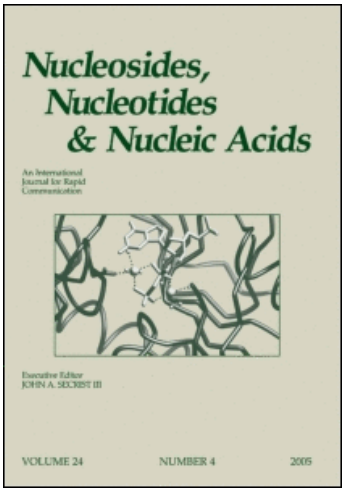

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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To cite this Article Jiang, Shucui , Fischione, Gemma , Guiliani, Patricia , Romano, Silvia , Caciagli, Francesco and DiIorio, Patrizia(2008) 'Metabolism and Distribution of Guanosine Given Intraperitoneally: Implications for Spinal Cord Injury', Nucleosides, Nucleotides and Nucleic Acids, 27: 6, 673 — 680
To link to this Article: DOI: 10.1080/15257770802143962
URL: http://dx.doi.org/10.1080/15257770802143962

Nucleosides, Nucleotides, and Nucleic Acids, 27:673–680, 2008

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770802143962

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METABOLISM AND DISTRIBUTION OF GUANOSINE GIVEN INTRAPERITONEALLY: IMPLICATIONS FOR SPINAL CORD INJURY

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□ *Intraperitoneal administration of guanosine to rats with chronic spinal cord injury stimulates remyelination and functional recovery. If guanosine produced its effects in the nervous system, it should enter it and elevate endogenous concentrations. [^{3}H]-guanosine (8 mg/kg) was administered intraperitoneally to rats and its distribution and concentration in different sites determined. Guanosine rapidly entered all tissues; its concentration peaked at about 15 minutes except in adipose tissue and CNS where it continued to rise for 30 minutes. Its chief metabolic product in all sites was guanine with over twice as much guanine as guanosine present in CNS after 30 minutes.*

Keywords Extracellular guanosine; pharmacokinetics; central nervous system

INTRODUCTION

Traumatic spinal cord injury (SCI) induces persistent perilesional demyelination[1] despite the presence of endogenous precursors of myelin-forming oligodendroglia that, under certain circumstances, can differentiate into mature oligodendroglia and remyelinate axons.[2,3]

Extracellular guanosine (GUO) has a number of trophic effects on cells.[4–7] For example, it stimulates proliferation of a variety of cells in culture[4,6,7] and enhances release of trophic factors such as nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and basic fibroblast growth factor (bFGF) from several cell types.[4] In vivo, five weeks after a

We thank Dr. Raelene Kinlough-Rathbone and Dr. Michel P. Rathbone for their helpful suggestions and critical appraisal of the manuscript. Shucui Jiang is recipient of a Chris Beehler Faculty Investigator Award from the Canadian Spinal Research Organization.

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moderate spinal crush injury in rats, a time at which no further spontaneous recovery occurs,[8] systemic intraperitoneal administration of GUO enhances locomotor recovery. This correlates with remyelination of denuded axons[9] due to proliferation of endogenous adult progenitor cells.[10] Since systemically administered purines are actively metabolized,[11,12] we questioned whether GUO acts peripherally by stimulating an unknown humoral effector that indirectly acts on the central nervous system (CNS), or whether GUO or a metabolic product crosses the blood-brain barrier and acts directly on cells in the CNS. Thus, we measured the distribution and metabolism of GUO after intraperitoneal (i.p.) injection.

MATERIALS AND METHODS

Experiments were in compliance with prevailing regulations and were approved by the Animal Research Ethics Board (AREB) of McMaster University.

