX is identical to MX except that it incorporates a ^{11}C instead of a ^{12}C in the same position. Following radiosynthesis, we conducted MRI and microPET studies in xenograft tumor models to quantitatively determine the *in vivo* pharmacokinetic profile of [^{11}C]MX.

2. Preparation of melanoma and glioma xenographs

Melanoma and glioma xenografts were prepared according to previously published procedures (29). Briefly, glioma (U87) and melanoma (WM164) tumor cells (5×10^6) were injected into the bilateral flanks of female athymic NRC nude mice (6–8 weeks of age). When the volume of tumor nodules reached 150–200 mm³, mice are randomly assigned to control or treatment groups (4 mice/group). All protocols used in the animal studies have been approved by Case Western Reserve University, Institutional Animal Care and Use Committee (Protocol 2007–028).

3. MRI or CT studies for localization of tumor regions in the animal models

High resolution MRI studies provide precise location of tumor tissues. For each imaging session, a pair of mice were used and placed on the same holder following anesthesia with 2.0% isoflurane delivered in oxygen gas with a nosecone. After initial localization scans, the two mice were simultaneously scanned with a T2-weighted turbo spin echo acquisition (TR/TE = 3000/60ms, resolution = 1mm x 200um x 200um). Each animal's respiration rate was monitored and adjusted to 50–60 breaths/min by adjusting the isoflurane level. The animal's core body temperature was also maintained at $37 \pm 2^\circ\text{C}$ throughout the scanning procedure by providing a warm air supply to the magnet core. MR images were acquired on a Bruker BioSpec horizontal magnet (7.0T; 30 cm bore) using a transmit/receive mouse volume coil. High-resolution anatomic MR images of the tumor region were acquired using contiguous multi-slice 2D spin echo and 3D gradient echo techniques.

Similarly, an ultra-high resolution micro-CT scanner from GammaMedica was also used to localize the tumor tissues. This Micro-CT scanner uses a microfocal x-ray source (10–220kV, 0.01–0.3 mA). A 2k x2k (16 bit) CCD camera is attached to 9°3-field II. The system uses a 7 axis positioning system. For a typical scan, the spatial resolution is 20 microns.

4. MicroPET studies

Following MRI studies, the mice were transferred to a Concord R4 microPET scanner under anesthesia. The same holder was used for microPET studies so that the tumor positions of the mice remain unchanged in order to facilitate image coregistration with MRI. Subsequently, dynamic microPET scans were performed over 60 min in a list mode, immediately after a bolus injection of ca. 500 μCi of [^{11}C]MX via the tail vein. Body temperature in the anesthetized animals was monitored using a rectal temperature probe and maintained at $37 \pm 2^\circ\text{C}$ with a heating lamp or a heating pad.

5. Quantitative image analysis

Following MRI and microPET studies, we conducted quantitative image analysis in order to evaluate the *in vivo* pharmacokinetic profiles of [^{11}C]MX in tumor tissues. We defined the tumor tissues as the region of interest (ROI) through coregistration of microPET images with MRI images. The co-registered images were used for quantitative image analysis to determine [^{11}C]MX uptake and retention associated with tumor tissues. The radioactivity concentration in the tumor regions is expressed in terms of standard uptake volume (SUV) [$(\mu\text{Ci/cc})/(\text{uCi/g})$] as a function of time.

