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Bioavailability and pharamcotherapeutics of nifedipine in human after single oral dose administration

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Abstract

To determine bioavailability and pharmaco-therapeutics of nifedipine in human after single oral dose administration. Six male healthy human subjects, participating in this complete crossover study, received 2 × 10-mg nifedipine capsules of reference and test batches. After drug administration, blood samples of the subjects were collected up to six hours and analyzed using HPLC. Mean AUC was observed as 1939.54 ng.h.ml-1 and l820.19 ng.h.ml-1 for Test and Reference batches respectively. Mean peak plasma concentrations were found to be 220.83 ng.ml-1 and 210.33 ng.ml-1 while time to peak plasma level were 1.25 h and 1.58 h for treatment and control batches respectively. Complete bioequivalence metrics are determined and thus this study may serve as a model for further bioequivalence studies in the country. The pharmacodynamic parameters such as blood pressure and pulse rate of the participating subjects were monitored throughout the study which, when examined for correlation with the drug blood levels, revealed a high degree of similarity in physiologic ranges and thus confirmed the earlier studies.

Key words: Nifedipine, bioequivalence, pharmacodynamic parameters.

Introduction

Nifedipine is an important member of widely used 1, 4-dihydropyridine group of calcium channel blockers. Nifedipine selectively inhibits calcium ion influx across the cell membrane of cardiac muscles without changing serum calcium concentrations (Zajc et al. 2005, Toal 2004, Godfrained et al. 1984, Katz et al. 1984). Nifedipine biopharmaceutic behavior in different populations differs very markedly. It necessitates research on bioavailability and pharmacodynamics of the drug in different populations. Much work has been done on nifedipine biopharmaceutics in different parts of the world including population samples from South Asia and Bangladesh. Bioavailability of nifedipine from oral, immediate release is 56%. Large inter- individual differences are observed in this respect (Wonnemann et al. 2006, Kleinbloesem et al. 1984). Reported values of the percent bioavailability range from 43% to more than 65% (Foster et al. 1983, Dai et al. 2001, Grundy and Foster 1996, Jakobson et al. 1979). Liver cirrhosis, age and ginkgo biloba affect the bioavailability of nifedipine (Kleinbloesem et al. 1985, Robertson et al. 1988).

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The basic idea behind this study was to initiate the biopharmaceutic work on nifedipine in local population. For this purpose, the comparative bioavailability of two different batches of the brand leader product Adalat® is evaluated. Besides the pioneering bioavailability work on the drug, a correlation between the blood levels of the drug and pulse rate, systolic and diastolic blood pressures were also aimed to investigate and establish.

The present study was, therefore, under taken to evaluate in vivo performance of nifedipine in local population, which has yet to date not carried out, to compare the bioavailability of two batches of the brand leader product Adalat® to confirm the pharmacodynamic relationship between nifedipine blood levels and therapeutic / pharmacological effects and to suggest a rational dosage regimen on the basis of blood levels observed in local population.

Materials and Methods

Human experimental protocol: The study was an open, single dose, complete two periods, crossover study. Volunteers received each of two treatment regimens once, after one-week washout period between doses. Drug administration began approximately at 7:30 a.m. on the study day. Each subject received 2 × 10mg nifedipine capsules of two different batches of Adalat® manufactured by Bayer Pharmaceuticals.

The specifications of the two batches are given below:

Batch-1: (Test batch) Batch No. 5801-R, Mfg. Date: 01-2008, Exp. Date. 01-2011

Batch-2: (Reference batch) Batch No. 5810-S, Mfg. Date: 05-2007, Exp. Date. 04-2010

Panel composition: The panel of subjects consisted of six healthy adult non-smoking male volunteers. They ranged in age 25 - 72 (mean = 34.67) years, weight 54 - 74 (mean = 59.83) kg and height 172.7 ± 1.8 cm.

Selection criteria: The selection of volunteers was carried out carefully. Only those subjects were selected for study program, who were devoid of any previous unwanted reaction to nifedipine and cardiovascular disease and hypertension. Previous physical examination, Clinical laboratory determination, previous health history confirmed normal kidney, liver, lungs and cardiac functions. All of the selected subjects were physically vigorous, having sound health and well aware of the whole experiment. They were also educated about unwanted effects produced by nifedipine in normal subjects. They gave their written, informed consent to take part in the study.