Spinal Cord Injury

Moderate spinal cord injury was induced in anesthetized adult female Wistar rats (200 ± 20 g weight, Charles River) exactly as previously described.[9,13]

Evaluation of Exogenous GUO Distribution

GUO 8 mg/kg in saline containing tracer amounts of [8-^{3}H]-GUO (0.4 ug/kg; sp. act. 7.3 Ci/mmol, MORAVEK, USA), was administered intraperitoneally (i.p.) to healthy rats (controls) and to rats submitted to spinal cord injury one month previously. Samples of plasma or brain, spinal cord, heart, liver, kidney, lung, and adipose tissue were collected before (time 0) and 7.5, 15, 30, 60, and 90 minutes after injection. Eighty-four rats were randomly assigned to two groups (control and injured rats). In both groups, animals were then equally subdivided (7 animals) for each time point. Blood was immediately centrifuged to obtain plasma to which perchloric acid was added to a final concentration of 0.4 M. Organ samples, about 200 mg (20–60 mg for spinal cord), were immediately homogenized in 1 ml of cold 0.4 M perchloric acid. Samples were centrifuged (4°C, 10,000 g, 10 minutes) and the supernates neutralized with KOH, then recentrifuged (4°C, 10,000 g, 5 minutes), filtered through 0.2 μm filters (Millipore, USA) and stored at –70°C until analysis.

Plasma and Tissue Levels of GUO and Its Derivatives Measured by HPLC-UV and CE Analysis

High Performance Liquid Chromatography (HPLC) and capillary electrophoresis (CE) yielded substantially equivalent values, differing by no more than 16%. Therefore, the data presented represent the means of values obtained by both analyses.

Ion-Pair HPLC-UV Analysis

GUO, guanine (GUA), xanthine (XAN), and uric acid were assayed by HPLC (Agilent Technologies, USA) using a reverse-phase analytical column (LiChroCART 125-4 LiChrospher 100 RP-18 5 μm; Merck, Germany), flow rate 1.5 ml/minute. A 15 minutes linear gradient was applied from 100% buffer A (60 mM KH_2PO_4 and 5 mM tetrabutylammonium phosphate, pH 6) to 100% buffer B (30% methanol plus 70% buffer A). Purines were measured with a diode array detector (Agilent Technologies) by absorption at 254 nm (290 nm for uric acid) and identified by migration times and UV spectra. Figure 1A is a representative chromatogram from HPLC-UV analysis of a plasma sample.

CE Analysis

HP^{3D}CE capillary electrophoresis used diode array detection and a 80.5 cm $\times$ 50 μm ID $\times$ 375 μm OD uncoated fused-silica capillary, effective length 72 cm to the detector window (Agilent Technologies) at 37°C, running buffer 10 mM borate pH 9.3. Samples were pressure injected (50 mbar for 40s) and separated at 22 kV, and detected at 254 nm. Purines were identified by migration times and by UV spectra. Figure 1C is a representative electropherogram of a plasma sample. CE resolved GUA better than did HPLC.

Plasma and Tissue Concentrations of [^{3}H] GUO and Its Derivatives Measured by Radio-HPLC

Reversed-phase liquid chromatography with on-line radiochemical detection (radio-HPLC) (FLO-ONE 500 TR, Packard Instruments, USA) was used to detect labeled compounds. Liquid scintillation cocktail (Ultima-Flo M, Packard) was pumped at 3 ml/minute, mixed continuously with the HPLC eluate and passed through a 500 μl detector flow cell. After background radioactivity subtraction, radio-chromatograms were integrated and the c.p.m. under each peak determined for GUO, GUA, XAN, and guanosine monophosphate (GMP). Figure 1B shows a typical HPLC pattern of radioactive substances contained in plasma following an i.p. pulse dose of

