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Pharmacokinetic Study of Copper (II) Acetylsalicylate

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Abstract This study was aimed at determination of pharmacokinetic parameters of copper (II) acetylsalicylate (CAS). Ten volunteers received a 60-mg dose of CAS. Blood samples were collected just before and after 0.25, 0.5, 0.75, 1.0, 1.5, 2.5, 3.0, 3.5, 4.0, 4.5, 5.5, 7.0, 10, and 12.0 h of administration of the drug. The plasma samples were analyzed for CAS and its metabolites by a validated high-performance liquid chromatography method having a suitable lower limit of quantification. The dose of 60 mg was well tolerated without any adverse effect. The maximum plasma concentration of CAS was found to be 0.38 mg L^{-1} with t_{max} of 0.72 h. The plasma half-life, clearance, and volume of distribution of CAS were 8.67 h, 66.30 L h^{-1} and 829 L kg^{-1}, respectively. The elimination of CAS, acetylsalicylic acid, copper salicylate, and salicylic acid follows the first order kinetics with r^2 0.979, 0.880, 0.991, and 0.998, respectively. The study provided for the first time the pharmacokinetic data for CAS after oral administration of CAS. The data were found to be useful in understanding the claimed enhanced anti-inflammatory activity of the drug as compared with that of acetylsalicylic acid.

Keywords Pharmacokinetics · Copper acetylsalicylate · Copper aspirinate · Copper · Biodistribution

Introduction

Acetylsalicylic acid (ASA) is being used for rheumatoid arthritis for more than a century. The main adverse effect (AE) associated with its extensive use is causation of peptic ulcer [1, 2]. In an attempt to counter this adverse side effect, Sorenson [3] did pioneering work

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and reported that copper (II) acetylsalicylate (CAS) is several times more active than ASA and also possesses antiulcer activity. In addition to the claimed enhanced anti-inflammatory activity, the antiulcer properties of CAS have been the subject of several research papers. Now, its potential as an ulcer-safe drug has been recognized, and the compound is being used in one way or the other for treatment of rheumatoid arthritis [4–9]. However, CAS could not have a place as a registered drug because of lack of essentially required clinical data. In Pakistan, a commercial preparation Nuhas® (Sigma Herbals, Lahore) containing CAS as an active ingredient is in use for the treatment of rheumatoid arthritis for the last several years. However, its pharmacokinetic study has not yet been reported. In the present paper, we report for the first time the pharmacokinetic study of the drug by use of a validated high-performance liquid chromatography (HPLC) method.

Study Participants and Methods

Study Participants

Ten healthy humans (five male and five female, aged between 18 and 25 years, median age of 21 with 10% of ideal weight, mean 55 kg, and build) were selected. The participants were educated about the type of study: the safety of the medicine and possible undesirable effects, etc., and consent was obtained. All the procedures followed were in accordance with the current revision of the Helsinki Declaration, and all the subjects used in this study gave informed consent.

The study was approved by the Ethics Committee of the University of Sargodha Medical College. The study was carried out at the affiliated hospital of the University of Sargodha under responsibility of Dr. Abdul Latif FRCS, the consultant at the hospital.

Study Design

The participants were kept on fast at least 10 h (over night) and were stopped from taking water 1 h before the administration of the drug. A single dose of 60 mg of the test product, CAS, was administered orally to the participants with 240 mL of water. The participants continued fasting for 5 h; however, they were allowed to take water 1 h after administration of the drug during this fast. After that, standard meals were served throughout the study.

Three to 5 mL of venous blood samples were collected from each participant by using disposable syringes, canulas, and butterflies under aseptic conditions. The participants were cooperative and gentle; however, three of the participants dropped out due to some personal reasons. Blood was collected from the antecubital vein. Heparin (Leo, Denmark) was used as an anticoagulant. The blood samples were collected in centrifuge test tubes and were arranged in the order on test tube racks and labeled accordingly with great accuracy. Blood samples were collected just before (blank) and after 0.25, 0.5, 0.75, 1.0, 1.5, 2.5, 3.0, 3.5, 4.0, 4.5, 5.5, 7.0, 10, and 12.0 h of administration of the drug.

