

Monosodium glutamate reduces ^{68}Ga -PSMA-11 uptake in salivary glands and kidneys in preclinical prostate cancer model

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ABSTRACT

We evaluated the ability of monosodium glutamate (MSG) to reduce salivary and kidney uptake of a prostate-specific membrane antigen (PSMA) radioligand without affecting tumor uptake.

Methods: LNCaP tumor-bearing mice were intraperitoneally injected with MSG (657, 329, or 164 mg/kg) or phosphate-buffered saline (PBS). Fifteen minutes later, mice were intravenously administered ^{68}Ga -PSMA-11. PET/CT imaging and biodistribution studies were performed 1 h post-administration.

Results: Tumor uptake was not statistically different between groups: 657 mg/kg (8.42 ± 1.40 %ID/g), 329 mg/kg (7.19 ± 0.86), 164 mg/kg (8.20 ± 2.44), PBS (8.67 ± 1.97). Kidney uptake was significantly lower in the 657 mg/kg group (85.8 ± 24.2 %ID/g) than 329 mg/kg (159 ± 26.2), 164 mg/kg (211 ± 27.4), and PBS groups (182 ± 33.5); $p < 0.001$. Salivary gland uptake was lower in 657 mg/kg (3.72 ± 2.12 %ID/g) and 329 mg/kg (5.74 ± 0.62) groups compared with PBS group (10.04 ± 2.52); $p < 0.01$.

Conclusion: MSG decreased salivary and kidney uptake of ^{68}Ga -PSMA-11 in a dose-dependent manner, while tumor uptake was unaffected.

KEYWORDS

Prostate-specific membrane antigen; ^{68}Ga -PSMA-11; monosodium glutamate; salivary glands; kidney

INTRODUCTION

Prostate-specific membrane antigen (PSMA) is an excellent prostate cancer target for theranostic applications. Many imaging agents showing high sensitivity/specificity for PSMA-expressing tissues have been developed (*1*). Some have been labeled with therapeutic radionuclides (i.e. ^{177}Lu , ^{225}Ac) and have had success in treating castration-resistant metastatic prostate cancer (*2,3*). The activity administered to patients is limited by toxicity to normal organs; high uptake is observed in lacrimal glands, parotids, submandibular glands, and renal cortex (*4*). The potential side effects of higher doses include hematotoxicity, xerostomia, and renal dysfunction (*2,3*). In particular, xerostomia with alpha-emitters is so severe that patients have discontinued treatment (*5*). A means of decreasing this toxicity without affecting tumor uptake would allow administration of greater activity with presumably greater tumoricidal effect.

Different pharmaceuticals including 2-(phosphonomethyl)pentanedioic acid (PMPA), a PSMA inhibitor, have been explored for nephroprotection (*6,7*). PMPA displaced renal activity of a PSMA radiotherapeutic in cancer models, but this was generally accompanied by a reduction in tumor uptake (*6,7*). Mannitol infusion reduced renal uptake of ^{68}Ga -PSMA-11 (*8*), but its effect on tumor uptake requires further investigations. Botulinum toxin was administered in the parotid gland of a patient and significantly decreased PSMA-ligand uptake (*9*). While this procedure is promising, it is invasive, costly, and may affect salivary gland function for weeks. In this study, we investigated monosodium glutamate (MSG) for reducing uptake of ^{68}Ga -PSMA-11 in salivary glands and kidneys, in LNCaP tumor-bearing mice. MSG is a well-studied food additive and can stimulate salivary flow (*10,11*). While PSMA is expressed in salivary glands and kidneys, part of PSMA-ligand uptake in salivary glands may be due to off-target binding, as uptake is not observed

in human studies with the radiolabeled J591 monoclonal antibody (*12-14*). As many PSMA ligands integrate glutamate for binding to PSMA, we hypothesized MSG could reduce non-specific accumulation in non-cancerous tissues.

MATERIALS AND METHODS

⁶⁸Ga-PSMA-11 Radiolabeling

The standard and radiolabeling precursor were purchased from ABX Advanced Biochemical Compounds. Radiolabeling was performed as previously published (*15*).

Cell Culture

LNCaP prostate adenocarcinoma cells (ATCC) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (Sigma-Aldrich), 100 I.U./mL penicillin, and 100 µg/mL streptomycin (Life Technologies) in a humidified incubator (37°C, 5% CO₂).

