Tissue Distribution of (Lipocurc™) Liposomal Curcumin and Tetrahydrocurcumin Following Two- and Eight-hour Infusions in Beagle Dogs

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Abstract. *This study interrogated whether different durations of intravenous infusions of lipocurc™ would alter curcumin metabolism, tissue distribution and whether treating necropsied tissues of Beagle dogs with phosphoric acid prior to measuring curcumin and its metabolite, tetrahydrocurcumin (THC), would stabilize the compounds allowing for accurate analytic measurements. Two cohorts comprising two male and two female dogs were infused each intravenously with 10 mg/kg lipocurc™ , either over two hours or over eight hours. Tissue data from each cohort was averaged from four dogs. Curcumin and THC distributed among all 13 tissues were examined at necropsy. The highest curcumin level was observed in the lungs followed by the liver. Tissue levels of curcumin in the lung, spleen and liver increased substantially following the eight-hour infusion compared to the two-hour infusion. The pancreas, kidney and urinary bladder also contained relatively high curcumin levels. Tissue partition coefficients for curcumin and THC were also higher for the eight-hour infusion than the two-hour infusion. The tissue THC/curcumin ratio varied in a tissue-specific manner and was lower for the eight-hour compared to the two-hour infusion. In conclusion, this raised the possibility that prolonged infusion of curcumin may facilitate distribution into tissues via a transporter-dependent mechanism and elevated tissue concentrations of curcumin may inhibit or saturate a putative reductase enzyme converting curcumin to THC. The addition of phosphoric acid stabilized the levels of curcumin and THC in some but not all the examined tissues, raising issues of tissue-specific curcumin and THC stability.*

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SignPath Pharma contributed to the cost of the study. Sabinsa Inc. manufactured and contributed the 99.2% pure synthesized curcumin.

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Deciding on clinical applications of a putative therapeutic compound requires information about its distribution in the organs and tissues of the body following administration. Such data allow identification of the most probable sites of therapeutic response and toxicity. To obtain this information it is essential to have an analytical procedure that is specific and sensitive, a compound or its metabolites that are unaffected by the analytical procedure, and sufficiently stable, to be accurately and reproducibly measurable when distributed in different tissues. The route of administration is critically important to a successful outcome. Oral administration of drugs is readily-accepted by patients, however it is also susceptible to limiting constraints such as solubility, gastro-intestinal degradation and ultimately limited bioavailability. Curcumin or diferuloylmethane, the drug of interest in this study, is considered to be an active disease preventative and a possible therapeutic compound based upon several thousand years of traditional use, and recent *in vitro* and animal model studies. However, it is insoluble in aqueous media, very unstable under conditions of ambient light, room temperatures, basic pH, and when administered orally is readily metabolized or degraded. The enzymatic reduction of curcumin to tetrahydrocurcumin (THC) as a major active metabolite is well-established. The identity of the enzyme reducing curcumin to THC in intestinal *E. coli* has recently been published (1). This enzyme, a medium-chain dehydrogenase/ reductase (MDR) belongs to a very large group of enzyme superfamilies found in all kingdoms of life, involved in metabolism, and multiple regulatory processes (2). Apart from its discovery in human

Table I. *Summary of treatment groups.*

Groups	Dose (mg/kg)	Concentration of curcumin (mg/mL)	Infusion rate mL/kg/hr	Duration of infusion (hr)	Number of beagle dogs on study	
					M	F
1. Part A, Lipocurc™	10	0.5	10	2	2	2
2. Part B, Lipocurc™	10	0.125	10	8	2	2