The blood samples were centrifuged at 3,000×*g* at +4°C for 3 min immediately after collection. Plasma were separated by using a micropipette with a sucker and stored at −20° C in polystyrene crystal tubes until analyzed.

HPLC Method

All the chemicals used for HPLC analysis were of analytical reagent grade and were obtained from E. Merck, Germany. Standard CAS and copper salicylate (CS) were prepared

according to reported methods [10]. The HPLC system consisted of: LC-10AT VP pump, UV-Vis detector SPD-10A VP, and SCL-10A VP system controller all from Shimadzu, Japan. The column used was Shim-Pak ODS 5 μm (4.6×250 mm). The mobile phase used was a methanol–acetic acid (20:01) mixture. The flow rate, detection wavelength, and injection volume used were 1 mL min^{-1}, 294 nm, and 20 μL, respectively.

The standard stock solutions of CAS, CS, ASA, and salicylic acid (SA) were prepared by dissolving 10 mg of the standard material in the mobile phase (100 mL) separately. An appropriate amount of each stock solution was added to blank plasma (1 mL) to obtain a concentration of 0.05 mg mL^{-1} of each analyte separately. To the above solution, acetic acid (1 mL) was added. The contents were vortex mixed for 90 s and centrifuged at 500×*g* for 3 min at +4°C. The upper layer was separated and used for analysis as the standard.

Method validation was carried out in the plasma sample as per the reported method [11]. The performance parameters thus obtained are given in Table 1. The lower limits of quantification (LOQs) in the plasma were found to be very low, with excellent precision and accuracy, and suitable for the present study. Other parameters also qualify the standard suitability criteria. The specificity of the method for CAS and CS was ascertained by measuring copper, using a graphite furnace atomic absorption spectrometer, in the eluates corresponding to these peaks. Some of the plasma samples were also analyzed for ASA and

Table 1 Validation Parameters of HPLC Analysis of Plasma

Parameter	CAS (mean of 10)	CS (mean of 10)	ASA (mean of 10)	SA (mean of 10)
Precision (CV, within day/between days)	0.05/0.1 at 0.012 μg mL^{-1}	0.08/0.14 at 0.13 μg mL^{-1}	0.06/0.1 at 0.06 μg mL^{-1}	0.12/0.18 at 0.04 μg mL^{-1}
	0.07/0.1 at 100 μg mL^{-1}	0.1/0.15 at 75 μg mL^{-1}	0.05/0.1 at 100 μg mL^{-1}	0.11/0.16 at 125 μg mL^{-1}
	0.05/0.11 at 200 μg mL^{-1}	0.09/0.13 at 150 μg mL^{-1}	0.04/0.09 at 200 μg mL^{-1}	0.13/0.17 at 250 μg mL^{-1}
Accuracy (% recovery)	95.0 at 0.012 μg mL^{-1}	80.20 at 0.13 μg mL^{-1}	96.50 at 0.006 μg mL^{-1}	92.83 at 0.04 μg mL^{-1}
	95.1 at 100 μg mL^{-1}	80.15 at 75 μg mL^{-1}	96.45 at 100 μg mL^{-1}	92.85 at 125 μg mL^{-1}
	95.2 at 200 μg mL^{-1}	80.0 at 150 μg mL^{-1}	96.56 at 200 μg mL^{-1}	92.90 at 250 μg mL^{-1}
LOD (ng mL^{-1})	6.0	65.0	30.0	20.0
LLOQ (ng mL^{-1})	12.0	130.0	60.0	40.0
Concentration range (μg mL^{-1})	0.012–200	0.13–150	0.06–200	0.04–250
Theoretical plates, N	2260	4659	5153	1072
Resolution, Rs[a]	1.97	1.01	1.69	–
Symmetry factor, As	1.0	0.9	1.1	1.0
Capacity factor, *k*	4.2	4.8	5.1	5.5
Specificity data, in terms of percent recovery, at 100 μg mL^{-1} of each analyte in six plasmas				
1	95.	80.15	96.55	92.8
2	95.05	80.2	96.5	92.83
3	95.07	80.16	96.45	92.72
4	95.12	80.19	96.54	92.84
5	95.08	80.15	96.6	92.81
6	95.09	80.18	96.5	92.8
CV (%)	0.0075	0.0025	0.012	0.005