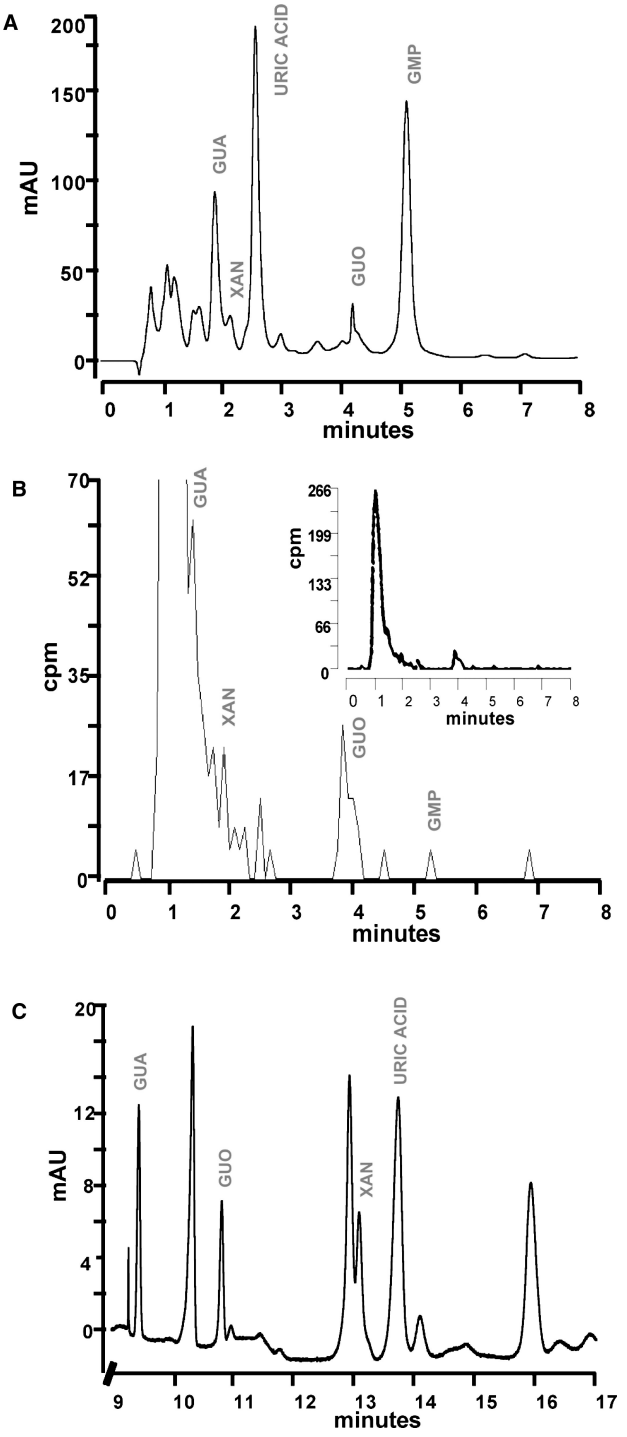

FIGURE 1 Representative chromatograms and electropherogram of a deproteinized plasma sample from a healthy female rat injected intraperitoneally with a guanosine solution (8 mg/kg) containing a tracer dose of [8-^{3}H]-guanosine (0.4 μg/kg). GUO and its derivatives (GMP, GUA, XAN, and uric acid) were detected by HPLC equipped with (A) a diode array detector or (B) on-line radiochemical detector for the evaluation of labeled purine counterpart or (C) by CE equipped with diode array detector.

[^{3}H]-GUO. Since GUO was tritied at position 8, its derivative uric acid, which lacks hydrogen at this position, could not be detected.

Statistics

Statistical significance was evaluated by a Student's two-tailed t-test using Prism (version 3.03) program (GraphPad Software, USA) with $p < 0.05$ considered to be significant.

RESULTS

Basal Plasma and Tissue Concentrations of GUO and Its Derivatives

GUO and its derivatives were measured in plasma and tissues from healthy and spinal-cord injured rats in basal conditions (time point 0, Figure 2A and Table 1). GUO concentration in plasma was 0.32 ± 0.04 μg/ml and ranged from 4.00 ± 0.96 μg/g tissue in liver to 6.3 ± 1.62 μg/g tissue in kidney. GUA and XAN concentrations were 0.80 ± 0.09 and 0.59 ± 0.07 μg/ml, respectively, in plasma. The concentrations of GUA and XAN ranged from 2.90 ± 0.43 and 6.04 ± 4.33 μg/g tissue respectively in liver to 5.9 ± 1.18 and 11.05 ± 1.84 μg/g tissue respectively in adipose tissue. Uric acid was measured only in plasma, where its concentration was 7.08 ± 0.8 μg/ml.

Plasma Concentrations of GUO and Its Derivatives after Exogenous GUO Injection

After i.p. administration of GUO (8 mg/kg), plasma levels slowly increased and doubled by 90 minutes (Figure 2A). Plasma concentrations of GUA, XAN and uric acid peaked 15–30 minutes after the i.p. injection of GUO, before slowly returning nearly to their respective basal values. The maximal increase was 3-, 1.8-, and 1.3-fold over the basal values for GUA, XAN and uric acid, respectively.