intestinal *E. coli*, no further documentation asserting its presence in animal or human tissues have been published. Hence, the inference that the enzyme termed NADH-dependent dihydrocurcumin reductase is present in animal and human tissues is indirectly supported by determinations of the presence of THC. Curcumin and its reductive metabolites may also be inactivated when conjugated with sulfates or glucuronides in the liver or the intestinal wall which effectively leads to reduced bioavailability. The negligible impact of curcumin as a therapeutic in over 69 clinical trials bears this out (3). For these reasons parenteral applications of formulated curcumin are preferred routes of administration. SignPath Pharma, Inc. is developing liposomal curcumin (Lipocurc™), an intravenous formulation of 99.2% pure synthesized curcumin, which upon intravenous administration avoids the pitfalls of the oral route and allows higher blood concentrations than those achieved by the oral route. However, there remain unknown drug-induced physiologic and pathologic effects, and changes in tissue distribution, metabolism, and excretion profiles following intravenous administration. Curcumin's concentration, dependent on rapid reactivity with multiple intracellular targets suggests that modifying the rates of infusion and the consequent quantitative exposure to curcumin, over time, could translate into a spectrum of clinical effects. Supporting this notion is the *bi*phasic dose-related activity of curcumin documented by proliferative stimulation of hippocampal stem and progenitor cells, and induction of autophagy and apoptotic mechanisms in malignant and normal cells to changes in membrane homeostasis. As an example, in pre-clinical animal studies in dogs acute red blood cell hemolysis following bolus injections of lipocurc™ was abrogated when the dose and infusion duration was extended to two hours (4).

Materials and Methods

Tissue concentrations of curcumin and THC were determined by LC-MS/MS with and without phosphoric acid (H_3PO_4) exposure, following two-hour or eight-hour intravenous infusions of lipocurc™ in two cohorts of two female and two male Beagle dogs, as shown in Table I. For bone marrow, due to the limited size of the sample, only curcumin and THC levels were determined in samples not stabilized with phosphoric acid. These data report the distribution and fate of lipocurc™ and THC in 13 sampled tissues (brain cortex, brain stem, hippocampus, striatum, heart, lungs, muscle, liver, kidney, pancreas, intestinal wall, urinary bladder and bone marrow), following the termination of an intravenous infusion of lipocurc™ at a dose of 10 mg/kg. Fifteen minutes following either the 2-hour or 8-hour infusion, blood, urine and bile samples were taken, prior to the dogs being necropsied and organs were removed. Multiple samples of tissue weighing approximately one gram were snap-frozen in the presence or absence of phosphoric acid (H_3PO_4). In a previous report in this same group of dogs, plasma curcumin, and THC pharmacokinetics, urinary and biliary excretion parameters were determined (5). In this report, tissue distributions are examined within the context of limited previous observations in the literature. For all tissue samples, the levels of curcumin and THC were determined using a method developed by the Bioanalytical Department at Nucro-Technics (6). Phosphoric acid was used to treat one set of samples based on preliminary studies indicating that phosphate increased the stability of curcumin and THC in the tissue matrix. Values that were below the limit of quantification were assigned a value of 0. As there were no consistent differences between the tissue levels of curcumin in male and female dogs, the average plasma concentrations from male and female dogs was analyzed as the mean±standard error (S.E.)

Results

The distribution of curcumin and THC in tissues and the ratio of THC to curcumin in plasma and tissues are presented in Tables II-V and illustrated in Figures 1 and 2. Curcumin and THC were widely distributed amongst the 13 tissues assessed including the bone marrow. The addition of phosphoric acid to plasma rendered a stabilizing effect on curcumin and THC and resulted in significantly higher concentration values compared to un-stabilized plasma samples. However, independent of this effect, changes in detection values with addition of phosphoric acid in tissues were variable, tissue-specific and overall more evident for THC. In brain tissues, phosphoric acid had a clear stabilizing effect, again more prominent for THC. The stabilizing effect of phosphoric acid was minor or absent in heart, kidney and other tissues. In contrast there is a consistency in plasma, bile, urine and other tissue levels of curcumin and THC stabilized with phosphoric acid. For bone marrow, due to the limited size of the sample, only curcumin and THC levels were determined in samples

