Differential Distribution of Intravenous Curcumin Formulations in the Rat Brain

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Abstract. *Background: The neuropathic side-effects of trauma, stroke or therapeutic radiation of the brain for life-threatening neoplastic diseases are the result of damage to normal tissues resulting in defects in cognition and memory. Based upon published preclinical data of curcumin activity application of parenteral curcumin formulations may prove to be to be promising chemotherapy for disorders following neuropathic insults. Studies in in vitro and animal models suggest curcumin may be an effective remediative agent for brain damage. The initial steps in curcumin development for clinical applications to neuropathic disorders are formulating it for intravenous administration, determining the formulated product passes the blood–brain barrier and reaches therapeutic amounts in damaged areas in the brain with tolerable safety. Following intravenous administration of liposomal curcumin, polymeric nanocurcumin and polylactic glycolic acid co-polymer (PLGA)–curcumin in rats, these formulations were observed to cross the blood–brain barrier using a sensitive HPLC assay. All three formulations localized in specific sites in the brain without observable adverse events. One hour following intravenous injection of 5 mg/kg nanocurcumin, or 20 mg/kg PLGA–curcumin, or liposomal curcumin, up to 0.5% of the injected material localized in the brain stem, the striatum, and the hippocampus with varied accumulation and clearance rates. Conclusion: These data indicate that curcumin does localize in putative damaged brain tissues and suggest therapeutic trials be explored with all three formulations in animal models with pre- and post traumatic states.*

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Trauma, stroke and radiation injury of normal cells in the central nervous system following therapeutic interventions for neoplasia can lead to acute transient and permanent damage, depending upon the dose and volume of healthy tissues affected. Permanent reactions may include memory impairment, decreased intellect, confusion and personality changes. Dementia and neurologic deterioration following whole-brain irradiation occur in 11% of one-year survivors to 50% of two-year survivors. Where radiation involves the hippocampus, executive function, memory recall, consolidation, mood and behavioral regulation, as well multiple functional interconnections with other sites in the brain controlling motor activity, may be perturbed. Hippocampus dysfunction following radiation is one cause of neuropathic disorders characterized by cognitive impairments, memory, and aberrant recall phenomena. Pharmaceutical remediation with curcumin following brain damage requires addressing careful dosing and scheduling with respect to the occurrence of damage. A variety of molecular targets including factors triggering neuropathic processes and replacing damaged or lost neurons *via* stimulation of neurogenesis and neuroplasticity (1) and replacement of malfunctioning neuronal circuits (2) are to be considered.

Application of curcumin may address these issues by stimulating hippocampal progenitor and stem cells (1). In an *in vivo* study, curcumin prevented stress-induced decreases of 5-hydroxytryptamine1A receptor subtype mRNA and brain-derived neurotrophic factor (BDNF) protein levels, and

stimulated neurogenesis through the serotonin-1-A receptor and BNDF (3). Hypothetically, with induced neuroplasticity and migration, these new neurons may replace damaged or destroyed neurons at other sites in the brain.

Pharmacokinetic study in normal disease-free rats and focusing upon drug distribution in the brain following intravenous administration are initial steps required to demonstrate the drug is able to passage across the blood–brain barrier, the brain parenchyma with little or no accompanying adverse events, and accumulate in therapeutic concentrations in putative pathogenic sites.

Curcumin appears to be an appropriate candidate drug for stroke, trauma and radiation-induced neuropathic applications based upon *in vitro* data with curcumin in brain-derived adult neural stem cells, where it stimulated neurogenesis, synaptogenesis, and migration (1). Additional animal and biochemical analyses revealed that curcumin also acts as an antioxidant (4) and has epigenetic effects on histone H3 and H4 acetylation (5, 6). These activities tend to promote neuronal differentiation in stem cells.

