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Vitamin C, omega-3 and paracetamol pharmacokinetic interactions using saliva specimens as determiners

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Abstract:
Background: With its low side effects profile and availability as an over-the-counter drug, paracetamol has been utilized extensively worldwide as an antipyretic and analgesic agent for decades. This is associated with the increasing concern over its ease of access and/or unawareness of the consumers to this issue of paracetamol-induced hepatotoxicity. Paracetamol-induced liver injury today is a big problem where most of the researchers are interested in the possible role of the naturally available antioxidants to ameliorate hepatotoxicity through kinetic interference. So the present study was designed to evaluate the effect of vitamin C and omega-3 on the pharmacokinetic property of paracetamol.
Methods: Six young (average age 29) healthy volunteers participated in the study. The study included three consecutive periods, each of which preceded by overnight fasting and separated by 6 day washout periods. The first period involved the ingestion of a single paracetamol dose. The second one included the ingestion of paracetamol and vitamin C concomitantly, and the final period included paracetamol plus omega-3. Saliva samples were collected and prepared for High-performance liquid chromatography analysis.
Results: There was a significant increase in saliva paracetamol level after 30 min of administration when given concomitantly with vitamin C compared with the remaining groups. No significant differences in the paracetamol concentration profile between the subjects for each group were observed at 60, 90, 120 and 150 min in all treated groups.
Conclusion: Concurrent administration of vitamin C with paracetamol increases significantly the C_{max} level (maximum measured concentration) in saliva and increases the extent of absorption and the possibility of drug-drug interaction and risk of side effects.

Keywords: omega-3, paracetamol, pharmacokinetics, saliva, vitamin C

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Introduction

With its low side effects profile and availability as an over-the-counter drug, paracetamol has been utilized extensively worldwide as an antipyretic and analgesic agent for decades. Its pharmacokinetics has been well described [1]. By contrast, its mechanism of action as an analgesic is yet to be elucidated [2].

Paracetamol has a rapid and almost complete oral absorption, with peak plasma concentration achieved at [0.5-1-1.5] h after ingestion of therapeutic dose. It undergoes the first-pass effect with 88% bioavailability. Furthermore, paracetamol exhibits low binding to plasma protein [1]. It is metabolized by the liver through glucuronidation (50%–70%), sulfation (25%–35%) and *N*-hydroxylation along with the CYP450 (CYP2E1) that produce the toxic metabolite *N*-acetyl-*p*-benzoquinone imine (NAPQI; 5%–10%), which accounts for paracetamol-induced hepatotoxicity. NAPQI can be readily detoxified by binding with glutathione through the sulfhydryl group [3].

Paracetamol has been in use for more than a century, but cases of paracetamol-induced hepatotoxicity have also been increasing, thereby eliciting concerns over the drug's ease of access and/or the lack of consumers' awareness about this issue [2]. With this global concern, many kinds of studies have been conducted to overcome or at least reduce the proble. Given the involvement of oxidative stress in paracetamol-induced liver injury, most of the researchers were interested in the possible role of naturally available antioxidants. Indeed, many of these antioxidants have been found to be hepatoprotective in animal models [4].

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Nonetheless, only a few studies have investigated such antioxidant effects on acetaminophen pharmacokinetics on animals or human models. Pingili et al. [5] revealed that paracetamol plasma concentration in Wistar rats increases upon co-administration of quercetin or chrysin over a period of 21 days.

In 2015, Qinna et al. investigated the pharmacokinetic changes occurring in paracetamol upon the administration of grapefruit juice and liquorice to healthy human volunteers. Saliva specimens showed a significant reduction in paracetamol C_{max} (maximum measured concentration) and AUC levels after consumption of the grapefruit juice [6].

In another study by Qinna et al. [7], a reduction in paracetamol oral bioavailability was demonstrated after the ingestion of multiple doses of grapefruit juice by Sprague-Dawley rats. Meanwhile, certain studies investigating possible pharmacokinetic changes in acetaminophen co-administered with antioxidants were conducted on healthy human volunteers [7], [8]. As such, the current researchers sought to investigate the effect of co-administering vitamin C (ascorbic acid) and omega-3 on paracetamol pharmacokinetics in saliva specimens.

Vitamin C was considered in this research because it is a well-known antioxidant supplied alone or in combination in many pharmaceuticals as an over-the-counter agent [9]. It is an essential vitamin that modulates the immune system and reduces or prevent atherosclerosis, cancer and common cold [10]. Moreover, vitamin C was investigated and proven to be hepatoprotective by Sabiua et al. in 2015, who found that acetaminophen-induced hepatotoxicity in Wistar rats can be improved by the co-administration of silymarin and vitamin C [11].