Table II. *Tissue distribution of curcumin and THC in untreated and tissues treated with phosphoric acid; 2-hour infusion with Lipocurc™ .*

Tissue	Levels (ng/g)			
	Curcumin	Curcumin+ H_3PO_4[1]	THC	THC+ H_3PO_4[1]
Cortex, brain	0.52±0.05	0.74±0.013	0.68±0.05	3.08±0.30
Hippocampus	0.00±0.00	0.09±0.09	0.75±0.09	6.46±1.82
Striatum	0.33±0.10	0.48±0.07	6.22±3.10	11.12±1.42
Brain stem	0.30±0.04	0.45±0.06	2.34±0.34	10.62±1.30
Heart	0.49±0.08	0.48±0.09	2.51±0.68	0.69±0.42
Lungs	86.82±24.99	22.86±2.14	17.75±5.11	9.43±2.67
Muscle	1.23±0.32	0.19±0.02	1.13±0.30	0.90±0.23
Spleen	0.48±0.08	0.08±0.04	1.35±0.32	1.60±0.42
Liver	4.28±1.90	1.82±0.45	0.52±0.15	0.98±0.18
Kidney	1.03±0.17	0.89±0.15	3.06±0.63	4.25±0.61
Pancreas	2.81±2.02	0.85±0.23	2.02±0.71	0.92±0.35
Intestinal wall	2.97±0.98	1.14±0.26	0.73±0.40	2.12±0.89
Urinary bladder	0.60±0.07[2]	0.69±0.12[2]	3.24±1.27[2]	2.26±0.51[2]

[1]Tissue samples were dipped in diluted phosphoric acid prior to freezing at –80˚C. Values are the mean±standard error of the mean, n=4. [2]n=3

Table III. *Tissue distribution of curcumin and THC in untreated and tissues treated with phosphoric acid; 8-hour infusion with Lipocurc™ .*

Tissue	Levels (ng/g)			
	Curcumin	Curcumin+ H_3PO_4[1]	THC	THC+ H_3PO_4[1]
Cortex, brain	0.72±0.18	0.81±0.015	0.06±0.04	0.49±0.12
Hippocampus	0.00±0.00	0.01±0.01	0.01±0.01	1.13±0.35
Striatum	0.15±0.02	0.49±0.08	1.12±0.11	3.14±0.26
Brain stem	0.41±0.10	0.58±0.04	0.83±0.08	3.02±0.37
Heart	0.67±0.15	0.75±0.17	0.51±0.08	0.03±0.03
Lungs	317.93±101.28	250.75±56.42	10.81±2.50	6.36±2.13
Muscle	3.25±1.31	0.79±0.24	0.06±0.05	0.16±0.05
Spleen	28.64±10.84	22.91±6.83	0.18±0.12	0.43±0.02
Liver	39.38±13.70	28.38±10.30	0.54±0.14	0.45±0.13
Kidney	2.71±0.65	2.77±1.04	1.32±0.18	2.04±0.32
Pancreas	1.88±0.62	2.84±0.76	0.34±0.19	1.34±0.52
Intestinal wall	1.79±0.53	0.84±0.17	0.31±0.31	0.21±0.12
Urinary bladder	0.84±0.20	0.87±0.34	1.37±0.42	1.11±0.41

[1]Tissue samples were dipped in diluted phosphoric acid prior to freezing at –80˚C. Values are the mean±standard error of the mean, n=4.

Table IV. *Ratio of THC/curcumin in plasma and tissues.*

Tissue	[THC]/[Curcumin][1]	
	2 hr	8 hr
Plasma	12.88	3.98
Cortex, brain	4.16	0.60
Hippocampus	71.78	113.00
Striatum	23.17	6.41
Brain stem	23.60	5.21
Heart	1.44	0.04
Lungs	0.41	0.03
Muscle	4.74	0.20
Spleen	20.00	0.02
Liver	0.25	0.08
Kidney	4.78	0.74
Pancreas	1.08	0.47
Intestinal wall	1.86	0.25
Urinary bladder	3.28	1.28

[1]The plasma concentrations used to calculate the ratio of [THC]/[curcumin] are defined in the legend of Table V.