Due to the insolubility of curcumin in aqueous media and its minimal bioavailability when administered by the oral route, we elected to license-in and develop liposomal curcumin, polymeric nanocurcumin, and polylactic glycolic acid co-polymeric (PLGA) curcumin: blood-soluble formulations for intravenous administration (7-9). The curcumin in these formulations was synthesized to 99.2% purity under good manufacturing practice conditions.

In pre-clinical studies in rats, we determined passage of these three formulations across the blood–brain barrier and intra-cerebral tissue pharmacokinetics following peripheral intravenous bolus injection. The objective was to determine curcumin distribution within the parenchyma and possible localization in specific brain regions associated with neurological disorders secondary to brain trauma.

Materials and Methods

Curcumin was synthesized to 99.2% purity by Sami Labs, Sabinsa Corporation, Bangalore, India and used in the liposomal, PLGA curcumin and nanocurcumin formulations. PLGA was bought from SurModic Pharmaceuticals, Inc. Birmingham, Alabama, and the PLGA-curcumin formulation was synthesized at the University of North Texas Health Sciences Center, Fort Worth, Texas. Nanocurcumin was synthesized at the Johns Hopkins Hospital Cancer Center using acrylic polymers bought from Sur Modics Pharmaceuticals, Inc. Birmingham, Alabama. Liposomal curcumin was synthesized at Polymun Scientific GmbH, Vienna Austria.

Sprague-Dawley rats weighing 250 g were purchased from Charles River, PQ, Canada and allowed to acclimatize to a 12-hour light-darkness cycle over seven days. They were given access to a commercial chow and tap water, and maintained strictly within the guidelines of institutional animal care. The investigational protocol was approved by the University of Western Ontario, Canada Animal Health Care Committee.

The caudal vein was chosen as a route for drug administration because it required minimal restraint and induced little stress. Liposomal curcumin was injected as a 20 mg/kg bolus without dilution. Nanocurcumin was solubilized in 0.9% normal saline and administered as a 5 mg/kg bolus, and PLGA-curcumin dissolved in double-distilled water was administered as a 20 mg/kg bolus. At time intervals following injection, blood samples were collected by cardiac puncture, immediately followed by euthanasia under ketamine anesthesia. Three to four rats were used for each dose and time intervals of one, two and four hours after intravenous injection. Following ketamine euthanasia, their brains were immediately removed, and portions of the cortex, left and right hippocampus, brain stem (medulla, pons, and midbrain), and striatum were dissected on an ice bed, weighed and prepared for HPLC analysis. Other brain regions, plasma, spleen, kidneys, liver were weighed, quickly frozen in liquid nitrogen and stored for further analyses.

The weighed specimens were homogenized in 20% phosphate-buffered saline. Two hundred microliters of each homogenized brain tissue were transferred to amber, labeled microcentrifuge tubes to which we added 200 μl of an internal standard (IS) of acetonitrile solution prepared at a final concentration of 0.15 μl acetonitrile per ml of phosphate buffer solution PBS. For curcumin standards, 180 μl of homogenized brain tissue from untreated rats were transferred to four amber, labeled microcentrifuge tubes, then 20 μl of known curcumin standard (0.5 μg/ml, 1.25 μg/ml, 2.5 μg/ml, and 5.0 μg/ml, respectively) were added to 200 μl of IS solution. For the blank negative control, 200 μl of homogenized control brain tissue were added to 200 μl IS. All samples, controls and standards with IS were vortexed for 10 minutes, and then centrifuged for 5 minutes at 6,000 rpm. After removing and filtering the supernatant of each sample through 0.22 μM nylon syringe filters, 50 μl of each sample were injected into the HPLC system.

For plasma determinations, 200 μl aliquots from each rat were transferred into amber, labeled microcentrifuge tubes to which 200 μl of IS were added. For curcumin standards, 180μl plasma was added to four microcentrifuge tubes and 20 μl of curcumin (0.5, 1.25, 2.5 and 5.0 μg/ml, respectively) with 200 μl of IS added. For the blank negative controls, 200 μl control plasma and IS solution were used. All samples, controls and standards with IS were vortexed for 10 minutes and then centrifuged for five minutes at 6000 rpm. The supernatants of each sample were passed through 0.22 μM nylon syringe filters, and injected in a final volume of 50 μl into the HPLC system.