Vitamin C also interferes with the pharmacokinetics of certain drugs such as by increasing furosemide bioavailability [12] and enhancing levodopa absorption in elderly patients with Parkinson's [13]. Omega-3 fatty acid was selected for parallel points; first, it demonstrates good anti-inflammatory, anti-malignant, anti-hyperlipidemia and antioxidant effects and is good for mental health [4].

Omega-3 is hepatoprotective against paracetamol-induced [14] and valproate-induced [4] liver injury. Moreover, it generates anti-inflammatory mediators from overexpressed COX2 human endothelial cells treated with aspirin [15]. With such human and animal studies proving the occurrence of interactions/and or pharmacokinetic changes upon the co-administration of vitamin C and omega-3, this study hypothesized that these agents may also alter paracetamol's pharmacokinetics.

Materials and methods

Chemicals and medications

Medications including paracetamol (1000 mg Doliprane^{R} , France), omega-3 (1000 mg Winzer co., England) and vitamin C (240 mg Hansel Co., Germany). All these drugs acquired from a local pharmacy (drugstore). High-performance liquid chromatography (HPLC Angstrom)-grade acetonitrile and (methanol, perchloric acid, potassium dihydrogen phosphate, and 85% phosphoric acid)Sigma- Aldrich, were obtained from the College of Pharmacy at the University of Basrah.

HPLC mobile phase preparation

The HPLC system used in this study was SELONLC-100 Angstrom; acetonitrile, methanol, and water mixed in a ratio of 12:12:76 were used to prepare 1 L of the HPLC mobile phase. The dissolved air was removed by sonicating the mobile phase for 20 min and equilibrating the column, which involved pumping the mobile phase at a 1.5–2.0 mL/min flow rate for approximately 20 min.

Calibration curve

A paracetamol stock solution was prepared by dissolving 10 mg of paracetamol in 100 mL methanol to achieve 100 μg/mL. The calibration curve was then constructed using frequent dilutions into 80, 40, 20 and10 μg/mL with $R^2 = 0.999$ and linear equation $Y = 7515X + 10183$.

Study design

The study involved six healthy and young (average age 29 years) volunteer participants; three of them were female and the rest were male. The purpose of the study was clearly defined to them, who then provided signed

informed consent. This study was approved by the Ethics Committee of the College of Pharmacy, University of Basrah. Clinical data including weight, height and drug and disease history were recorded, and exclusion criteria were established to finalize the enrolment process. All the participants were informed and taught the correct and precise method of saliva collection before the commencement of the study.

This research included three consecutive 150 min periods that were each preceded by overnight fasting of the volunteers and separated by 6 day washout periods. Six blood samples were subsequently collected for paracetamol assessment in saliva during each period. The first period involved the ingestion of a single 1000 mg paracetamol dose. The second one included the oral administration of 1000 mg paracetamol and 240 mg vitamin C. In the final period, 1000mg paracetamol and 1000 mg omega-3 were taken orally.

Saliva was collected in labeled test tubes before ingestion and at 30, 60, 90, 120 and 150 min after each ingestion. Then, 200 μL of saliva samples were mixed with 200 μL of acetonitrile. Vortex and centrifugation were performed at 3000 rpm for 10 min. Finally, 20 μL of the resulting supernatant was injected into the HPLC system for analysis. Maximal plasma concentration and time to reach such concentration (T_{max}) were obtained directly from plasma concentration-time profile.

Pharmacokinetic analysis

The pharmacokinetic parameters for paracetamol concentrations in saliva were calculated using six time points for drug measurement of C_{max}, T_{max} (time to maximum concentration), elimination rate constant, area under the concentration curves to last collection time (AUC) in order to study the effect of time, drug and matching for significant considerations with multiple comparison using statistical hypothesis testing.

Statistical analysis

Data are expressed as mean ± SD, and differences between groups were assessed using two-way analysis of variance with Bonferroni's multiple comparison test as post hoc analysis associated with the area under the curve measurement using GraphPad Prism (GraphPad Software, La Jolla, CA, USA) version 6.0 software and Excel for T_{max} and C_{max} pharmacokinetic parameter measurements.

Results

Table 1: Pharmacokinetic parameters of paracetamol, paracetamol + vitamin C and paracetamol + omega-3 groups (mean ± standard deviation).