Table V. *Tissue partition coefficients (Kp) for curcumin and THC following 2 and 8 hour infusions.*

Tissue	Kp [tissue]/[plasma][1]			
	Curcumin, 2 hr	THC, 2 hr	Curcumin, 8 hr	THC, 8 hr
Cortex, brain	0.0046	0.0015	0.0780	0.0119
Hippocampus	0.0006	0.0031	0.0010	0.0274
Striatum	0.0030	0.0054	0.0472	0.0760
Brain stem	0.0028	0.0051	0.0559	0.0731
Heart	0.0030	0.0003	0.0723	0.0007
Lungs	0.1419	0.0045	24.1570	0.1540
Muscle	0.0012	0.0004	0.0761	0.0039
Spleen	0.0005	0.0008	2.2071	0.0104
Liver	0.0113	0.0005	2.7341	0.0109
Kidney	0.0055	0.0020	0.2669	0.0494
Pancreas	0.0053	0.0004	0.2736	0.0324
Intestinal wall	0.0071	0.0010	0.0809	0.0051
Urinary bladder	0.0043	0.0011	0.0838	0.0269

[1]The plasma concentrations used to calculate the plasma [THC]/[curcumin] ratios were an average of the plasma concentration measured at the end of the infusion period and 15 minutes post infusion and were for 2 and 8 hr curcumin concentrations, 161.11 and 10.38 ng/mL, respectively and for 2 and 8 hr THC concentrations, 2075.15 and 41.30 ng/mL, respectively.

not stabilized with phosphoric acid. Following the two-hour infusion, the tissue distribution was 13-254-fold higher for curcumin in the lung (22.86 ng/g) compared to other tissues. The next highest tissue was the liver (1.82 ng/g), with distribution in other tissues ranging from 0.08 to 1.14 ng/g. The concentration ratio of THC/curcumin (Table IV) varied according to the specific tissue and similar to plasma, was greater than 1 for many tissues. The ratio was particularly high in brain tissues and lower in lung and liver. The 2-hour and 8-hour infusion levels of curcumin and THC were 0.06±0.06 ng/g and 2.81±0.6 ng/g, and 0.87±0.2 ng/g and 0.00±0.00