6. Bioassay of AP sites following TMZ treatment

In parallel to the imaging studies, mice of the same batch were also treated with TMZ using the same dose. At 4 hours or 24 hours following TMZ treatment, i.e the same time points as used in the imaging studies, the tumor tissues were harvested and the AP sites were measured using ARP (aldehyde reactive probe) reagent. The assay was performed as previously described with minor modifications (30–32). Briefly, athymic mice carrying human melanoma xenograft were treated intraperitoneally with TMZ (80 mg/kg). Tumors were collected at 4 hours and 1 day after treatment and time-dependent AP sites were measured. After extracting by phenol (Fischer Scientific, Fair Lawn, NJ) and chloroform (Sigma-Aldrich, St Louis, MO), DNA (10 μg) was incubated with 15 μl of 1 mM ARP (Dojindo Laboratories, Kumamoto, Japan) in 150 μl PBS solution at 37 °C for 15 min. DNA was then precipitated with 400 μl ice-cold ethanol (100%) at –20 °C for 20 min and washed with 70% ethanol. DNA was dried at room temperature for 30 min and then resuspended in TE buffer to achieve a final concentration of 0.3 $\mu\text{g}/100 \mu\text{l}$. The ARP-labeled DNA was then heat-denatured at 100 °C for 5 min, quickly chilled on ice and mixed with an equal amount of 2 M ammonium acetate. The DNA was then immobilized on BA-S 85 nitrocellulose membrane (Schleicher and Schuell, Dassel, Germany) using a minifold II vacuum filter device (Schleicher and Schuell, Dassel, Germany). The membrane was baked at 80°C for 1 hr and incubated with 0.25% BSA/PBS containing streptavidin-conjugated horseradish peroxidase (BioGenex, SanRamon, CA) at room temperature for 40 min with gentle shaking. ARP-labeled AP sites were visualized by chemiluminescence (Amersham Corp, Piscataway, NJ) followed by quantitative densitometry using NIH ImageJ software.

Results and Discussion

1. *In vivo* studies in melanoma xenografts

Because TMZ, like dacarbazine, has been used in the treatment patients with metastatic malignant melanoma, we first conducted microPET studies in human (WM164) melanoma xenograft tumor model. In each experiment, two mice bearing melanoma xenografts were used, one treated with TMZ (80 mg/kg) to induce DNA damage and the other used as negative control. Prior to microPET scans, the mice were placed in a 7T MRI scanner and T2-weighted MRI images were acquired following the above-mentioned protocol. While kept in the same position under anesthesia, the mice were then transferred to the microPET scanner. Ten minutes after TMZ treatment, [^{11}C]MX (ca. 2 mCi/kg) was then administered through tail vein injection, which was immediately followed by microPET scans for 60 min. The images from MRI scans are shown in Figure 2A–B. The composite images from

microPET scans are shown in Figure 2C–D. The co-registered images are shown in Figure 2E–F.

Following microPET and MRI scans, images from both modalities were co-registered for quantitative measurements of [^{11}C]MX concentration in each tumor region. As shown in Figure 3, [^{11}C]MX readily entered the tumor tissue at early time points. In the mice treated with TMZ, tumor tissues showed an increased uptake of [^{11}C]MX compared to tumor tissues in non-TMZ treated mice. At 10 min post injection of [^{11}C]MX, for example, the radioactivity concentration in the TMZ-treated mice was 1.6-fold higher than that in the non-treated mice.

2. *In vivo* studies in flank glioma xenograft

Malignant glioma is another type of cancer that can be effectively treated with TMZ because TMZ can readily penetrate the blood-brain barrier. We thus conducted imaging studies in nude mice of glioma xenografts following a similar protocol as described in the above-mentioned imaging studies. Thus, two mice of glioma xenografts were used in each experiment, one treated with TMZ (80 mg/kg) and the other used as negative control. Prior to microPET studies, the mice were placed in 7T MRI scanner to acquire T2 weighted high resolution MRI image. While kept in the same position under anesthesia, the mice were then transferred to microPET scanner. Ten minutes after TMZ treatment in mice, [^{11}C]MX was then administered to both treated and non-treated mice, which was followed immediately by 60 min of microPET scan.

Following image coregistration and quantitative analysis, the radioactivity concentrations of [^{11}C]MX were calculated (decay corrected) and plotted as a function of time. As shown in Figure 4, the retention of [^{11}C]MX was higher in TMZ-treated tumor regions than in non-treated tumor regions, suggesting that AP-site formation is elevated following TMZ treatment.