Subjects were excluded, from participation, if there was a medical history or evidence upon physical examination of hypertension and cardiovascular disorder or use of an addicting drug substance.

Procedure: Subjects were instructed to be drug free, including OTC drugs for twelve days before and during the study. The selected volunteers attended the Clinical laboratory twice, for in vivo studies, with a washout period of one week between the doses. Volunteers reported the lab in fasting state for last ten hours on each day of the study. After dose administration victual was allowed in systematic plan. Foodstuff was permitted in the form of standardized meals served at two hours (breakfast) and six hours (lunch) after dosing, respectively. The meals consisted of the following in both studies:

1. Break fast: Cheese, sandwiches and a cup of milk.
2. Lunch: Escallop, rice, carrots, wheat bread and milk tart dessert.

The volunteers received 200 ml of drinking water at 2, 4 and 6 h after drug administration and 100 ml of orange juice with their meals. During the study, subjects were requested to remain in a sitting position for

three hours after drug administration. Subjects remained in the laboratory throughout the study each day, where they were carefully monitored for the appearance of any side effects or unwanted reactions until released from the facility eight hours after the dosing.

Blood sampling: Exactly the same procedure and schedule for blood sampling was adopted on both the occasions. An indwelling venous cannula with heparin lock was inserted in a suitable forearm vein and heparinized venous blood sample were collected and transferred into heparinized vacutainers according to the following time schedule:

Before drug administration (0 h) and at 0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00, 4.00, 6.00, 8.00, 10.00 and 12.00 h after dose administration. The 0 hour samples were sufficient to yield 8 ml plasma; all subsequent samples yielded 4 ml plasma. Blood samples were centrifuged immediately and from each sample two aliquots of plasma were transferred to labeled tubes and stored at -20 °C pending nifedipine assay.

Prevention from photolysis: Due to the documented light sensitivity of nifedipine to routine light sources, drug dosing, blood sampling, and plasma harvesting procedures were performed in windowless rooms lit with sodium lamp or subdued lighting. No white fluorescent lighting was used. To prevent from direct sun lighting, most possible procedures were carried out at night. To prevent photo-degradation during blood sampling, vacutainers were wrapped immediately with aluminum foil, placed on ice and transferred to a room with yellow lights.

Haemodynamic response: Heart rate, systolic blood pressure (SBP) and diastolic blood presume (DBP) were recorded before the dose, after at least 15 minutes of rest at sitting posture, and immediately before drawing each blood sample until 6 hours. The same technical staff throughout the study period took measurements.

Analysis of blood samples, Analytical method: Nifedipine plasma levels were measured by a specific high performance liquid chromatographic (HPLC) method (Snedden et al. 1984). Mobile phase consisted of phosphate buffer and acetonitrile (1:1, pH 6.8). The mobile phase was passed through a Bonda pack C-18 column at flow rate of 1.3 ml/min. UV detector at 238 nm detected the drug. The nifedipine was extracted from the plasma by ethyl acetate under vacuum which proved to be simple efficient and reproducible.

To a 3 ml stopper vial, 1 ml plasma was taken and extracted with 3 ml ethyl acetate using vortex, first time with 2 ml ethyl acetate and second time with 1ml ethyl acetate. Ethyl acetate layer was collected into another separator stopper tube. 2 ml of this ethyl acetate extract was pipette out in 5 ml beaker. It was dried under vacuum. Accurate volumes of acetonitrile and phosphate buffer pH 6.8 were mixed gently with the ratio of 1:1 (v/v) to prepare mobile phase. Dried substance was reconstituted in 0.5 ml mobile phase. An appropriate aliquot was injected into 50 µl injector of HPLC system. The HPLC apparatus consisted of a pump and a spectrophotometeric variable detector (Shimadzu, Japan), data modulator (CBM 101, Shimadzu, Japan) and HPLC syringe (Pyrex, England). Chromatographic separation was performed using reverse phase C-18 HPLC column (Waters, USA). The mobile phase consisted of accurate volumes of acetonitrile and phosphate buffer pH 6.8 with the ratio of 1:1 (v/v). Flow rate was 1.3 ml/minute at ambient temperature. Quantitation was achieved by comparing peak-height ratio of nifedipine in plasma to those prepared by spiking blank plasma samples with various concentrations of nifedipine.