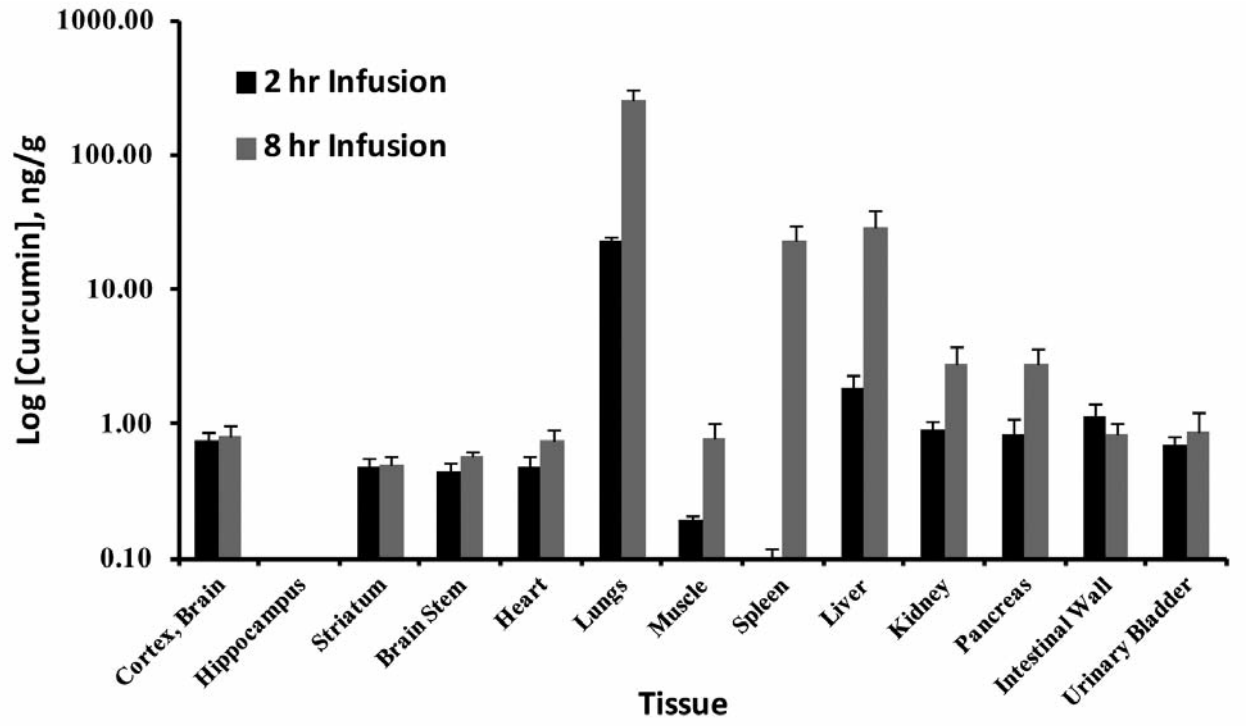

Figure 1. *Tissue distribution of curcumin following 2- and 8-hour infusions of Lipocurc™ . Values are presented as the mean±standard error of four determinations.*