4. *In vivo* blocking experiment

For quantitative imaging studies, it is important to demonstrate whether the measured signal is specific for binding of [^{11}C]MX to AP sites. Thus, we conducted *in vivo* blocking experiment in tumor mice with unlabelled MX as a further test of whether the difference in radioactivity concentrations between TMZ-treated and untreated tumors is due to specific binding of [^{11}C]MX to AP sites. For this purpose, 4 tumor-bearing mice were treated with TMZ (80 mg/kg) for 10 min. Following the treatment, two mice were injected with unlabelled MX (2–10 mg/kg) and two were injected with vehicle control. Thirty minutes later, 2 mCi/kg of [^{11}C]MX was administered to each mouse and microPET imaging was performed. In each case, a vehicle-treated and an unlabeled MX-treated mouse were imaged side-by-side. Dynamic acquisition was carried out for 60 min in list mode. As shown in Figure 5, the concentration of tumor-associated radioactivity in TMZ-treated mice significantly decreased following treatment with unlabelled MX. This study suggested that the increased retention of radioactivity in the tumor regions was due to specific binding of [^{11}C]MX to AP sites.

To further approve that [^{11}C]MX binds specifically to AP sites, we conducted another *in vivo* binding competition assay using an AP site binding agent, termed F422, which is structurally unrelated to MX. Our preliminary studies have shown that F422 binds potently to AP sites similar to MX (Figure 6). Thus, the same xenografts bearing melanoma tumors were treated with TMZ (80 mg/kg) for 10 min. Following the treatment, one mouse was injected with unlabelled F422 (10 mg/kg) and the other was injected with vehicle control. Thirty minutes later, 2 mCi/kg of [^{11}C]MX was administered to each mouse. MicroPET imaging was then conducted with a vehicle-treated and unlabeled F422-treated mouse side-by-side. Dynamic acquisition was carried out for 60 min in list mode. As shown in Figure 7, the concentration of tumor-associated radioactivity in TMZ-treated mice significantly decreased following treatment with unlabelled F422. This study further demonstrated that the increased retention of radioactivity in the tumor regions was due to specific binding of [^{11}C]MX to AP sites.

5. Correlation of time course of AP formation between *in vivo* microPET studies and *in vitro* ARP-based biochemical assays

To validate the imaging results, we determined the time course of AP site formation and correlated the *in vivo* microPET studies with the *in vitro* ARP-based biochemical assays at different time point. Thus, each group containing a total of three tumor mouse xenografts was subject to TMZ treatment using the same dose (80 mg/kg). One was used for longitudinal imaging at 4 hours and 24 hours and the other two were sacrificed at 4 hours or 24 hours post TMZ treatment for ARP assays. [^{11}C]MX uptake as determined by microPET studies was found to be proportional to the amount of AP sites as determined by ARP-based biochemical assays in the tumor tissues that were harvested following TMZ treatment. As shown in Figure 8, levels of AP-site formation in xenograft tumor tissues were determined separately at 4 hours and 24 hours after mice received a single injection of TMZ (80 mg/kg). Both microPET studies and biochemical assays showed that the levels of TMZ-induced AP sites in tumor tissue were consistently higher at 4 hours than that at 24 hours after TMZ treatment. The same ratios of AP sites between 4 hours and 24 hours were observed in the microPET studies and ARP assays. These studies suggested that [^{11}C]MX-PET can be used as an imaging marker of AP formation.