[a] Between the adjacent peaks

SA and CS by a reported method [12] for validation purposes, and a good correspondence ($t<t_{95,\ 5}$) was found between the two methods. The reported method could not detect CAS.

Specimen Analysis

The test plasma sample (1 mL) was thawed quickly under cold water, then promptly but briefly vortex mixed and processed as for the standard preparation.

The plasma samples were analyzed by the HPLC method validated above, and concentrations of CAS and its metabolites CS, ASA, and SA were determined. The typical chromatograms of the blank and sample plasma containing all the four species are shown in Fig. 1.

Pharmacokinetic Analysis

Concentration–time curves were plotted, and the following parameters were determined: AUC_{0-t}, the area under the curve from time zero to time t; $AUC_{0-\infty}$, the area under the curve from time zero to time infinity using the formula $AUC_{0-\infty} = AUC_{last} + Ct/ke$; $t_{1/2} = 0.693/ke$, the half-life of the drug; C_{max}, the peak drug concentration; t_{max}, the time to peak drug concentration; $Cl = Dose/AUC_{0-\infty}$; $V_d = Cl/ke$. The area under the concentration–time curve was calculated by the linear trapezoidal method. The terminal rate constant, ke, was determined by regression analysis of at least three data points in the terminal phase. The statistical analysis was performed by use of Statgraphics® 5.1.

Results and Discussion

Safety

The single dose of 60 mg CAS was well tolerated without any AE. Clinical assessment continued throughout the study, and no AE was reported except that two of the participants

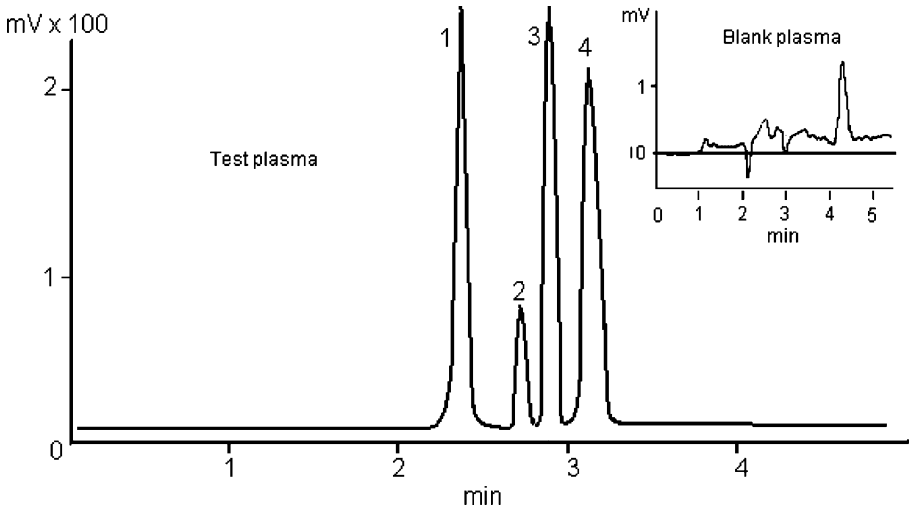

Fig. 1 Chromatograms of the blank and test plasma showing separation of CAS (*1*), CS (*2*), ASA (*3*), and SA (*4*)

Table 2 Pharmacokinetic Data after a Single Oral Dose of 60 mg CAS and Its Metabolites

Parameter	CAS	ASA	SA	CS
t_{max}, h	0.72	1.41	2.13	3.02
C_{max}, mg L^{-1}	0.38	0.178	0.022	0.13
$t_{1/2}$, h	8.67			
$AUC_{0-\infty h}$, h mg L^{-1}	0.91	0.45	0.31	2.35
V_d, L kg^{-1}	829			
Cl, L h^{-1}	66.30			

reported symptoms of slight nausea 15 min after drug administration. Three of the participants dropped out due to some personal reasons.