Data analysis: The relevant individual profiles observed in the present work were processed for bioavailability parameters by using residual technique. Using the non-compartmental approach the plasma levels of nifedipine were employed to compute their individual disposition kinetics. For the calculation of mean residence time of the drug, the following equation was employed:

$$MRT = AUMC_{0-\infty}/AUC_{0-\infty}$$

Where, MRT = Mean residence time (h)

AUC = Area under the curve ($ng.h.l^{-1}$)

AUMC = Area under the first moment curve ($ng.h^2.l^{-1}$)

A computing program was developed for the computation of above mentioned parameters.

Results and Discussion

Blood level data: Plasma levels ($ng.l^{-1}$) and bioequivalence metrics of nifedipine after oral administration of 20 mg of the dose are reported in Table 1 and 3. Figures 1 and 2 show the individual and mean plasma level profiles of both brands in all of the subjects on logarithmic scale. Pharmacodynamic data of systolic and diastolic pressures and pulse rate are given in Table 2.

Table 1. Mean ± SEM plasma levels of nifedipine after oral administration of 20 mg dose of 2x10 mg capsules from two different batches of adalat®

TIME (h)	Mean plasma levels ± SEM ($ng.l^{-1}$)		
	Test product	Reference product	% Difference
0.25	116.17±27.16	69.18±13.87	-40.46
0.50	176.50±29.51	130.67±15.20	-25.97
0.75	196.67±22.50	168.00±22.31	-14.58
1.00	202.17±16.88	189.83±26.33	-6.10
1.50	197.00±15.87	207.83±29.02	5.49
2.00	184.00±19.59	208.00±28.45	13.04
3.00	156.67±22.98	190.17±24.78	21.38
4.00	133.05±22.20	147.93±27.16	14.88
6.00	104.00±19.78	123.83±15.81	26.36
8.00	78.66±25.87	80.57±13.88	1.91
10.00	51.83±23.33	59.84±24.51	8.01
12.00	28.50±16.20	37.61±12.59	9.11

Bioequivalence study: Mean plasma levels (ng/ml) of nifedipine along with SEM for both, under study, batches of Adalat® capsules after oral administration of 20 mg dose are given in Table 1. The table also reports the percent difference in the mean values at every sampling time. The mean plasma level versus time profiles, shown in Figure 1, provides a comparative visualization of the two batches. According to Figure 1, the lower and upper bars of test and reference batches touches each other, it reveals the insignificant difference in pattern of bioavailability of the two batches all over the sampling times. Maximum difference in the plasma levels was found on first two sampling times where the test batch showed a significant superiority in respect to higher plasma level. However; in the forthcoming samples, difference was not so profound and the mean difference all over the timings was only 4.99%.

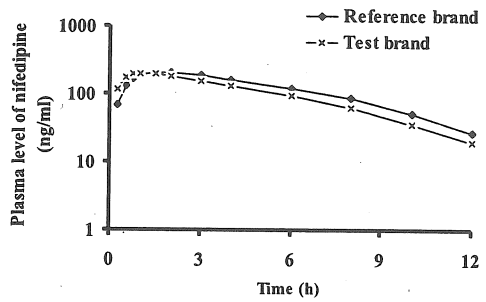

Figure 1. Plasma level of nifedipine after oral administration of 20 mg doses of 2x10 mg capsules from Reference and Test batches of Adalat® on logarithmic scale.

Bioequivalence metrics is given in Table 3. Mean AUC for reference and test batches falls within the conventional bioequivalence range of 80 – 125 %. The reference and test batches of Adalat® were, therefore, bioequivalent with respect to the extent of absorption of nifedipine.

The individual volunteer's plasma concentration-time profiles (Figures 2) for nifedipine depicts significantly different pattern in semi-logarithmic graph. This may be due to inter-individual differences / inter-subject variability for nifedipine plasma concentration, but the mean profiles reveal the same pattern for test and reference batches which confirm the bioequivalence and high degree of inter subject variability.

Mean AUC values were found to be very close for both the reference and test batches. Mean AUC for nifedipine capsule 2×10 mg were 989.15 ± 124.60 and 894.98 ± 90.62 ng.h.ml^{-1}, observed after oral administration of reference and test batches respectively (Table 3). The table also reports the percent difference for each subject in control and treatment status.