These results also showed that formation and repair of AP sites are relatively slower than initial excision of damaged DNA bases as determined by Jaruga and Dizdaroglu in 1996 related to oxidative DNA damage and repair (33). In that work, cultured human lymphoblast cells were treated with H_2O_2 to induce oxidative DNA damages. Following treatment with H_2O_2 , DNA samples were analyzed by gas chromatography/isotope-dilution mass spectrometry (GC/MS) at different time points to investigate the cellular repair of DNA base damage products. They determined that the excision rates of these damaged DNA bases are relatively fast with half-lives ranging from 11 min to 62 min. However, the excision rate of the damaged DNA bases does not directly correlate with the rate of AP-site formation and repair. After treatment of TMZ, O^6 -methylguanine (O^6mG), 7-methylguanine (N^7mG), and 3-methyladenine (N^3mA) DNA adducts are formed. The O^6mG DNA adduct is a cytotoxic and genotoxic lesion mainly excised by O^6 -methylguanine DNA-methyltransferase (MGMT) (34). N^7mG , the dominant lesion formed by TMZ, and N^3mA DNA adducts are

removed by MPG. Following excision of N7mG and N3mA DNA damage products, AP sites are formed and subsequently repaired through BER pathway. The rate of AP-site formation and repair is a multi-protein process and independent of DNA base excision. While the excision rate of damaged DNA bases can be fast, the rate of AP-site repair can be relatively slow. Back in 1994, we have studied the kinetics of DNA repair after exposure to N-methylnitrosourea (MNU), a DNA-methylating agent (35). In those studies, we specifically evaluated the formation and removal of N7mG DNA adduct, which precede formation of AP sites and subsequent BER repair. In both MGMT-CD2 transgenic and non-transgenic littermates, the removal rates of N7mG DNA adduct in various tissues such as thymus, spleen, and liver were found relatively slow compared to removal of O6mG DNA adduct, which precedes MGMT-mediated repair and does not involve AP site formation. At 24 hours after MNU exposure, less than 50% of N7mG were removed, a large portion of N7mG were still detected. Consequently, the downstream formation and repair of AP sites are expected to be as slow as removal of N7mG DNA adduct. This is consistent with what we have observed *in vivo* after exposure to TMZ, where the level of AP sites at 24 hours post treatment is still significantly high. In fact, the prolonged repair of AP sites as demonstrated in our work makes it possible to develop therapeutic agents that bind to AP sites and block BER repair in order to enhance cytotoxicity of TMZ or other DNA-methylating agents. Direct detection and quantification of AP sites will thus provide a platform technology for efficacy evaluation of a variety of DNA-targeted chemotherapies.

Conclusion

We have demonstrated that MX can be radiolabelled with positron-emitting carbon-11 and used as radiotracer for PET imaging of AP sites *in vivo*. Radiosynthesis of [^{11}C]MX was achieved through radiomethylation for microPET studies. Based on the microPET studies, AP-site formation was found to be elevated in melanoma xenograft tumor model following TMZ treatment. AP-site formation was also elevated in glioma xenograft tumor model following TMZ treatment. [^{11}C]MX bound to AP sites *in vivo* specifically as determined by *in vivo* blocking experiments. In addition, correlation of microPET studies with ARP-based biochemical assays suggested that [^{11}C]MX-PET is an appropriate imaging marker of AP site formation.

These data form the basis of future experiments designed to measure AP site formation with other chemotherapeutic agents. In both preclinical and clinical settings, visualizing persistent AP sites will predict both drug effect and drug efficacy. The goal is to maximize persistence of AP sites to increase the apoptotic signal emanating from unrepaired DNA damage and Topo II binding. Given that these processes will differ among agents, imaging of these processes will likely represent a significant advance over empiric dose schedules.

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Figure 1.

Radiosynthesis of $[^{11}\text{C}]\text{MX}$ starting with hydroxylamine protected with a t-butoxyl carbonyl (Boc) group. $[^{11}\text{C}]\text{CH}_3\text{I}$ was prepared *in situ* by reduction of $[^{11}\text{C}]\text{CO}_2$ generated by an on-site cyclotron. Following radiomethylation under basic condition, the so obtained Boc-protected $[^{11}\text{C}]\text{MX}$ was deprotected using HCl to yield $[^{11}\text{C}]\text{MX}$ as a HCl -salt.

Figure 2.

