Effect of valproate and antidepressant drugs on clozapine metabolism in patients with psychotic mood disorders

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Abstract:

Background: The aim of the present study was to appraise retrospectively the influence of valproate (VPA) and antidepressants (ADs) on the steady-state plasma concentrations of clozapine (CLZ), the prototype of various second-generation antipsychotics (SGAs), norclozapine (NCLZ, its main metabolite), and their ratio (NCLZ/CLZ).

Methods: Sixty-seven psychotic patients with a prevalent diagnosis of bipolar disorder (BD) were studied. We then analyzed data altogether and subdivided them into four groups, according to pharmacological treatments: #1 CLZ (n=21), #2 CLZ plus antidepressants (ADs) (n=13), #3 CLZ plus VPA (n=16), and #4 CLZ plus ADs plus VPA (n=17).

Results: Firstly, significant positive correlations between CLZ plasma parameters (ng/ml) and the drug daily dosages (mg/kg body weight) (n=67) were observed (Spearman, r_{CLZ}=0.49, r_{NCLZ}=0.43, P<.001; r_{ratio}=0.31, P=.011). We then normalized by given doses CLZ and NCLZ plasma levels, natural log transformed them, and carried out ANOVA factor analyses followed by pairwise comparisons, performed on the four groups and the three CLZ parameters. We identified significant drug effects on: 1) CLZ plasma levels, significantly higher in group #2 vs. group #1, 2) NCLZ/CLZ ratio, lower in group #2 vs. group #1 and #3. Significant drug × gender interactions were observed in group #3, showing higher NCLZ levels and NCLZ/CLZ ratios in men compared to women.

Conclusions Despite its inherent limitations, this observational study confirms the significant increase of plasma CLZ concentrations and reduction of NCLZ/CLZ

ratio when this drug is co-administered with ADs (group #2), an effect apparently counteracted by VPA (group #4). The drug × gender interactions in patients taking both CLZ and VPA (group #3) warrant further prospective study.

Keywords: Clozapine, Valproate, Antidepressants, Clozapine Plasma Parameters.

Introduction

Although several novel second-generation antipsychotics (SGAs) are currently available,[1] the prototypical clozapine (CLZ) remains the gold standard treatment for both positive and negative refractory symptoms in schizophrenia.[2-7] This is mainly attributable to the proven greater capacity of this drug to prevent patients' relapses, as well as to improve their social and occupational adjustments.[8-11.]

However, CLZ is not without adverse effects. The most relevant ones are neutropenia and agranulocytosis,[12] the later occurring in about 0.5% of treated patients, with a mortality rate of 3%,[13] thus requiring mandatory periodic blood tests. Too high daily dosages, a reduced renal clearance, characterized by abnormal metabolic profiles, as shown by dramatically increased plasma concentrations of the desmethylated metabolite norclozapine (NCLZ), have been linked to the augmented incidence of agranulocytosis,[12,14] as well as to the higher probability at developing seizures, delirium, confusion,[15-19] and/or inflammation states.[20] Therefore, with no doubt, the assessment of plasma levels of CLZ and NCLZ would represent a helpful tool for the management of patient's compliance, presence of adverse effects, unusual metabolic activities, pharmacological interactions, toxicity, possibly arising during the treatment with this drug. In effect, CLZ therapeutic monitoring would contribute to attain the rationalized planning of daily dosages and, possibly, the optimization of clinical responses.[15, 21, 22]

A high inter-individual variability of steady-state CLZ plasma levels has been observed even at the same given daily dosage,[19,23, 24] a finding which makes

difficult to predict the circulating concentrations of this drug, also implying its complex biotransformation and clearance.[25, 26] Some authors pointed out the current limits of CLZ therapeutic monitoring and the need of additional investigations.[2]

Indeed, present knowledge on CLZ metabolism regulation is still incomplete: in particular, there is little information on the disposition and biotransformation of this drug with respect to other relevant side effects, such as obesity, diabetes, and myocarditis, that may lead to its discontinuation. [1, 27]

Consequently, the measure of CLZ plasma levels represents a current challenging issue in clinical psychiatry, and the full elucidation of factors potentially able to alter CLZ biotransformation would allow to apply a more robust and valuable therapeutic monitoring.