To confirm that the increased GUO and its metabolites were derived from the exogenously administered GUO, we studied the distribution and metabolism of [^{3}H]-GUO (0.4 μg/kg) added as a tracer (about 0.005% of the total GUO). [^{3}H]-GUO, [^{3}H]-GUA and [^{3}H]-XAN were found in plasma, indicating some rapid metabolism of exogenous GUO (eGUO) after i.p. administration. In plasma, the pattern of change of the [^{3}H]-labeled and unlabeled substances was similar (Figures 2A and 2B), demonstrating that use of [^{3}H]-GUO radiotracer is a valid approach to study the bioavailability, biodistribution and kinetics of eGUO.

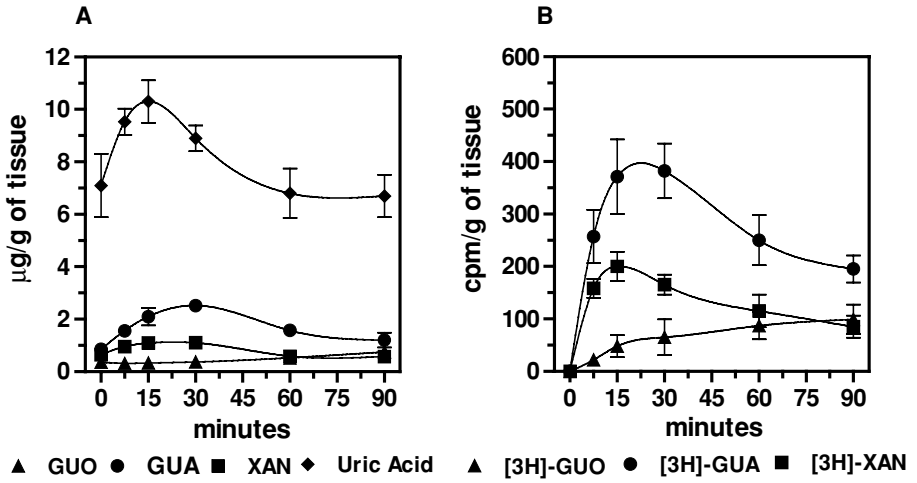

FIGURE 2 Concentrations of labeled and unlabelled guanosine and its metabolites in plasma of female rats. At time 0, a guanosine solution (8 mg/kg) containing a tracer amount of [^{3}H]-guanosine (0.4 μg/kg) was administered intraperitoneally in healthy animals. At the indicated time points, (A) unlabelled purines GUO, GUA, XAN, and uric acid were assayed by HPLC and CE. Data are expressed as the mean ± SEM of values obtained by both analyses from seven animals. (B) Radiolabeled purines (– [^{3}H]-GUO, – [^{3}H]-GUA, and – [^{3}H]-XAN) were assayed by HPLC equipped with an on-line radiochemical detector. The values are the mean ± SEM of samples from seven rats.

TABLE 1 Kinetics of distribution and metabolism of guanosine (8 mg/kg) i.p. administered to rats