Axial view of melanoma xenografts acquired by 7T MRI and microPET using [^{11}C]MX. [^{11}C]MX was injected iv (2 mCi/kg) to mice anaesthetized with 1.5–2.0% isoflurane and a 60 min PET scan was performed using a Concord microPET scanner. A–B, MRI images of non-treated mouse (A) and the TMZ treated mouse (B). MicroPET images (C–D) were superimposed onto the corresponding slices of the MRI scans (E–F). PET images shown correspond to the activity between 80–110 min. The tumor regions are shown in the circles.

Figure 3. Radioactivity concentration as a function of time obtained in TMZ-treated tumor regions and non-treated tumor regions as determined in melanoma xenografts. [^{11}C]MX (2 mCi/kg) was injected i.v. to mice anaesthetized with 1.5–2.0% isoflurane and a 60 min PET scan was performed. Note that retention of [^{11}C]MX in TMZ-treated melanoma tumor regions was higher than in the non-treated tumor regions during the 60-min scan. The radioactivity concentration is expressed in average ($n=4$, where n is the number of tumors imaged and analyzed) in standard uptake volume [$(\mu\text{Ci/cc})/(\mu\text{Ci/g})$] (SUV) (decay corrected).

Figure 4.

Radioactivity concentrations as a function of time in TMZ-treated tumor regions and non-treated tumor regions as determined in flank glioma xenografts. [^{11}C]MX (2 mCi/kg) was injected i.v. to mice anaesthetized with 1.5–2.0% isoflurane and a 60 min PET scan was performed. Similarly, retention of [^{11}C]MX in TMZ-treated glioma xenograft tumor regions was higher than in the non-treated tumor regions during the 60-min scan. The radioactivity concentration is expressed in average ($n=4$, where n is the number of tumors images and analyzed) in standard uptake volume [$(\mu\text{Ci/cc})/(\mu\text{Ci/g})$] (SUV) (decay corrected).

Figure 5.

Left: Time-radioactivity courses of [^{11}C]MX in the glioma tumors before and after blocking by unlabelled MX. Right: Average SUV between 0–60 min ($n=4$, where n is the number of tumors imaged and analyzed). Decrease of radioactivity concentration indicates *in vivo* blocking of AP binding sites by unlabeled MX (p value = 0.003).

Figure 6.

AP sites increased in melanoma cells (A375) after treatment with TMZ in a dose-dependent manner. Using ARP assay, formation of AP sites in A375 melanoma cells were measured. Cells were treated with TMZ (0–1500 μM) alone or TMZ plus MX (12.5 mM) for 24 hr for a dose-dependent assay (black), or treated with TMZ and MX (12.5 mM) for 24 hr, or treated with TMZ and F422 (12.5 mM) for 24 hours. Co-treatment with MX or F422 reduced the ARP detected AP sites, suggesting that F422, same as MX, competed with ARP in binding to AP sites induced by TMZ.

Figure 7.

Left: Time-radioactivity courses of [^{11}C]MX in the melanoma tumors before and after blocking by F422 compound. Right: Average SUV between 0–60 min ($n = 2$ tumors). Decrease of radioactivity concentration indicates *in vivo* blocking of AP binding sites by F422 compound (p value = 0.001).

Figure 8.

Quantification of AP sites at different time points following TMZ treatment in a xenograft tumor mouse model. [^{11}C]MX (2 mCi/kg) was injected i.v. to mice anaesthetized with 1.5–2.0% isoflurane and a 60 min PET scan was performed. A. a representative CT scan showing the tumor regions; B. a representative microPET scan showing the whole-body radioactivity uptake, C. fused PET/CT images for quantification of [^{11}C]MX uptake. D. kinetics of [^{11}C]MX uptake as a function of time at 4 hours and 1 day after TMZ treatment, with p value = 0.001; E. averages of radioactivity concentration in the tumor regions over the 60 min scan and correlation with those determined by ARP-based biochemical assays following 4 hours and 1 day treatments of TMZ.