Pharmacological interactions are among the main causes of variation of CLZ plasma concentrations. Indeed, beyond the divergent views on the relative advantages of CLZ on polypharmacy,[28,,29] as a matter of fact, this SGA is frequently used in combination with other psychotropic compounds. Combinations of SGAs and/or FGAs are, for instance, used for improving compliance or for CLZ augmentation strategies in schizophrenic patients.[30-32] Further, CLZ is often co-administrated in schizoaffective and mood disorders with treatment-refractory psychotic symptoms[33] while suggesting the helpfulness of therapeutic drug monitoring (TDM) also in these conditions.[34]

Valproate (valproic acid, VPA) is a drug frequently co-prescribed with CLZ to reduce the risk of seizures or as a mood stabilizer (MS) in BD.[19, 35, 36] Data on

the effect of VPA on CLZ metabolism are still conflicting,[37-46] and such controversies have been related to the complexity of CLZ metabolism.[19]

A recent study showed the propensity of VPA at enhancing CLZ metabolism[46] by applying a multivariate mathematical model. In addition to VPA, antidepressants (ADs), like selective serotonin reuptake inhibitors (SSRIs), may also be used in association with CLZ in the treatment of mood disorders with psychotic symptoms: in most studies, they were found to reduce CLZ metabolism.[47-50] Given the contradictory data available, our study aimed at appraising the influence of VPA and ADs, administered separately or together, by a retrospective and naturalistic investigation on the plasma steady-state a concentrations of CLZ and NCLZ (ng/ml), together with their ratio (NCLZ/CLZ), in a cohort of patients suffering from psychotic mood disorders. The parameter ratio is considered to be an index of CLZ metabolism.[51]

Material and Methods

Subjects

Patients were selected during outpatient examination or hospitalization at the Psychiatry Unit of the "Santa Chiara" Hospital and University Center, University of Pisa. Patients' inclusion criteria were as follows: an established diagnosis of a mood or schizoaffective disorder carried out by skilled psychiatrists according to DSM-IV R criteria[52]; treatment with a fixed CLZ dosage for at least 15 days from the beginning of the therapy or from appreciable changes in daily dosages, considering the approximate CLZ and NCLZ half-lives are 12 and 22 hours, respectively [53]; the accessibility to records of CLZ and NCLZ plasma levels

measured at steady state; a history of good health conditions before and during the monitoring period, as shown by results of blood and urine tests. Patients following a concomitant treatment with a stable dose of ADs, VPA, or both compounds were included. Exclusion criteria were as follows: history of alcohol or drug abuse; neurological and neurodegenerative CNS disorders; altered routine laboratory tests; concomitant treatments with MSs other than VPA, such as carbamazepine and lamotrigine; concomitant treatments with other drugs such as antibiotics, anti-inflammatory and painkiller drugs. Smoking habit was also included. Smokers were included in this study when receiving higher CLZ dosages than required. Patients' caffeine assumption did not exceed 400 mg/die.[23, 25, 54, 55]

All patients presented severe psychotic symptoms or a high risk of relapse, defined by delirium, paranoia, significant social/cognitive impairment, very low quality of life, and elevated propensity to suicide. All patients had signed an informed consent form for the use of blood withdraws for the analysis of CLZ plasma levels.

Data from medical records reporting a CLZ treatment in association with benzodiazepines (BDZs) and/or lithium were included in the analysis, due to their minor pharmacological interaction with CLZ metabolism.[37, 51] We also included patients taking haloperidol, which was prescribed to increase compliance or to augment CLZ efficacy, at doses not exceeding 5 mg/day.

Measurement of clozapine and norclozapine concentrations in plasma by HPLC

Blood sampling, plasma preparation, and measurements of CLZ and NCLZ levels

were performed as previously described.[56] CLZ and NCLZ plasma levels, reported in patients' database as ng/ml, were used as such or transformed into their ratio, plasma NCLZ to CLZ (NCLZ/CLZ). We used the ratio as an index of the drug metabolism..