Absorption was found quite rapid after administration of nifedipine 2×10 mg capsules, with a mean C_{max} of 210.33 ± 28.44 ng.ml^{-1} at T_{max} 1.58 ± 0.17 h for reference batch and C_{max} 220.83±22.17 ng.ml^{-1} at T_{max} 1.25 ± 0.30 h for test batch.

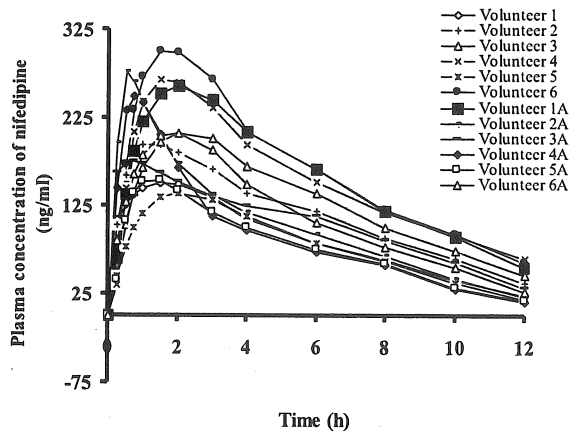

Figure 2. Individual volunteer's plasma concentration-time profiles for nifedipine after oral administration of 20 mg doses of 2 × 10 mg capsules from reference and test (A) batches of adalat® on logarithmic scale

Table 2. Plasma levels versus haemodynamic parameters.

Time (h)	Plasma level ($ng.ml^{-1}$, Mean ± SEM)	Diastolic Pressure (mm Hg, Mean ± SEM)	Systolic Pressure (mm Hg, Mean ± SEM)	Pulse Rate (min^{-1}, Mean ± SEM)
0.00	0.00	82.58±2.9	117.4±2.2	74.42±2.9
0.25	92.67±16.9	77.25±2.7	116.5±3.13	66.33±3.2
0.50	153.58±16.1	71.92±2.9	113.2±2.76	63.83±3.0
0.75	182.33±15.1	68.75±3.6	109.1±3.98	65.58±2.6
1.00	196.00±14.4	67.33±2.7	107.2±3.02	66.58±2.8
1.50	202.42±15.1	67.25±2.6	105.6±2.69	68.67±3.1
2.00	196.00±16.2	68.92±3.4	108.6±3.15	70.00±3.6
3.00	173.42±16.2	72.17±2.6	108.0±2.64	67.25±3.9
4.00	133.78±13.8	73.45±2.5	109.2±2.54	69.79±2.8
6.00	110.92±12.2	75.76±2.7	110.2±2.76	70.83±2.6
8.00	79.62±13.9	78.98±2.3	111.2±3.12	71.12±3.1
10.00	55.42±11.3	80.23±2.1	113.2±32.45	72.76±4.7
12.00	33.18±18.1	81.54±2.5	114.2±4.84	74.54±2.5

Pharmacodynamic equivalence study: Besides the bioequivalency of the two products tested by plasma level comparison for their mono-variant and multi-variant characteristics, the two products were also compared for their pharmacodynamic effects of nifedipine (pulse and blood pressure). Table 2 reports the haemodynamic parameters; systolic blood pressure, diastolic blood pressure and pulse rate at blood sampling times after the administration of the dose.

Similar to plasma level profile case, inter-individual variability in pharmacodynamic effects were also observed. Obviously this may be attributed to the different plasma drug levels in the subjects, since the Pharmacological effects of nifedipine are well documented for their ccrrelation with serum levels (Kleinbloesem et al. 1984). However, here the variability is not as significant as it is in the blood levels of the drug. Observing the haemodynamic effects (Table 2) of the two nifedipine products, it is evident that these are almost same in their intensity and pattern and bear no significant difference in these respects. With both batches, the pulse rate peaked at about 1.5 h after the administration of nifedipine and a significant drop in systolic and diastolic blood pressure was observed for 2 h after the administration of the drug. In conclusion, relative therapeutic efficacy in terms of pharmacodynamic effects on pulse, systolic and diastolic blood pressures were similar for the two nifedipine batches.

The peak plasma concentration and duration to reach at this peak level shows similarity with respect to the extent of absorption. So both reference and test batches are concluded as bioequivalent.