Pharmacokinetic parameters	Study groups (mean ± standard deviation)				
	Paracetamol group	Paracetamol + vitamin C group	Paracetamol + omega-3 group	Paracetamol group:paracetamol + vitamin C group	Paracetamol group:paracetamol + omega-3 group
C_{max}, μg/mL	2.942 ± 2.088	6.291 ± 0.752	3.943 ± 2.18	p < 0.007	p < 0.423
T_{max}, min	30 ± 30	15 ± 21.213	15 ± 21.213	p < 0.572	p < 0.572
AUC (total peak area), μg min/mL	369.7 ± 84.21	513.5 ± 63.04	391.7 ± 70		

The concentration-time profiles of paracetamol in saliva when given alone or concomitant with vitamin C or omega-3 are summarized in Figure 1, while the kinetic parameters are shown in Table 1. There was a significant increase in saliva paracetamol concentration 30 min after administration when it was administered concomitantly with vitamin C when compared with the other groups. C_{max} was significantly increased to 6.291 ± 0.752 μg/mL in the paracetamol + vitamin C group compared with the levels of 2.942 ± 2.088 and 3.943 ± 2.18 μg/mL in the paracetamol only and paracetamol + omega-3 groups subsequently. Conversely, no significant differences in the paracetamol concentration profiles of the subjects were observed at 60, 90, 120 or 150 min in any group. Indeed, T_{max} was not significantly altered when paracetamol was administered concomitantly with vitamin C or omega-3. Regarding the AUC level, our findings demonstrate that the AUC of the paracetamol

+ vitamin C treated group was significantly higher than both paracetamol only and paracetamol + omega-3 groups.

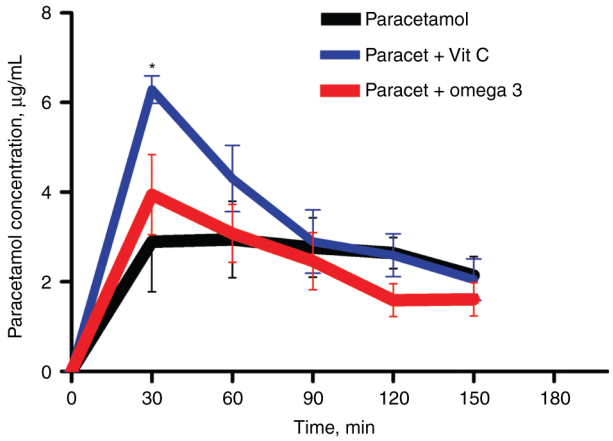

Figure 1: Mean ± SD saliva levels of paracetamol given alone and in combination with vitamin C or omega-3 in six volunteers.
* represents a significant difference when compared with the other groups at the same time point ($p < 0.05$); Paracet+Vit C: paracetamol + vitamin C; Paracet+omega 3: paracetamol + omega-3.

Discussion

Routine use of paracetamol in combination with different drugs and nutrients gives rise to significant pharmacokinetic interactions. Substances such as ascorbic acid enter the body almost daily, and paracetamol is used frequently, which may lead to several favorable and unfavorable kinetic interactions as well as increased or decreased pharmacological response. In the present study, the concurrent administration of vitamin C with paracetamol increased the paracetamol's C_{max} significantly, while the T_{max} in the saliva was not significantly different. The highest significant concentration of paracetamol was obtained 30 min after intake in comparison to paracetamol alone or after intake of omega-3.

Explaining such increment is somewhat difficult given the limited amount of available research in this area. Indeed, to date, no published study has dealt specifically with confirming the effect of vitamin C on the pharmacokinetic parameters of paracetamol in human being. The mechanisms that may possibly be responsible are pH dependence of drug absorption that could affect water solubility, but in general, paracetamol does not ionize unless above pH 8. Moreover, in all physiological situations, it exists entirely in the form of neutral molecules [16]. As paracetamol is absorbed from the small intestine by passive diffusion, the rate-limiting point is the gastric emptying into the intestines. Any factor that will attenuate emptying such as food will decrease its absorption, and any factor that will increase gastric movement will increase absorption [17], [18]. Furthermore, it has been found that vitamin C administration accelerated and quickened gastric emptying and attenuated mucosal oxidative stress. This led to an increase in the absorption rate of paracetamol in our study as well as a significant increase in its concentration within 30 min of intake with vitamin C [19].

The available data concerning the effect of both paracetamol and vitamin C on P-glycoprotein (P-gp) are conflicting and do not allow for the determination of a clear explanation of our findings regarding the roles of transporters on paracetamol absorption. A small number of studies have demonstrated that paracetamol makes P-gp upregulation [20]. Induction of intestinal P-gp by paracetamol in rat intestine and human LS174T cells was reported in Ghanem et al. [21]. In contrast, another study reported the inhibitory activity of paracetamol on P-gp [22], whereas a different study regarding cell lines and cell cultures showed that P-gp expression on the cell surface was unaltered by vitamin C treatment. [23] Thus, the interaction at P-gp levels between vitamin C and paracetamol is excluded as it does not appear to offer a clear explanation for our result.