Statistical analyses

All demographic, clinical, and laboratory data were presented as mean ± standard deviation (SD), variation range (min and max values), or medians, as required. The Spearman's test was carried out for correlations between CLZ and NCLZ plasma levels and CLZ dose and reported as CLZ daily dose (mg/kg body weight; *bw*).[54] Correlations were followed by linear regression analyses to yield R-squared determination coefficients (r^2). For correlation and linear regression tests, plasma CLZ and NCLZ levels were directly evaluated as ng/ml, without normalization for a given dose.[54]

To evaluate the influence of different pharmacological treatments, patients were divided into four groups: #1 CLZ alone (n=21); #2 CLZ plus ADs, n=13); #3 CLZ plus VPA (n=16); #4 CLZ plus ADs plus VPA (n=17). For comparisons, CLZ and NCLZ were considered as ng/ml/(mg/kg bw), or plasma levels at a given dose,[54] while the NCLZ/CLZ ratio was treated as such. Since CLZ plasma parameters are skewed to the right and not normally distributed, we decided to transform them into natural logarithms (*ln*), in order to apply parametric statistics. This was essentially done to better synthesize results as well as to use the factorial ANOVA model, which was considered more suitable for our aims. Specifically, we utilized factorial ANOVA followed by pairwise comparisons to study the influence of drug, gender, age, and smoking habit and their possible interactions

on CLZ, NCLZ, and ratio as *ln*.

All statistics were carried out using the SPSS Statistical Package for Social Sciences (SPSS-20.0, Chicago, Ill., USA)[57] and the Graph-Pad Prism version 5.0 (Graph-Pad Software, San Diego, USA).[58] The two-tailed statistical significance was set at P=.05.

Results

Sixty-seven patients were included in the investigation. From records, 80% of patients were outpatients. Table 1 summarizes patients' demographic data, administered CLZ daily dosages, and the antipsychotic plasma parameters, normalized for dose-weight or not. There was a preponderance of women (about 2:1, W:M). Patients' age was considered in respect to the study of Haring et al. (1989) and its potential effect on CLZ metabolism[23]: subjects were thus also divided into those aged below 35 and above 35 years, respectively, lower and equal or higher than the mean age of present cohort. An equal proportion of subjects aged below and above 35 years defined our cohort.

Patients' clinical features and treatment subgroups

Fifty-nine patients received a diagnosis of BD (20 BDI; 13 BDII ; 20 non-otherwise specified, NSBD and six mixed state), while some patients were suffering from major depression (n=5, MD) and schizoaffective disorder (n=3, SZD). Table 2 presents the main features of patients within each of four groups. BD1 was a frequent diagnosis in group #3 (CLZ plus VPA), while an equal number of patients with BD1 and BD2 was found in group #1 (CLZ alone).

Diagnosis of mixed state, depression, or schizoaffective disorder occurred in group #2 (CLZ plus ADs) and #3, whilst BDNS was prevalent in group #4 (CLZ plus VPA plus ADs). CLZ daily doses (mg/day) or daily dose corrected for weight ((mg/kg)/day) in the four groups are also reported: lower mean doses were administered to patients taking ADs, thus belonging to group #2 and #4, a difference which did not achieve, however, statistical significance.

Table 2 also shows gender, age, and smoking habit distribution in each of the four groups of patients in respect to treatment. Table 2 also displays age values divided into below and above 35 years. Information on smoking behavior and treatment duration was available, respectively, for 63 and 40 of the 67 subjects. Group #1 and #3 were balanced for gender, smoking behavior, and age, whereas group #2 and #4 were composed mainly by women younger than 35 years in group #2, older than 35 in group #4; group #4 was also defined by a higher number of smokers (11 S: 6 NS) than the others.

In terms of pharmacological treatment, 33 patients were taking VPA (daily doses 200-1250 mg, mean ± SD: 588 ± 267 mg) and 34 were not taking VPA. Thirty patients were taking ADs, either SSRIs or tricyclics (TCAs), in combination with CLZ alone (n=13) or with CLZ plus VPA (n=17). The SSRIs were as follows: fluvoxamine (n=11, 150 mg/day), paroxetine (n=7, 45 mg/day), sertraline (n=4, 75 mg/day), and fluoxetine (n=3, 20 mg/day). The TCAs were trimipramine (n=7, 90 mg/day), imipramine (n=4, 150 mg/day), clomipramine (n=1, 100 mg/day), and amitriptyline (n=1, 150 mg/day).

Twenty-two patients were taking haloperidol. More than half of group #1 patients, two of group #2, four of group #3, and three of group #4 were also

treated with FGAs. Nineteen patients were receiving doses lower than 5 mg/day of haloperidol (2.5 ± 1.5 mg/day (mean ± SD)).

No significant correlation was observed herein between age, body weight, and CLZ plasma parameters.