Curcumin standard and emodin as the HPLC internal standard were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Bejing, China). This curcumin standard was compared with GMP grade curcumin synthesized by Sabinsa Inc., Bangalore, India. For curcumin determinations in plasma and tissues, the method adopted by Li *et al*. was used (10).

This method closely follows US Good Laboratory Practice guidelines. The HPLC analysis was carried out on a Water HPLC System consisting of Waters 1525 binary pumps, 2487 dual absorbance detector and Waters Breeze Software, 3.3 version (Waters, MA, USA). The curcumin measurements were determined using the Waters HPLC system with an Inspire C18 column (4.6×100 mm, 5 μm particle size; Kikma Technologies, Bejing, China). The mobile phase was composed of acetonitrile and 5% acetic acid (75:25, v/v) at a flow rate of 1.0 ml/min. The mobile phase was filtered through a 0.45 μM nylon membrane filter and ultrasonically degassed prior to use. The detection wavelength was 420 nm. The analysis time was 5 minutes per sample at room temperature.

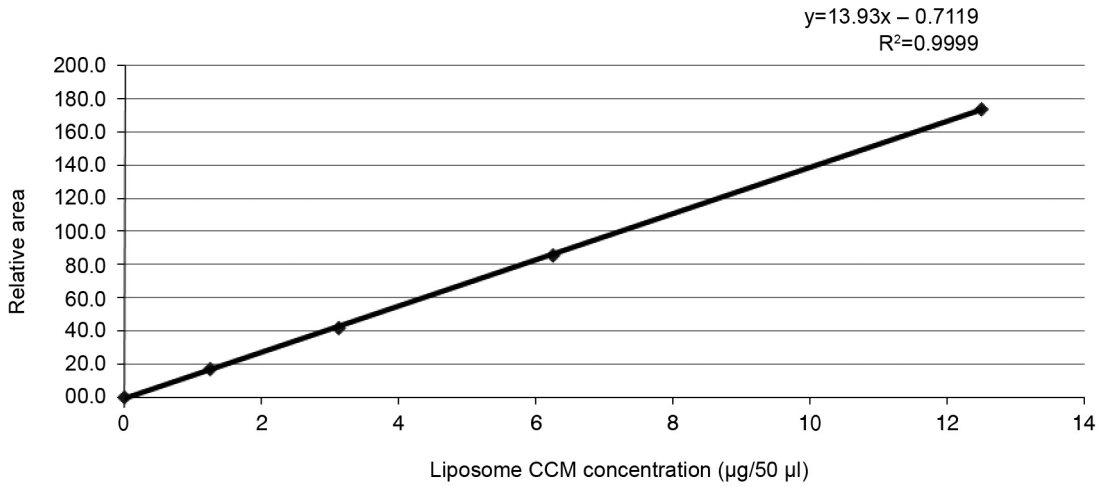

Figure 1. *Liposome curcumin (CCM) standard curve for use in HPLC analyses.*

Results

The relative area of plasma liposome curcumin standard was linear within the range of 1-12 µg/5.0 µl of the sample as determined by HPLC (Figure 1). In the absence of any prior or concomitant chemical or physical manipulation, for the liposomal formulation, the tissue:blood curcumin ratio in the hippocampus and the pooled-brain was greater than unity at the 4-hour time interval. Similarly, the striatum:blood ratio at two hours for PLGA–curcumin indicated that tissue measurements were not simply reflecting vascular curcumin and that these formulated compounds do in fact pass the blood–brain barrier (Tables I, II and III). Localization and clearance rates of the three formulations varied, suggesting that the individual formulation compositions may have modulated the cerebral pharmacokinetic characteristics.

Curcumin was detectible primarily in the hippocampus, brain stem and striatum at levels less than 0.5% of the total injected dose.