Paracetamol is normally metabolized in the liver (95%) by the glucuronidation and sulfation processes. However, a large dose may lead to the saturation of the glucuronide and sulfate, which points that a highly reactive intermediate NAPQI is formed by the cytochrome P-450 enzyme. NAPQI can form adducts with proteins, which in turn causes DNA fragmentation, mitochondrial dysfunction, oxidative stress and subsequent liver failure. These effects appear after the supply of cellular glutathione was saturated [24]. Vitamin C is an important cofactor involved in different biochemical functions and acts as a reducing agent through electron donation. Vitamin C efficiently scavenges superoxide and hydroxyl and traps free radicals, directly or indirectly, by quenching a chain-starting catalyst and regenerating vitamin E [25], [26]. As humans have a limited

capacity for sulfate formation in the body, vitamin C prolongs the half-life of paracetamol, by competing for the availability of sulfate.

In general, pretreatment with vitamin C significantly prevents liver damage. Its protective effect against lipid peroxidation could be related to its antioxidant property, which assists in maintaining membrane integrity through free radical scavenging. [27]

At the elimination level, there is a possible interaction of clinical importance between paracetamol and vitamin C, in which vitamin C caused a rapid and prominent decrease in the elimination rate of acetaminophen, leading to increased serum concentration. This finding was in line with the results of the present work [28]. Overall, different *in vivo* studies have indicated that paracetamol interactions with different nutraceuticals or plant foods such as citrus fruits were found to induce Uridinediphosphate-glucuronosyltransferase (UGT) and affect paracetamol kinetics compared with a devoid diet with a 2 week washout period [29], [30]. For paracetamol, the relative contribution of gastric absorption appeared higher in the non-fasting state compared with fasting. [31]

In the present study, the potential effects of omega-3 supplementation on saliva paracetamol concentrations showed no significant difference as compared with the paracetamol only and paracetamol + vitamin C groups. In general, many articles have reported that concomitant administration of food and natural products had little effect on the extent of absorption of paracetamol and did not alter its metabolism [32].

Actually, there are conflicting reports investigating the protective role of omega-3 on paracetamol intoxication. The ameliorative effect of omega-3 in paracetamol toxicity seems to be associated with higher detoxification as a glucuronide conjugate [33]. In contrast, another study showed that omega-3 was not hepatoprotective against paracetamol toxicity; instead, it aggravates liver injury [34]. Possible mechanisms through which omega-3 protects the liver against paracetamol-induced liver damage include its membrane-stabilizing action on the hepatocytes by serving as an antioxidant, rising the threshold of hepatocytes susceptible to the damaging action of free radical [14], [35].

In spite of these conflicting observations, flavonoids might interfere with the bioavailability of different drugs through different mechanisms, such as with cytochrome P450 competition, esterases enzymes and transporters, such as multi-drug resistance, P-glycoprotein, breast cancer resistance protein and organic anion transporter. [36]

The relevant paracetamol concentrations were assessed in saliva, which is feasible because of the simplicity of the collection of samples compared with using blood, no required medical expertise and lower potential for adulteration with the presence of good correlation, and the pharmacokinetic characteristics are more predictable compared with blood than urine and hair [37]. Assessment of oral fluid drug levels are affected by different factors such as salivary flow, pH, and physicochemical properties of the compounds. However, blood samples are the gold standard for quantitative assessment of drugs, as blood drug levels are more correlated to pharmacological effects [38]. Indeed, as a non-complicated, non-invasive, and inexpensive technique with low contamination risk, salivary sampling can be used as a surrogate for plasma sampling. According to salivary excretion classification system [39], paracetamol falls in class I category where its high intestinal permeability (fraction absorption (F_a = 1) and low protein binding (low fraction unbound F_u = 0.99) permit salivary excretion [40]. Idkaidek and Arafat have demonstrated that paracetamol has a good correlation between saliva and plasma concentration with a coefficient of 0.99 and saliva/plasma concentrations ratios of 1.45–1.50 [41].

In conclusion, concurrent administration of vitamin C with paracetamol increases significantly the C_{max} level in the saliva of six healthy volunteers and increase the extent of absorption as well as the possibility of drug-drug interaction and risk of side effects. Conversely, the co-administration of omega-3 with paracetamol did not have a significant impact on their pharmacokinetic parameters.

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Informed consent: The purpose of the study was clearly defined to all individuals included in this study and signed informed consent was obtained.

Ethical approval: The present study has been done after obtaining informed consent and achievement College of Pharmacy, University of Basrah ethical agreement in accordance with the tenets of the Helsinki Declaration (as revised in 2013): (7-39-2001 in 24/9/2017).

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