Dose-level correlations

Figure 1 (A,B) depicts the significant Spearman's correlations between plasma levels (ng/ml) of CLZ, NCLZ, and dose, CLZ mg/kg *bw*, in the whole study group. Significant and positive correlations were found for CLZ and NCLZ plasma levels. Correlations remained significant for CLZ and NCLZ plasma levels when patients were analyzed considering their treatment without (n=34) or with VPA (n=33), separately (data not shown). Patients treated with (n=30) and without ADs (n=37) also showed positive correlations between CLZ, NCLZ plasma levels, and given dose (data nor shown).

ANOVA factorial test: effect of the different drug treatments on CLZ metabolism

Figure 2 (A-D) shows CLZ parameters, corrected by the given dose (mg/kg), obtained in the various groups. After natural log transformation of data, the ANOVA factorial test, followed by pairwise comparisons, revealed a significant main effect of drug on 1) CLZ plasma levels (*ln*), F(3.59) = 2.77, P=.049, significantly higher in group #2 than group #1 (P=.025) after the Bonferroni *post-hoc* test, 5.32 ± 1.20 vs. 4.30 ± 0.90; 2) ratios (*ln*), F(3.59) = 4.56, P=.006, significantly lower in group #2 vs. group #1 (P=.001) and #3 (P=.016) after Bonferroni *post-hoc* test, −1.94 ± 0.99 vs. −1.0 ± 0.46 and −1.15 ± 0.76.

ANOVA factorial tests: interactions between different treatments and

individual variables

Despite possible biases due to unmatched groups or missing values, we examined the impact of age, gender, and smoking behavior on plasma CLZ parameters in the four groups, by means of the ANOVA factorial model. Although treatment duration represents a potentially relevant influencing factor of plasma CLZ variation, it was not possible to evaluate its impact herein, since precise information was available for 40 patients only.

No significant drug × age interaction was detected, suggesting the hierarchical influence of AD treatment vs. age on CLZ plasma parameters. Figure 3 (A,B) depicts NCLZ plasma levels, corrected by the given dose (mg/kg), and NCLZ/CLZ ratio values obtained in group #3, where a significant factorial ANOVA analysis was reported on *ln* transformed data and in respect to gender. Results revealed significant drug × gender interactions on 1) NCLZ levels ((*ln*), $F(3.59) = 4.01$, $P=.012$), higher values in men than women in the CLZ-VPA group (group #3), 4.19 ± 0.95 vs. 2.82 ± 0.65, $P=.004$; 2) ratios (*ln*), $F(3.59) = 3.20$, $P=.03$, lower value in men than women, -1.57 ± 0.39 vs. -0.61 ± 0.79, in group #3, $P=.007$, corresponding to higher NCLZ/CLZ ratio in men than women (Fig. 3B).

The factorial analysis applied for smoking behavior showed no significant drug × smoking interaction ($P>.05$).

Discussion

The management of treatment-refractory psychotic patients still represents a major challenge in psychiatric care.[59] Although newer SGAs have been

introduced into clinical practice,[60] CLZ is considered the first-choice therapeutic strategy in such severe conditions.[6] However, despite its recognized higher effectiveness compared with that of other SGAs, the use of CLZ is mainly limited by its side effects and the need of monitoring white blood cell counts.[60] The availability of CLZ TDM would considerably contribute to a more appropriate use of CLZ. Daily dosages ranging from 300 to 600 mg, together with a window of CLZ plasma levels, ranging between 250 and 420 ng/ml, have been associated with a higher probability to obtain satisfactory clinical responses in schizophrenic patients without significant side effects, [21, 22, 61-64] although this is not universally accepted .[27] Similarly, the reported high inter-individual variability of the drug's plasma levels somehow continues to limit their predictive impact during treatment. This variability has been attributed to a number of factors essentially linked to pharmacogenomics, epigenetics, and patients' lifestyle[65] . This underlines the need to obtain a more detailed understanding of the variables affecting CLZ plasma levels: a complete and appropriate monitoring of the SGAs can pave the way toward ever more tailored and personalized antipsychotic treatments.