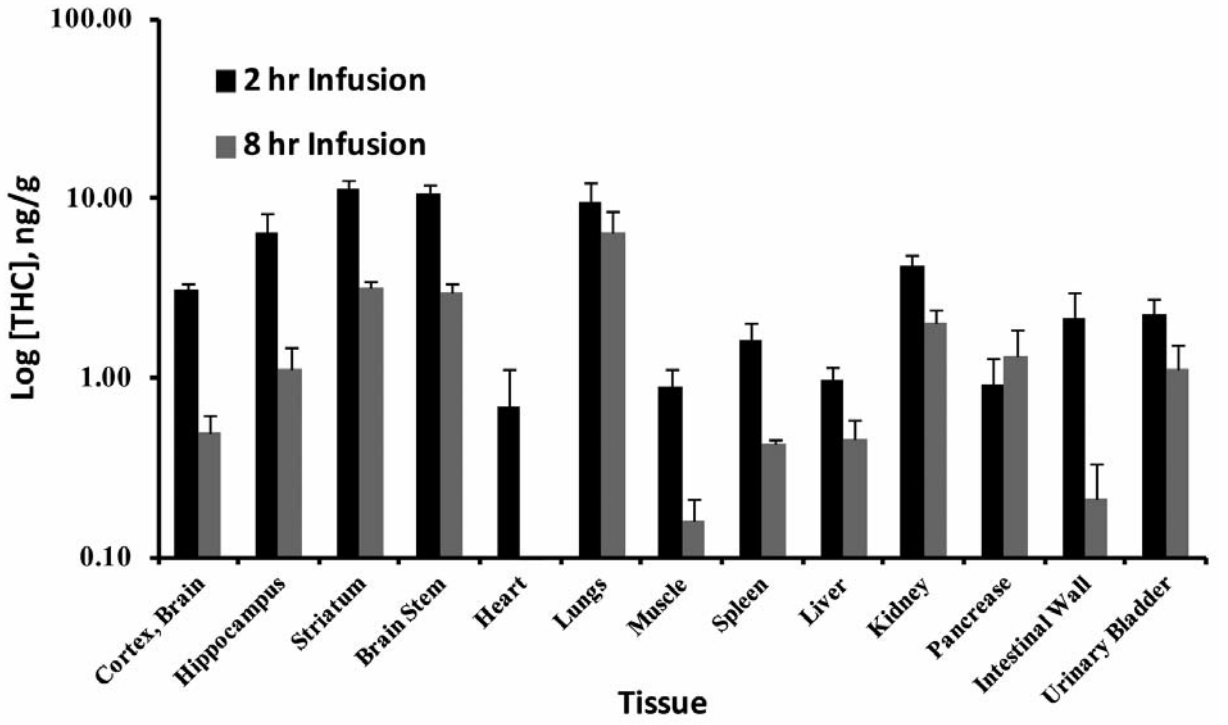

Figure 2. *Tissue distribution of THC following 2- and 8-hour infusions of Lipocurc™ . Values are presented as the mean±standard error of four determinations.*

ng/g, respectively. Following the eight-hour infusion of 10 mg/kg Lipocurc™ at a low infusion rate and concentration, the distribution of curcumin and THC were altered significantly. Under these conditions, the lung and liver again had the highest and second-highest levels of curcumin. However, there were now clearly increased concentrations of THC in the lungs, liver and spleen. Comparing the two-hour with the eight-hour infusion, curcumin levels were 22.86 *vs*. 250.75 ng/g, in the lung, 1.82 *vs*. 28.38 ng/mL in the liver and 0.08 *vs*. 22.91 ng/mL, in the spleen. The highest level (250.75 ng/g) of curcumin observed in the lung following a 10 mg/kg total dose, is equivalent into a tissue concentration of 0.68 μM on the premise that 1 gram tissue is equivalent to 1 mL of volume. In other tissues, curcumin levels ranged from 0.01-2.84 ng/g. The levels of THC in other tissues were comparable or lower to those observed with the two-hour infusion.

Discussion

This study of parenteral curcumin revealed significant differences in tissue curcumin, and its metabolite THC following two different infusion rates of the same dosage in dogs. These differences may be due to differing metabolic profiles, lipid content of the tissues, or acid-base conditions for each tissue. The distribution to the lung was significantly higher than the rest of the body tissues. The reason for this high distribution of curcumin compared to THC and the remainder of tissues is unknown. Presumably it may be due to curcumin's high lipophilicity, a lower rate of reductive metabolism to THC (*i.e*. decreased activity of NADH-dependent dihydrocurcumin reductase (1), or conversion to other metabolites in lung tissues. The increased tissue incorporation of curcumin in the lung, liver, and spleen following the eight hours of infusion is consistant with the assumption that prolonged infusion of curcumin enhances tissue uptake and elimination from the plasma and simultaneously if sufficient accumulation occurs, it may impact on the reductase enzyme. This observation is not entirely surprising, since in a recent published study of curcumin–loaded PLGA particles using an HPLC method, curcumin was preferentially localized in the spleen and secondarily in the lung of Sprague-Dawley rats (7). The differences in distribution may be ascribed to the carrier, analytical method, questionable stabilization of curcumin, local tissue factors in the species tested and the observation that curcumin's metabolism and excretion following intravenous infusion in dogs is, in contrast to rats, following intravenous administration where it is actively metabolized in the liver and transported into the biliary system. Comparison of the tissue partition co-efficients (Kp) sheds additional light on the tissue distribution of curcumin and THC in dogs (Table V). Following two0- and eight-hour infusions, at 10 mg/kg total dose, the majority of the Kp values for curcumin and THC were below one: suggesting a poor distribution of curcuminoids into tissues. This may be relevant to explain the low oral bioavailability of curcumin and limited beneficial therapeutic effects (3, 8). Low Kp values have also been observed in rodent studies and ranged from 0.06-0.25 in the rat (3, 8). Exceptions to this were the liver and lung with curcumin Kp values of 1.6 for the lung at two hours and 21.3, 2.6 and 1.9 for the lung, liver and spleen, respectively with eight hours of infusion. The Kp values are higher for curcumin than for THC, suggesting that either curcumin has greater accessibility than THC to tissues or that THC is more effectively removed from tissues than curcumin, or differences in enzyme distribution and relatively decreased THC being produced. The Kp values are higher among all tissues for both curcumin and THC following the eight-hour infusion compared to the two-hour infusion. This latter point highly supports an enhancement of the tissue distribution of curcuminoids with longer infusion. In the literature, curcumin has been reported to inhibit the transporter-mediated efflux of drugs from cells (9, 10). At the mechanistic level, this may indeed explain the increased uptake of curcumin into tissues with a longer infusion and inability to attain steady-state plasma levels. Essentially, as infusion proceeds, curcumin levels build-up in tissues and begin to progressively inhibit efflux, resulting in greater cellular sequestration over time, the extent of which in any one tissue is dependent on the balance between uptake and efflux transporter activity. The higher levels of THC in tissues at eight hours may be a consequence of the metabolism of the higher tissues levels of curcumin. Thus, the rapid clearance of curcumin from the circulation is dependent not only on metabolic clearance but also on tissue transporter (uptake and efflux)-mediated clearance. In conclusion, infused liposomal curcumin susceptibility to a complex of reductive and conjugating enzymes produces different metabolites. The major metabolite with biological activity affecting multiple cellular targets is THC. Pharmacokinetic and distribution data of curcumin and THC in plasma and tissues reflect their net contribution to therapeutic effects. This leads to ambiguous issues of causal relationships between the parenteral drug curcumin, its metabolite THC, and biological-effects. There is a crude proportionality between the amounts determined in plasma and tissues and the aggregate of therapeutic effects of the sum of the molecules, however outstanding tissue localization of curcumin or THC is an important key to disorders of these same tissues. In addition, tissue differences in metabolism, and the modifying effects of infusion rates on distribution and metabolite formation offer additional clues to choosing dosage schedules and clinical targets. An additional area of ambiguity in determining distribution in tissues and plasma of animal models and humans is the need for stabilization of curcumin to prevent spurious data (5).