Competition Binding Assay

LNCaP cells were washed with PBS and harvested by trypsinization. Cells (200,000/well) were seeded onto a 24-well poly-D-lysine coated plate for 48 h. Growth media was replaced with PBS buffer (20 mM 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid, 2% bovine serum albumin, pH 7.5) 1 h prior to the study. ¹⁸F-DCFPyL (0.1 nM), synthesized according to published procedures (*16*), was added to wells containing MSG (1×10^{-2} to 1.3×10^{-7} M), in triplicate. The assays were incubated for 1 h at 37°C with agitation. After aspiration, cells were washed twice with cold 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid-buffered saline. To harvest cells,

400 μ L trypsin was added to each well. Samples were counted using a Wizard2 2480 (PerkinElmer) automatic gamma counter. K_i value was calculated using nonlinear regression in GraphPad Prism 7 (GraphPad Software) with a K_d value of 0.49 nM for ^{18}F -DCFPyL.

PET Imaging and Biodistribution

Animal experiments were approved by the Animal Ethics Committee of the University of British Columbia. Immunodeficient NOD.Cg-*Prkdc^{scid}Il2rg^{tm1Wjl}*/SzJ male mice were obtained in-house. Mice were subcutaneously inoculated with 5×10^6 LNCaP cells (100 μ L; 1:1 PBS/Matrigel), with tumors grown for 4-6 weeks. Mice were injected intraperitoneally with MSG (657, 329, or 164 mg/kg) or PBS. After 15 min, mice were anesthetized with isoflurane (2.5% in 2.0 L/min O_2) and injected intravenously with ^{68}Ga -PSMA-11 (5.34 ± 0.95 MBq for PET/CT, 1.86 ± 0.71 MBq for biodistribution studies).

PET/CT imaging was conducted on a Siemens Inveon microPET/CT. Following a 45 min uptake period, mice were sedated with isoflurane and placed on scanner. Body temperature was maintained by a heating pad. A CT scan was obtained for localization and attenuation correction (80 kV tube voltage, 500 μ A current, 3 bed positions, 34% overlap, 220° continuous rotation). This was followed by a 10 min PET acquisition 1 h post-injection of ^{68}Ga -PSMA-11. PET was acquired in list mode, reconstructed using 3-dimensional ordered-subsets expectation maximization (2 iterations) followed by a fast maximum a priori algorithm (18 iterations) with CT-based attenuation correction. Images were analyzed using Inveon Research Workplace software (Siemens Healthineers).

For biodistribution studies, 1 h following radiopharmaceutical injection, mice were anesthetized with isoflurane and euthanized by CO₂ inhalation. Blood was collected via cardiac puncture. Organs/tissues were harvested, rinsed with PBS, blotted dry, and weighed. The radioactivity in tissues was assayed by gamma counter and expressed as percentage of injected dose per gram of tissue (%ID/g).

Statistical Analysis

Analysis was performed with R 3.4.0 (R Foundation for Statistical Computing). Outliers identified by one round of Grubb's test ($\alpha < 0.01$) were removed. When data distribution was normal according to the Shapiro-Wilk test ($\alpha < 0.05$), groups were compared with Welch's *t*-test, or else by Wilcoxon's rank sum test. Holm's correction was used for multiple comparisons. A significance level of $\alpha < 0.05$ was used. PBS controls repeated for each experiment (different batches of radiotracer), were aggregated in a single group for analysis.

RESULTS

⁶⁸Ga-PSMA-11 was synthesized in 66.3±8.8% decay-corrected radiochemical yields with > 99% radiochemical purity and 82.6±44.3 GBq/μmol molar activity (n=6).

Biodistribution results are shown in Figure 1 and Supplemental Table 1. There were no statistical differences for tumor uptake between the four groups: 657 mg/kg (8.42±1.40 %ID/g), 329 mg/kg (7.19±0.86), 164 mg/kg (8.20±2.44), PBS (8.67±1.97). Kidney uptake was significantly lower in the 657 mg/kg group (85.8±24.2 %ID/g) compared with 329 mg/kg (159±26.2), 164 mg/kg

(211 ± 27.4), and PBS groups (182 ± 33.5); $p < 0.001$. Difference between 329 mg/kg and 164 mg/kg groups was significant ($p < 0.05$), but not when they were respectively compared with PBS. Salivary gland uptake was lower in 657 mg/kg (3.72 ± 2.12 %ID/g) and 329 mg/kg (5.74 ± 0.62) groups compared with the PBS group (10.04 ± 2.52); $p < 0.01$. There were no significant differences between 164 mg/kg (14.2 ± 4.27 %ID/g) and PBS groups, or between 657 mg/kg and 329 mg/kg groups. Muscle uptake was generally lower in MSG treated groups.