Of note, even therapeutic ranges have been found to change according to different patients' diagnoses. Indeed, CLZ low plasma levels (25 ng/ml) are considered the threshold for an effective response in patients with Parkinson's disease and L-dopa-induced psychosis.[66, 67] Doses ranging between 25 and 100 mg/day may improve psychotic symptoms in borderline personality disorder.[68] Furthermore, dosages lower than 250 mg/day have been reported effective in BD patients.[69-71]

Bipolar patients were also found to respond more rapidly to CLZ than schizophrenic patients .[69] Other studies have reported improvement at relatively lower CLZ daily dosages or plasma levels when another SGA is co-administered.[72-74]

Furthermore, although not definitely proven,[75] the metabolite NCLZ has been shown to elicit specific pharmacological CNS actions, and, in particular, it behaves as an agonist of muscarinic M1 receptors, thus suggesting that it may contribute to the therapeutic effect of the parent compound.[76-81] CLZ and NCLZ plasma/serum levels also reflect the successful passage across the brain-blood barrier of these compounds and the effective occupation of neuroreceptors.[82] Consequently, the CLZ metabolism indexNCLZ/CLZ [51,83] or the reciprocal CLZ/NCLZ,[84] might perhaps be considered an optimal indicator of patients' response.

Interestingly, some authors described a relationship between the measure of NCLZ ratio values and working memory performances in treated patients.[85] Ratio values change as a function of blood withdraw timings, for instance, when samplings are obtained shortly after a relevant change of daily dosage and/or just after the last drug administration. These indexes are also affected by the analytical method used, or the procedure employed to extract CLZ and NCLZ from the biological matrix[51,56,61,86] or by the interactions with other drugs.[45, 84, 87]

VPA is one of the drugs most used in combination with CLZ.[19] VPA is metabolized by CYP2C9, a CYP450 isoform involved also in CLZ metabolism. Studies on the pharmacological interactions between VPA and CLZ, mostly

performed in schizophrenic patients, provided contrasting data: two reports described a moderate increase of CLZ levels (39% and 20%) after at least one week of steady dose treatment,[37, 40] without reaching statistical significance. Wong et al.[43] observed increased CLZ plasma levels and a decrease of NCLZ/CLZ ratio in Chinese schizophrenic patients treated with VPA. By contrast, Finley and Warner[38] noted that VPA induced CLZ metabolism in a small sample of patients (n=4). Another study reported a 15% decrease of CLZ levels after VPA addition,[39] while, in a case report, plasma CLZ concentration was decreased after VPA administration.[88] Others, in a large group of patients, showed no effect of VPA on CLZ levels, similarly to that observed in a cohort of BD patients.[41] As reviewed by Varma et al.,[19] opposite results were reported in smoker and non-smoker patients, as nicotine is an inducer of VPA metabolism .[44] A more recent and comprehensive investigation carried out in 151 patients supports the potential of VPA as an inducer of CLZ metabolism, by using a multivariate mathematical model to exclude confounding factors.[45, 46]

The present observational study addresses the question of the influence of both VPA and ADs on CLZ and NCLZ plasma levels, without searching the "true" effect of co-administered drugs on CLZ metabolism, but rather exploring their ensuring effect on psychotic patients, mainly suffering from BD.

Our study reports on various findings. Firstly, we confirmed the presence of a significant positive correlation between CLZ daily doses and CLZ or NCLZ plasma levels, in agreement with naturalistic and prospective studies.[23, 51, 54, 89-93] Our dose-level correlation coefficients are within values previously reported, even if there are significant differences among them. This may be due

to the different compliance or individual variables among the patients examined. In addition, normalization or not of daily dosage for body weight, or the different sample sizes are also potential sources of variance.

To appraise the presence of possible pharmacological interactions of CLZ with ADs, VPA, or both drugs and to better synthetize statistics and ensuing results, we thus normalized CLZ and NCLZ for given doses and transformed all the three CLZ plasma parameters into natural logarithms (*ln*).[89] This permitted to perform parametric factorial ANOVA analyses and compare them and the four treatment groups. Only patients from group #2 (taking CLZ plus ADs) reported significant differences: in particular, they displayed higher CLZ plasma levels together with a reduced NCLZ/CLZ ratio, confirming the AD-dependent competitive inhibition of CLZ metabolism[37, 49,50] Not surprisingly, CLZ augmentation by the SSRI fluvoxamine has been proposed as a safe strategy to reach CLZ therapeutic effects at lower doses.[50]