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References

1 Hassaninasab A, Hashimoto Y, Tomita-Yokotani K and Kobayashi M: Discovery of the curcumin metabolic pathway involving a unique enzyme in an intestinal microorganism. Proc Natl Acad Sci USA *108(16)*: 6615-6620, 2011.
2 Knoll M and Pleiss J: The medium-chain dehydrogenase/reductase engineering database: a systematic analysis of a diverse protein family to understand sequence-structure-function relationship. Protein Sci *17(10)*: 1689-1697, 2008.
3 Clinical Trials. Gov (curcumin), 2012.
4 Helson L: Intravenous infusion of curcumin and a calcium channel blocker. Patent application number: 20110117186, 2011
5. Helson L, Bolger G, Majeed M *et al*: Infusion Pharmacokinetics of Lipocurc™ and its Metabolite Tetrahydrocurcumin in Beagle Dogs. Anticancer Res *(32)10*: 4365-4370, 2012
6. Nucro-Technics Bioanalytical Report: Project # 253395, 2012.
7 Tsai Y-M, Chien C-F, Lin L-C and Tsai T-H: Curcumin and its nano-formulation: The kinetics of tissue distribution and blood-brain barrier penetration. Int J Pharmaceutics *416*: 331-338, 2011.
8 Suresh D and Srinivasan K: Tissue distribution and elimination of capsaicin, piperine and curcumin following oral intake in rats. Indian. J Med Res *131*: 682-691, 2010.
9 Tang X-Q, Bi H, Feng J-Q and Cao J-G: Effect of curcumin on multidrug resistance in resistant human gastric carcinoma cell line SGC7901/VCR. Acta Pharmacologica Sinica *8*: 1009-1016, 2005.
10 Shukla S, Zaher HM, Harta A, Bauer B, Ware JA and Suresh VA: Curcumin inhibits the activity of ABCG2/BCRP1, a multidrug resistance-linked ABC drug transporter in mice. Pharm Res *26*: 480-487, 2009.

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