PET imaging studies were performed in mice pre-injected with PBS or 657 mg/kg of MSG (Figure 2). High uptake in LNCaP tumors was observed, with the remainder of the radioactivity cleared through the renal pathway. Compared to PBS group we observed lower uptake in salivary glands and faster excretion (more urinary output) in MSG-treated mice. The difference in clearance rates led to higher contrast images for MSG-treated mice.

The binding affinity (K_i value) of MSG to PSMA was 0.90 ± 0.58 mM ($n=3$), as measured using a cell-based competition assay with ^{18}F -DCFPyL (Figure 3).

DISCUSSION

The development of PSMA radiotheranostic agents have had a significant impact on prostate cancer management (17). Small molecule inhibitors were developed as alternatives to monoclonal antibodies, primarily for their fast targeting properties and clearance profiles (17). However, radiolabeled PSMA inhibitors show undesired uptake in salivary glands (17). While this does not hinder diagnostic interpretation, it imposes a limit on the maximum-tolerable dose for therapy.

We investigated the effect of MSG on ^{68}Ga -PSMA-11 salivary uptake. The doses of MSG chosen for this study corresponded to one-tenth, one-twentieth, and one-fortieth of the median lethal dose for mice (18).

We observed significant decrease in kidney (50% for 657 mg/kg) and salivary uptake (43-53% for 329 and 657 mg/kg) of ^{68}Ga -PSMA-11 for MSG-treated mice compared to control. Uptake in kidney and salivary glands for the 164 mg/kg group was higher than PBS group, but this was not statistically significant. Notably, tumor uptake was not statistically different between the four groups. At > 329 mg/kg, MSG blocked renal and salivary uptake without affecting tumor uptake. PET imaging corroborated biodistribution results, notwithstanding enhanced contrast for MSG-treated mice, which can be explained by accelerated clearance of ^{68}Ga -PSMA-11 and/or reduced non-specific accumulation.

Kratochwil et al. demonstrated that PMPA can reduce renal uptake of ^{125}I -MIP-1095 without affecting LNCaP tumor uptake; however, a 16 h latency period was required for tracer binding/clearance before PMPA administration (6). Chatalic et al. showed that co-injection of PMPA with $^{111}\text{In}/^{177}\text{Lu}$ -PSMA I&T could improve tumor-to-kidney absorbed dose ratio, but this was accompanied by a reduction in tumor uptake (7). The high affinity of PMPA to PSMA (reported K_i : 0.28 nM) may complicate dose optimization (19). Conversely, MSG has poor binding affinity to PSMA (K_i : 0.90 ± 0.58 mM), which may benefit this application.

Although MSG has been linked to headaches and “Chinese restaurant syndrome”, human studies have shown MSG to be safe; based on a no adverse event limit of 3,200 mg/kg/day for neurodevelopmental toxicity, the European Food Safety Authority recommended an acceptable daily intake of 30 mg/kg/day (18,20). Fernstrom administered a single dose 12.7 g MSG orally in humans without side-effects, with return of plasma glutamate levels to baseline after 3 hours (21). The MSG quantity required to achieve effective off-target blocking of ^{68}Ga -PSMA-11 in humans is not known, as human physiology may differ. Whether sufficient quantities can be practically administered in human subjects will require further investigation.

CONCLUSION

MSG can decrease salivary and renal uptake of ^{68}Ga -PSMA-11 without affecting tumor uptake in mice, presumably by competing with off-target binding sites. This could potentially be used to increase the therapeutic index of glutamate-based radioligands. Further work is needed to assess whether this blocking effect is sufficiently durable to protect these organs from longer-lived radioisotopes, to understand the mechanism of action and to assess if the same effect can be translated to humans.

CONFLICTS OF INTEREST

None to declare.