Our ANOVA results, together with the strong positive correlations between CLZ daily doses and NCLZ plasma levels or ratio in patients taking ADs (authors' observation, data not shown), suggest to investigate also these parameters as possible predictors of an effective and safe CLZ augmentation strategy in mood disorders with psychotic features. No appreciable difference was found between CLZ plasma parameters measured in patients treated or not with VPA, accordingly to our previous study[41]. However, group #4 taking VPA plus ADs showed greater ratio values than group #2, while suggesting the potential of VPA at somehow counteracting AD-mediated inhibition of CLZ metabolism. Indeed, if there had been a VPA-mediated inhibitory effect on CLZ metabolism and a

synergic VPA-AD action, we would have expected lower NCLZ/CLZ ratio values in group #4, at least comparable to those reported in group #2. Moreover, group #4 was composed of older subjects than group #2 supposed to have higher CLZ levels.[23] In a previous study, we had evaluated the influence of patient-related variables such as age, gender, or smoking behavior on CLZ plasma parameters, e.g., CLZ and NCLZ plasma levels, their sum and ratio, while reporting moderate effects of gender and smoking on CLZ metabolism. The action of smoking on sum (CLZ + NCLZ) reported herein confirms decreased CLZ plasma levels, as already reported in a meta-analysis carried out in a larger cohort of patients.[94] By using a different approach and applying parametric statistics, we observed significant drug × gender interactions only in group #3 taking CLZ plus VPA, a group matched for age, gender, and smoking habit. This finding needs, however, replication and further study, since the observed drug × gender interaction could also be due to clinical factors, such as different clinical history or treatment duration in the two sexes. As observed for patients co-treated with ADs, the significant variations of NCLZ and ratio in VPA-medicated subjects suggest that these two parameters deserve additional investigation on their possible use for CLZ TDM.

Conclusions

The overall findings of the present study carried out in patients suffering from psychotic mood disorders, mainly BD, confirm the significant increase of plasma CLZ concentrations and reduction of NCLZ/CLZ ratio when this drug is co-administered with ADs, while VPA appears to counteract this effect. In patients taking both CLZ and VPA a drug × gender interactions was revealed, with men

showing higher NCLZ levels and NCLZ/CLZ ratio than women. This is an intriguing result that needs to be further investigated in future studies aimed at improving and tailoring antipsychotic treatments.

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None

Conflict of interest:

None declared

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Figure legends

Figure 1: Spearman correlations between n clozapine (CLZ) daily dosage (mg/kg *bw*) and: A) CLZ, and B) norclozapine (NCLZ). (***): Significant positive correlation, P<.0001; (*): significant positive correlation, P<.05. R^2: R-squared determination coefficients after linear regression analysis. The straight line represents the linear regression fit.

Figure 2: Clozapine (CLZ) plasma parameters in the 4 groups of patients, in respect to pharmacological treatments. Data are the mean ± SD (median). A) CLZ plasma levels, ng/ml/(mg/kg), were: group #1, 106.3 ± 92.57 (84); group #2, 389.2 ± 474.8 (183.4), group #3, 128.7 ± 95.37 (102), group #4, 136.9 ± 94.48 (105); B) norclozapine (NCLZ levels), ng/ml/mg/kg, were: group #1, 52.07 ± 73.53 (32.8); group #2, 46.08 ± 51.87 (18.12); group #3, 52.18 ± 62.14 (30); group #4, 30.11 ± 19.68 (25); and C) the ratio NCLZ/CLZ was: group #1, 0.4067 ± 0.21 (0.39); group #2, 0.224 ± 0.23 (0.16); group #3, 0.458 ± 0.616 (0.29); group #4, 0.298 ± 0.202 (0.23).

Figure 3: NCLZ/CLZ ratios and NCLZ plasma levels in women and men from the 4 treatment groups. Data are the mean ± SD (median). A) Women norclozapine/clozapine (NCLZ/CLZ) ratios were mean ± SD (median): group #1, 0.47 ± 0.24 (0.45); group #2, 0.25 ± 0.26 (0.16); group #3, 0.22 ± 0.08 (0.23); group #4, 0.29 ± 0.23 (0.21); men ratios were mean ± SD (median): group #1, 0.31 ± 0.11 (0.33); group #2, 0.13 ± 0.04 (0.14); group #3, 0.76 ± 0.86 (0.48); group #4, 0.32 ± 0.09 (0.33). B) Women NCLZ plasma levels, ng/ml/mg/kg, were: group #1, 60.4 ± 84.0 (38); group #2, 48.4 ± 57.3 (20); group #3, 20.2 ± 12.8 (20.9); group #4, 26.9 ± 17.0 (21.6); men NCLZ plasma levels were: group #1, 38.7 ± 55.0 (16); group #2, 38.3 ± 35.0 (18); group #3, 93.3 ± 77.0 (49); group #4, 37.8 ± 25.0 (34.6).