Population characterization as fast and slow metabolizers: In both of the studies of present work on six adult, healthy, male volunteers originating from Pakistani population, mean results show high AUC and C_{max} values for nifedipine 2×10 mg capsule (Adalat®) after single oral dose administration. The comparative high AUC and C_{max} of this region to other population could be due to ethnic origin, environmental factors and/or nutritional habits.

Another important variability which can be seen obviously in Figure 2 is the inter-individual variation in plasma nifedipine concentration. In the light of our observed values, as well those reported by literature, it can be concluded that there exist a very significant inter-subject variability in nifedipine plasma concentration through out its time course in the body to achieve a successful therapy without reaching up to the levels that could produce undesirable side effects leading to poor patient compliance (Hoyo Vadillo et al. 1989). Since the Pharmacological effect of nifedipine is related to plasma concentration we strongly recommend the individualization of dosage regimens. An adequate application of biopharmaceutics and pharmacokinetics should give the basis for a rational dosage regimen design.

It is important to know that the minimal plasma nifedipine concentration that produces a significant vasodilating effect is 15 $ng.ml^{-1}$, thus the therapeutic level must he kept above this threshold.

The earlier studies have proposed the existence of two distinct phenotypes, fast and slow, of nifedipine metabolizers which can be identified on the basis of AUC values (Kleinbloesem et al. 1984, Kleinbloesem et al. 1985, Kleinbloesem et al. 1986, Kleinbloesem et al. 1987a). In their report, 83% of the studied subjects were fast metabolizers and remaining 17% were slow. Early studies also communicated mean AUC values duly corrected by dose factor (Foster et al. 1983, Renwick et al. 1988). Mean values that correspond to a higher frequency of fast metabolizers were found to be between 12.5 ± 1.78 and 14.8 ± 7.2 $ng.h.ml^{-1}.mg$. Reported value in literature indicates that only 8.3% subjects were fast and 91.7% were found to be slow metabolizers of nifedipine (Hoyo Vadillo et al. 1989). The proportion of fast and slow metabolizers in present work (8:92) is contrary to the proportions reported by Kleinbloesem (17:83). This may be accounted for differences in genetic built up of different populations on which the two workers researched. In another report, 75% of the population was observed as slow metabolizers (Gutierrez et al. 1986).

In our population, the value of AUC per unit of dose was found to be 894.88$ng.h.ml^{-1}.mg$ which are much higher than the previously reported values. On this base, we can conclude that our population consists of slow metabolizers. All the subjects revealed high values for AUCs and hence we suggest a thorough and extensive study on large population sample. Comparison of our observed data to other reported data shows significantly high AUC and $t_{½}$ values. The biological basis for the higher AUC and longer half-life in this region could be either due to:

- A genetically determined difference in cytochrome P450 III A3, arising from a difference in enzyme expression.
- Increased plasma protein binding; because nifedipine is highly bound to plasma proteins and therefore α-acid glycoprotein concentration in this region could give higher plasma concentration. However, nifedipine is a high clearance drug and therefore, an increased plasma protein binding would give a large apparent volume of distribution and a shorter half-life. The longer half-life detected indicates a decrease in metabolism not an increase in protein binding or
- The presence of a component of spicy diets which inhibits cytochrome P450 III A3.

- The possibility of an inhibitory dietary component which could be consistent with the high

AUCs of nifedipine, in early study, was investigated by studying the pharmacokinetics of nifedipine in Caucasians on a normal western diet and following 3 days of consumption of a spicy curry diet (Hoyo Vadillo et al. 1989). There was no evidence of any trend towards an increased AUC of nifedipine following the curry diet or other changes consistent with the ethnic differences. If an inhibitory spice component has a half-life greater than one day, then steady state would not have been approached in the Caucasians and this could have resulted in the failure to detect an increase in the AUC. Thus, if the postulated inhibitory component had a half-life of 10 days, the Caucasians would have reached less than 20% of the steady state concentration that would be present in the South Asians, all of whom had retained their original spicy practices. Nevertheless the absence of any suggestion of an increase in the AUC of nifedipine indicates that the consumption of a spicy diet is unlikely to be the source of the ethnic differences detected. An alternative explanation of the data is that the Western diet contains an inducer of cytochrome P450 III A3, a possibility that cannot be excluded from this discussion. In early study, it was concluded that South Asians population who has lower ability to metabolize nifedipine than Caucasians (Ahsan et al. 1991). They also suggested lower doses of nifedipine to South Asians, to obtain therapeutic effect without excessive side effects.