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FIGURES

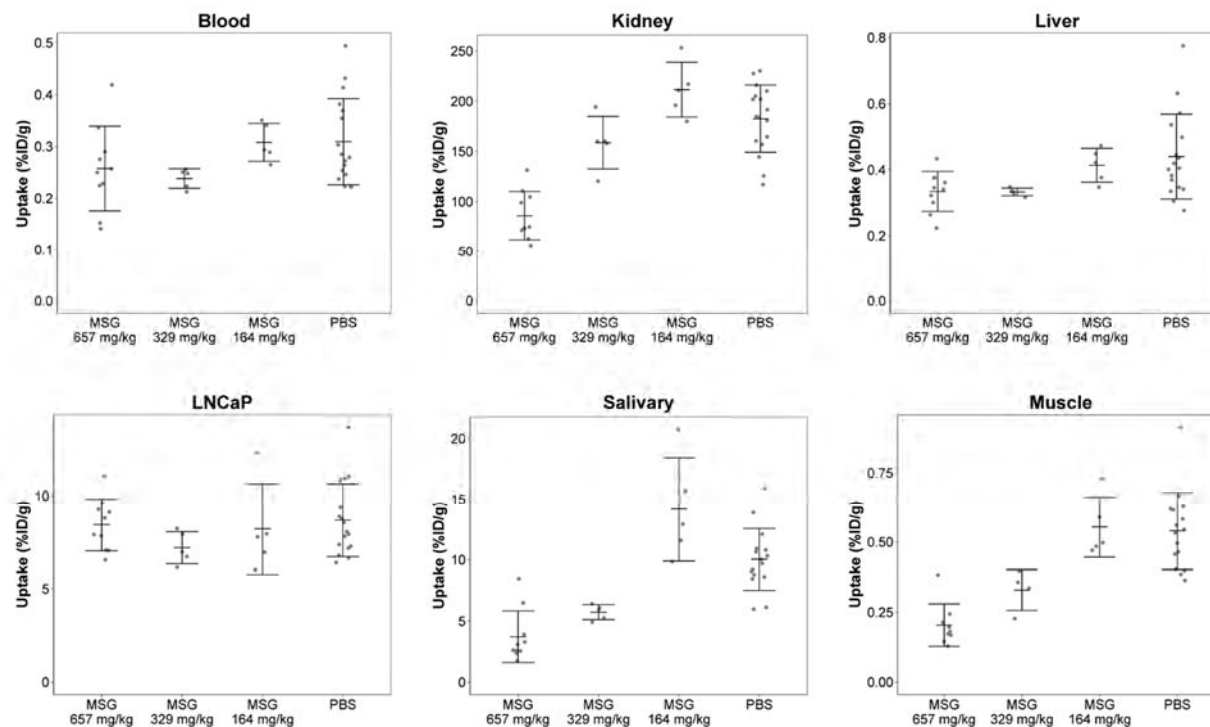


Figure 1. Biodistribution of ^{68}Ga -PSMA-11 in selected organs at 1 h post-injection. Mice received MSG (657, 329, or 164 mg/kg) or PBS intraperitoneally 15 min prior to tracer administration.