Liposomal curcumin concentrations in the hippocampus and striatal regions peaked at two hours. Pooled brain regions other than the striatum and the hippocampus had lesser or no detectible curcumin, suggesting preferential deposition. Clearance rates relative to the injection time at these two sites also varied. At two hours, the striatum had twofold greater curcumin content than the hippocampus, and following peak concentrations, clearance in the striatum was greater than that in the hippocampus (Table I).

In nanocurcumin distribution studies, due to a limited supply only 5 mg/kg total curcumin was injected into each rat. Nevertheless, there was notable accumulation in the brainstem. Further, the localization in the hippocampus was slightly greater than that observed with the liposomal preparation, with overall slower clearance rates from the striatum (Table II). In this study, the nanocurcumin clearance from the plasma between one and two hours was less than that observed with liposomal curcumin. The decline in brain tissue curcumin levels did not proportionally follow the fall in plasma. It is logical to assume curcumin levels observed at one hour would be higher than at two hours. Following nanocurcumin administration, higher levels of curcumin in the hippocampus than in the striatum as compared to liposomal curcumin were noted.

For PLGA–curcumin at 20 mg/kg, the intravenous dosage of curcumin was 2 mg/kg when corrected for the ratio of PLGA to curcumin (Table III). Uptake was highest in the striatum, followed by the hippocampus and the brainstem. A high brain tissue:plasma ratio was observed at 2 hours post-intravenous injection. Four hours post-injection, the plasma curcumin level decreased by 50%, while both curcumin levels in striatum and brain stem were undetectable. Hippocampus curcumin levels remained relatively constant at the fourth hour post-injection.

Discussion

Following intravenous bolus administration of curcumin formulations, passage across the blood–brain barrier of rats was demonstrated. Passage across the blood–brain barrier was not surprising based upon previous studies with nanocurcumin given intraperitoneally in mice (4). The impact of the solubilizing components on drug diffusion, transport and clearance from the brain parenchyma memains unclear, as are the fates of the liposomal, polymeric and PLGA chemical constituents. However, they could contribute to the variances in localization and clearance differences noted among the three formulations.

Table I. *Mean brain tissue and plasma curcumin levels in rats following intravenous administration of liposomal curcumin at 20.0 mg/kg.*

	Curcumin level		Brain/Plasma ratio	
	1-h post-injection	2-h post-injection	1-h post-injection	2-h post-injection
Striatum (ng/g)	165.0±72.2	12.0±7.4	1.00	0.81
Hippocampus (ng/g)	83.0±35.0	41.0±40.0	0.50	2.61
Brain stem (ng/g)	43.0±38.1	62.0±0. 6	0.23	4.01
Plasma (ng/ml)	166.0±15.0	15.4±13.2	……	……..

Values are expressed as the mean±SEM (n=4 at each time interval).

Table II. *Mean brain tissue and plasma curcumin levels in rats following intravenous administration of nanocurcumin at 5.0 mg/kg.*

	Curcumin level		Brain/Plasma ratio	
	1-h post-injection	2-h post-injection	1-h post-injection	2-h post-injection
Striatum (ng/g)	69±9.05	48.18	0.018	0.024
Hippocampus (ng/g)	80±11.9	54.41	0.020	0.027
Brain stem (ng/g)	120±22.78	235.08	0.030	0.120
Plasma (ng/ml)	3913±924	1980.01	…….	…….

Values are expressed as the mean±SEM n=2 for the 1-hour group and n=1 for the 2-hour group. Total curcumin as the active principal ingredient injected was 1.6% or 0.08 mg/kg due to limited supply of Nanocurc® at the time of this study.

Table III. *Mean brain tissue and plasma curcumin levels in rats following intravenous administration of PLGA-curcumin at 20 mg/kg.*

	Curcumin level		Brain/Plasma ratio	
	1-h post-injection	2-h post-injection	1-h post-injection	2-h post-injection
Striatum (ng/g)	23.3±13.4	0	2.8	……..
Hippocampus (ng/g)	5.2±8.2	5.1±5.2	0.63	1.1
Brain stem (ng/g)	5.3±5.4	0	0.65	……..
Plasma (ng/ml)	8.2±4.5	4.5±4.6	…….	…….