	guanosine				guanine				xanthine			
	0 min	7.5 min	15 min	30 min	0 min	7.5 min	15 min	30 min	0 min	7.5 min	15 min	30 min
Liver												
μg/g	4.00	12.50*	11.20*	9.00	2.90	17.32*	27.09*	30.10*	6.04	17.66	19.21*	16.15
± *SEM*	0.96	2.97	3.63	4.26	0.43	4.33	3.72	5.22	4.33	5.25	3.96	7.01
Kidney												
μg/g	6.30	12.23*	14.25*	13.42*	2.60	22.42*	30.61*	28.70*	7.20	19.60*	22.35*	20.50*
± *SEM*	1.62	2.03	2.43	1.57	0.73	3.88	3.45	3.04	4.02	3.61	5.07	2.67
Heart												
μg/g	5.10	9.80	12.21*	11.05*	3.40	23.25*	39.55*	41.25*	9.10	15.80*	17.40*	17.41*
± *SEM*	1.62	2.19	1.54	1.30	0.63	4.26	3.03	5.88	1.12	2.66	1.91	3.04
Lung												
μg/g	5.22	8.30	8.80	7.60	3.30	21.85*	42.00*	40.70*	7.89	15.24	16.50*	14.52
± *SEM*	1.69	1.61	1.48	2.67	0.83	4.64	4.39	5.20	2.92	2.61	2.23	1.86
Adipose Tissue												
μg/g	5.74	8.13	10.66	12.52*	5.90	19.60*	28.40*	33.30*	11.05	15.44	8.46*	19.83*
± *SEM*	1.73	1.81	2.02	2.55	1.18	3.29	2.77	4.33	1.84	2.24	2.74	3.06
CNS												
μg/g	5.36	7.31	8.94*	10.50*	3.80	13.54*	24.13*	30.25*	9.50	13.25	16.05*	18.21*
± *SEM*	0.71	1.42	1.31	1.92	0.92	2.88	2.26	4.41	1.33	1.42	2.05	1.64

At time 0, guanosine (8 mg/kg) was intraperitoneally administered in animals. At the indicated time points, GUO and its metabolites (GUA and XAN) were assayed by HPLC and CE analysis in samples of liver, kidney, lung, heart CNS and adipose tissue. Data are expressed as the mean ± SEM from seven animals. *$p < 0.05$ relative to time 0.

There was no significant difference between the plasma concentrations of either [^{3}H]-GUO or unlabelled GUO and its metabolites in rats with spinal cord injury and unoperated healthy rats.

Tissue Distribution of GUO and Its Derivatives

The concentration of GUO in segments of spinal cord from the lesion sites was not different from that in other parts of the spinal cord, or from spinal cord from uninjured rats or brain. Therefore values for brain and spinal cord in injured and uninjured animals were pooled as CNS.

After i.p. administration of GUO, the concentration of GUO in liver, kidney, heart and lung peaked after 7.5–15 minutes (Table 1). The maximal increase ranged from 1.8- to 2.8-fold in lung and liver respectively. Interestingly, the GUO concentrations increased in CNS and adipose tissue, and at the end of the observation period were about twice the basal values. The time course of changes in the GUO metabolites GUA and XAN was very similar in all the tissues, the maximal level being reached by about 15–30 minutes after GUO injection. At those times, the concentration of GUA increased by about 9-fold in liver and kidney and 10–12 fold in heart and lung, whereas XAN levels were about 2- to 3-fold higher than basal. Subsequently, the metabolite concentrations slowly decreased, but remained above basal values for 90 minutes (data not shown). The concentration of GUA and XAN in the different tissues was very similar, except that GUA concentrations were much higher in heart and lung than in other tissues.

There was no significant difference between brain and spinal cord in the concentration of GUO, GUA or XAN over time. Moreover, these concentrations were similar in spinal cords of rats that had received crush injury and in spinal cords of uninjured control rats.

DISCUSSION

The amount of GUO used in our previous biological experiments, 8 mg/kg, is sufficient to affect the concentration of GUO and its metabolic products in plasma and in several tissues. Thus, for example, the over-basal eGUO rapidly increased in plasma, reaching 80% of its final maximum after 7.5 minutes and peaking 30 minutes after i.p. injection, thereafter remaining constant for the next hour.

Our data indicate that despite active purine metabolism in the peripheral tissues,[11–12] eGUO and its metabolite GUA enter the system and measurably increase the concentration of GUO and GUA in the CNS. Importantly, our previous observation that remyelination occurs in response to GUO cannot be attributed to its preferential uptake at the lesion site, since eGUO was evenly distributed in the CNS. Immediately after injection of

GUO, the ratio of GUO:GUA was 2:1, but after 30 minutes the GUO:GUA ratio was about 1:3. Meanwhile the total GUO + GUA continued to increase in the CNS (brain and spinal cord) and adipose tissue at least up to 30 minutes after GUO injection. Thus, either GUO or GUA or both could exert their biological effects at a locus within the CNS. Whether the biological effects of eGUO on remyelination can be attributed to GUO or to its metabolic product GUA is under investigation.

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