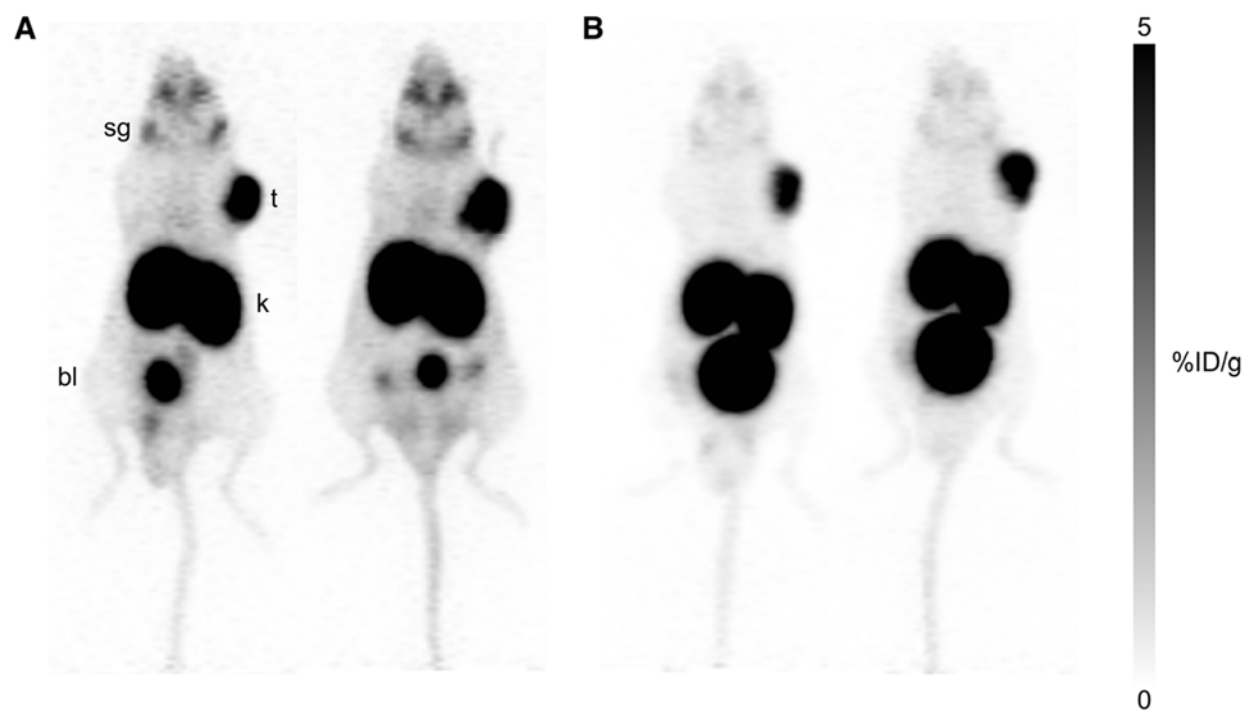


Figure 2. PET maximum intensity projection images of 4 mice with ^{68}Ga -PSMA-11 at 1 h post-injection. LNCaP tumor-bearing mice received (A) PBS or (B) MSG (657 mg/kg) intraperitoneally 15 min prior to tracer administration. t=tumor; k=kidney; sg=salivary glands; bl=bladder.

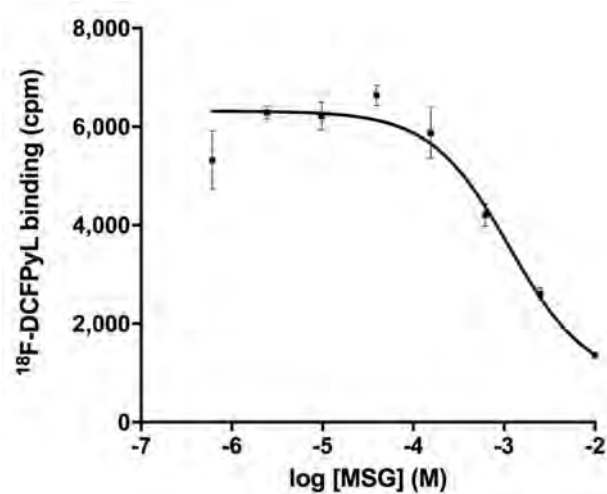


Figure 3. Representative competition binding assay for MSG against ^{18}F -DCFPyL in LNCaP cells. The K_i value for this assay was 0.95 mM.

Supplemental Table 1. Effect of MSG on biodistribution of ^{68}Ga -PSMA-11 in LNCaP tumor-bearing mice at 1 h post-injection.

	MSG 657 mg/kg		MSG 329 mg/kg		MSG 164 mg/kg		PBS	
	mean\pmSD	n	mean\pmSD	n	mean\pmSD	n	mean\pmSD	n
Blood	0.26 \pm 0.08	10	0.24 \pm 0.02	5	0.31 \pm 0.04	5	0.31 \pm 0.08	17
Fat	0.65 \pm 0.20	10	1.14 \pm 0.25	5	2.49 \pm 0.96	5	2.25 \pm 0.75	17
Spleen	4.31 \pm 1.98	10	7.91 \pm 3.44	5	19.5 \pm 6.19	5	22.2 \pm 7.91	17
Liver	0.33 \pm 0.06	10	0.33 \pm 0.01	5	0.41 \pm 0.05	5	0.44 \pm 0.13	17
Pancreas	0.33 \pm 0.05	9	0.57 \pm 0.11	4	1.21 \pm 0.33	5	1.44 \pm 0.39	17
Adrenals	2.45 \pm 1.20	9	5.30 \pm 3.61	3	8.67 \pm 2.16	5	7.47 \pm 2.36	13
Kidneys	85.8 \pm 24.2	10	159 \pm 26.2	5	211 \pm 27.4	5	182 \pm 33.5	17
Muscle	0.20 \pm 0.08	9	0.33 \pm 0.07	4	0.55 \pm 0.11	5	0.54 \pm 0.14	16
Tumor	8.42 \pm 1.40	10	7.19 \pm 0.86	5	8.20 \pm 2.44	5	8.67 \pm 1.97	17
Salivary glands	3.72 \pm 2.12	10	5.74 \pm 0.62	5	14.2 \pm 4.27	5	10.0 \pm 2.52	16



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