Values are expressed as the mean±SEM, n=3/group. For PLGA–curcumin, the purity of free curcumin was 99.2% and the relative ratio of free curcumin to PLGA copolymer was 1:9, hence the intravenous dose of free curcumin in the PLGA–curcumin formulation was 2 mg/kg.

While the dosages were different in these studies, the important observation is that these three curcumin formulations pass the blood–brain barrier, a first obstacle to treatment of neuropathic disorders. Because of the low availability of drug at the time of this study, a limited number of rats, and sampling times for each formulation was tested. Detectible differences among the three formulations in different sites reflect the net effect of influx, residence time, and efflux properties. Vascular anatomy or blood flow at different sites in the rat may be considered a contributing factor, however, the high striatum and brain stem levels in the liposomal- and nanocurcumin-treated rats, respectively, may not extrapolate to humans since studies correlating local blood flow with high resolution anatomy in patients did not disclose any relative increase in vascularity in the hippocampus, striatum and brain stem compared to the rest of the brain (11).

The mechanisms contributing to focal distribution and uptake of these three curcumin formulations in specific brain regions, remain cryptic and cannot be explained by passive processes that depend upon physicochemical characteristics such as lipophilicity, or molecular weight. Curcumin may not be unique in its pharmacokinetic profile or specific cerebral localization patterns when compared with a number of different neurotropic compounds. However, the specific distribution of curcumin is suggestive evidence of potential efficacy for curcumin in the treatment of neuropathic disorders secondary to brain damage from trauma, stroke or radiation. This is particularly applicable to hippocampus neuronal stem and progenitor cells, where low concentrations of curcumin (500 nM) stimulate, and high concentrations (10 μM) inhibit neurogenesis and neuroplasticity (1).

In experiments with rats, after intravenous injection of liposomal curcumin, polymeric nanocurcumin, and PLGA–curcumin, passage through the blood–brain barrier and distribution to putative neuropathic disease-associated sites suggest that normal traumatized adult brain cells may be functionally improved following curcumin exposure, depending the initial extent of damage. Embryonic neural stem cells in the dentate gyrus of the hippocampus if irreversibly damaged by irradiation for example, may not be responsive to curcumin. Evaluating animal models with different degrees of radiated hippocampus, brain stem or striata should allow determination as to what extent of radiation damage is ameliorated by curcumin, and which of the three formulations may be optimum.

Conclusion

These data indicate that following intravenous injections, curcumin formulations composed of liposomes, acrylic polymers (nanocurcumin), and PLGA cross the blood–brain barrier and preferentially localize in the hippocampus, the striata, and brain stem. These observations of curcumin localization at specific brain sites at concentrations which may have antioxidant, anti-inflammatory, positive neurogenesis and neuroplasticity activity support clinical applications for remediation of neuropathic disorders following trauma, stroke and therapeutic radiation affecting normal brain tissues. Data concerning scheduling and dose–response characteristics of each of the formulations are required in post-trauma animal models to plan an evidence-based treatment paradigm for eventual clinical trials.

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Conflict of Interest

Lawrence Helson is CEO and President of Sign Path Pharma, Inc., a commercial developer of parenteral curcumin formulations. Muhammad Majeed is CEO of Sabinsa Inc., a manufacturer of GMP grade 99.2% pure curcumin.

Contributions

SC conceived the project, carried out the injections, necropsies and co-wrote the manuscript, LH organized the project, and co-wrote the manuscript. EL assayed the blood and tissue curcumin levels, MM supplied the GMP grade curcumin, JKV, AR, conceived and produced PLGA-curcumin, AM and DP conceived and produced nanocurcumin, JAS carried out the initial animal toxicology and dose finding studies of liposomal curcumin.

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