Determination of CAS and Its Metabolites in Plasma Samples

The separation and determination of CAS, CS, ASA, and SA was carried out successfully by the use of the validated HPLC method. The retention times for CAS, CS, ASA, and SA, in spiked plasma were 2.6, 2.8, 3.0, and 3.2 min, respectively. Similar peaks with identical retention times were found in the samples collected from the subjects of this study. The LOQ values for CAS, CS, ASA, and SA were 12, 130, 60, and 40 ng mL^{-1}, respectively (Table 1). The between-days precision near the limit of detection (LOD), in terms of the coefficient of variation (CV), ranged from 0.1 to 0.18, and accuracy, in terms of percent recovery, was found to be greater than 92% for CAS, ASA, and SA and greater than or equal to 80% for CS (Table 1). Specificity data along with other performance parameters (Table 1) clearly established the validity of the HPLC method for this study.

Pharmacokinetic Analysis

The pharmacokinetic data following the single oral administration of 60 mg CAS is given in Table 2. The plasma concentration–time curves are shown in Fig. 2. It was noted that about 1.5% (i.e., $AUC_{0-\infty} \times 100/\text{Dose}$) of unconverted CAS, of the oral dose, reaches systemic circulation. This behavior was found to be similar to that of ASA indicating

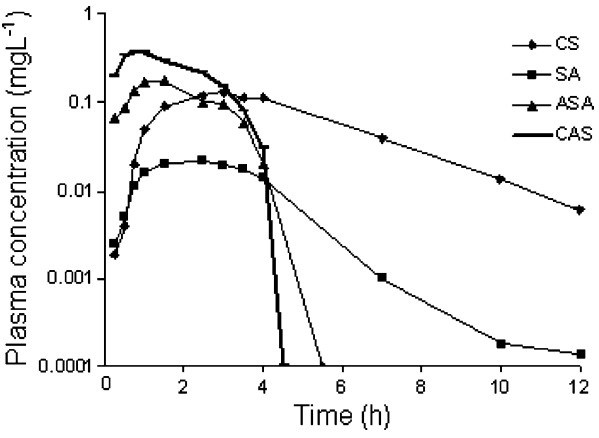

Fig. 2 Plasma concentration–time curves of CAS, ASA, SA, and CS

similar bioavailability after oral administration [13]. The extrapolated AUC_{∞} was less than 10% in case of CAS and ASA (as the metabolite). The $AUC_{\infty h}$ of the metabolites including ASA, SA, and CS (0.45, 0.31, and 2.35 h mg L^{-1}, respectively) indicate that significant amounts of these species remain available in plasma for longer periods of time, with the concentration of CS being the highest. Whereas, after the administration of 900 mg ASA, the level of ASA in plasma rises rapidly to reach a maximum, with only small amounts remaining after 2 h [13].

The C_{max} was found to be 0.38 mg L^{-1} at a t_{max} 0.72 h, which is about 0.6% of the administered dose. In the case of aspirin, normally, it is about 4% of the dose between 14 and 15 min after the administration of 900 mg [13]. The $t_{1/2}$ was 8.67 h, which is ideal for once-a-day dosing. The V_d and Cl values for CAS were 829 L kg^{-1} and 66.30 L h^{-1}, respectively. The large V_d may be due to the uptake by a specific tissue or membrane as highly lipophilic compounds are known to distribute into lipids in cell membranes and fat stores; these effectively form slow-release depots of the drug and prolong the plasma levels [14, 15]. The relatively high clearance may lead to low exposure and low plasma average concentrations during chronic dosing.

Theses findings offer an understanding of the enhanced anti-inflammatory activity of CAS.

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