Another report shows that nifedipine metabolism may be altered both by environmental and genetic factors which may act at different sites (Rashid et al. 1995, Yu et al. 2001). The main effect of grapefruit juice appears to be on the metabolism of oral nifedipine, possibly in the intestinal wall. On the other hand the main cause of the reported ethnic differences in nifedipine metabolism appears due to a much lower systemic clearance in the South Asians.

Pharmacodynamics: Heart rate, systolic and diastolic blood pressures were observed throughout the study (Tables 2).This table does not report and show only the separate values for the two batches but also reports and shows the means which can be attributed directly as intrinsic characteristics of the nifedipine. The heart rate was increased significantly (compared with the predose level) between 15 minutes and 6 h after each dose for nifedipine 2×10-mg capsules. A significant decrease in both systolic and diastolic blood pressure was found after 15 minutes from the dose administration, until 6 h.

Beside the pharmacodynamic bioequivalency of nifedipine as discussed in the last section, we tried to look into any other correlation between the time course of blood levels and pharmacological effects of the drug. The mean ± SEM data for plasma levels of the drug and haemodynamic parameters (pulse rate, systolic and diastolic blood pressures) in all the 12 subjects is reported in Table 2. Although the Pharmacological effects of nifedipine are well documented for their correlation with serum levels, we could not find at this dose level a significant or high correlation between the blood levels and haemodynamic parameters (Kleinbloesem et al. 1984). Body regulatory mechanism does not allow these functions to fluctuate beyond some limits. Pharmacological effect of nifedipine starts appearing after blood concentration reaches to 15ng.ml^{-1} level. Thus the actual quantitative and statistically significant correlation could only be examined only up to some above this level. When the levels cross a many-fold of this level, body regulatory mechanism starts working actively and

resists changes beyond the limit. This is the reason for which we could not find a good correlation constant in any of the three parameters and blood levels of nifedipine. However a very exact inverse pattern-correlation of blood levels with systolic and diastolic blood pressures are very evident from their haemodynamic profiles shown in Table 2.

Table 3. Bioequivalence metrics of nifedipine after oral administration of 20 mg dose of 2x10 mg capsules from two different batches of adalat®

	C_{MAX}	AUC	T_{max}	AUC_{tail}	AUC_{inf}	$AUMC_{tail}$	$AUMC_{inf}$	MRT
	Capsules of test batch							
1	261.00	1245.0	2.00	1184.40	2429.40	7106.38	10805.57	4.45
2	277.00	921.8	0.50	1000.00	1921.75	6000.00	8263.50	4.30
3	176.00	829.8	0.75	2825.00	3654.88	16950.00	19263.00	5.27
4	250.00	753.2	0.75	81.08	834.33	486.49	2045.37	2.45
5	154.00	662.8	1.50	697.25	1360.13	4183.49	6003.55	4.41
6	207.00	957.1	2.00	479.64	1436.76	2877.83	5549.77	3.86
Mean	220.83	894.9	1.25	1044.56	1939.54	6267.37	8655.13	4.12
STDEV	49.57	202.6	0.67	954.94	999.27	5729.64	5961.00	0.94
SEM	22.17	90.62	0.30	427.06	446.89	2562.37	2665.84	0.42
	Capsules of reference batch							
1	151.00	729.38	1.50	463.69	1193.07	2782.12	4778.68	4.01
2	194.00	933.75	1.00	1053.10	1986.85	6318.58	8949.96	4.50
3	208.00	1004.0	2.00	965.28	1969.28	5791.67	8820.61	4.48
4	269.00	1224.8	1.50	1013.33	2238.08	6080.00	9621.75	4.30
5	140.00	661.12	2.00	466.29	1127.41	2797.75	4723.44	4.19
6	300.00	1381.9	1.50	1024.54	2406.42	6147.24	10103.24	4.20
Mean	210.33	989.15	1.58	831.04	1820.19	4986.23	7832.95	4.28
STDEV	63.59	278.61	0.38	284.95	536.94	1709.72	2432.07	0.19
SEM	28.44	124.60	0.17	127.43	240.13	764.61	1087.65	0.08

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