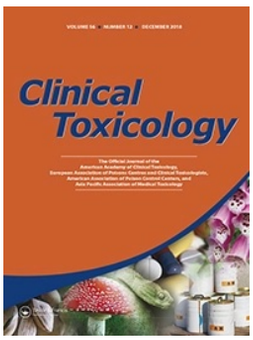

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BASIC RESEARCH

In vitro study of pharmacobezoar formation in simulated acetaminophen overdose

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ABSTRACT

Objectives: There have been few studies of pharmacobezoar formation, but they can be an important contributor to overdose toxicity. Pharmacobezoars may explain the delayed peak or "double hump" pharmacokinetics, which were noted in previous case reports with delayed toxicity of acetaminophen (APAP). We validated the presence of APAP bezoar formation in a controlled modified *in vitro* environment simulating acute APAP overdose.

Methods: This study involved the APAP and control groups (ferrous sulfate and chlorpheniramine). The APAP study group contained three subgroups of APAP with different dosage, i.e., 25 g (50 tabs)/37.5 g (75 tabs)/50 g (100 tabs). The positive control group containing ferrous sulfate, i.e., 15 g (50 tabs), has been reported previously to form pharmacobezoars in overdose. The negative control group containing chlorpheniramine, i.e., 200 mg (50 tabs), has not been reported to form pharmacobezoars in previous case studies. Tablets from each study group were placed into a separate pig stomach. Each stomach contained 28 ml USP standard simulated gastric acid. The stomach was placed in a plastic box filled with water maintaining at 37 °C. Each test group was examined for 4 h in the stomach. The primary outcome was the presence of clump formation. Positive clump formation was defined as tablets sticking together and the ability to maintain shape upon dissecting the pig stomach and lifting with fingers. Tablet clumps would then undergo dissolution testing with subsequent analysis of dissolution profiles.

Results: Formation of tablets clumps was confirmed in APAP overdose in the *in vitro* environment. Clumps were noted to be present in the 37.5 g and 50 g APAP groups, while 25 g APAP was unlikely to form clumps. The dissolution profile of clump demonstrated slower release without reaching plateau at 60 min, as compared to corresponding individual tabs of APAP. f1 and f2 analyses showed the dissolution profile of clump was different compared to that of referenced individual tab.

Conclusions: APAP clump formation was confirmed in acute overdose of 37.5 g or more. Dissolution tests revealed delayed and steady release of tablet residue from the clumps, which could explain prolonged or delayed toxicity in large APAP overdose.

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Acetaminophen; pharmacobezoar; *in vitro*; massive overdose

Introduction

Pharmacobezoars are concretions of tablets/medication that form and persist in the gastrointestinal (GI) tract. Pharmacobezoar formation has been demonstrated with some medications such as aspirin and iron sulfate in overdose [1]. Its presence might alter subsequent management because of prolonged absorption and mechanical effects, e.g., obstruction to GI tract [2,3].

Pharmacobezoar formation may occur when there is evidence of continuing absorption noted by delayed or multiple peak drug concentrations [4]. There are few reports of pharmacobezoar formation from endoscopy, imaging, intra-operative, or autopsy findings.

The exact mechanism of pharmacobezoar formation is unknown but is likely multifactorial. This might include amount, physical or chemical property of the drug, delayed gastric emptying and anatomical abnormalities [5]. Acetaminophen (APAP) is of particular interest in view of being the commonest type of analgesic overdose in many countries [6]. Hendrickson et al. have also reported three cases of delayed peak in APAP overdose, which could be attributed to formation of bezoar altering pharmacokinetics [7].

Our study aims to investigate the ability of APAP to form clumps using an *in vitro* model. This has the potential to inform on characteristics that may lead to pharmacobezoars in APAP overdose.

Study design and methods

This study involved the study group of interest (APAP) and control groups (ferrous sulfate and chlorpheniramine).

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The APAP group consisted of three subgroups of APAP (Medipharm's endopain II, 500 mg per tab) with different dosages: (i) 25 g (50 tabs), (ii) 37.5 g (75 tabs), and (iii) 50 g (100 tabs). The positive control group used ferrous sulfate ($FeSO_4$, APT Pharma, Burlingame, CA) tablets: 15 g (50 tabs of 300 mg tab). This has been reported previously to form pharmacobezoars in overdose [3]. Chlorpheniramine (Chlorpyrimine, Atlantic Laboratories Corporation Ltd., Bangkok, Thailand) 200 mg (50 tabs of 4 mg tab) was used as the negative control group. This amount has not been reported to form pharmacobezoars previously. The experiment was repeated six times for each group.

Tested drugs were put into pig stomach freshly bought from the wet market. Each stomach contained 28 ml USP standard simulated gastric acid (USP-SGA) without pepsin. The fresh pig stomach with USP-SGA is an original method developed for this experimental study. This was chosen to try reproducing conditions present in the human stomach. The use of 28 ml USP-SGA is based on the estimation of fasting gastric volume in previous human physiology study [8]. The USP-SGA was prepared by the toxicology reference laboratory at Princess Margaret Hospital, Hong Kong. The stomach was then placed in a plastic box (35 cm $L \times 30$ cm $W \times 18$ cm H) filled with water maintaining at 37 °C. Each group of medications were placed in an individual pig stomach for 4 h. The equipment setup is shown in Figure 1. It consisted of a plastic box, heater probe with digital thermometer, timer and USP-SGA containing pig stomach.

Figure 1. Equipment setup.

Dissolution testing

After 4 h in the pig stomach, each clump formed would undergo dissolution testing. Utilizing an UV spectrophotometer, fragments of clump with an equivalent dose of 5 g APAP were retrieved and compared with a corresponding 10 individual tabs of APAP. The dissolution testing was repeated three times in each APAP group. The dissolution testing is the standard test performed by the pharmaceutical industry to provide critical *in vitro* drug release information for both quality control purposes, i.e., to assess batch-to-batch consistency of solid oral dosage forms such as tablets, and drug development, i.e., to predict *in vivo* drug release profiles [9].

The dissolution equipment set up is shown in Figure 2. The dissolution assay employed the USP version 41(2018) APAP tab monograph. Automated retrieval of dissolution medium was done at scheduled intervals, namely at 5, 10, 15, 20, 30, 40, 50, and 60 min. Subsequent analysis of the sampled dissolution medium revealed the actual amount of dissolved APAP at each respective time point. Results were presented as mean percentage of dissolved APAP at the specific time point and plotted on a graph. Dissolution profiles between clump and corresponding individual tabs of APAP were evaluated using difference factor (f1) and similarity factor (f2), which were developed by Moore and Flanner [10].

The difference factor (f1) calculates the percentage difference between the two curves at each time point and is a measurement of the relative error between the two curves. The similarity factor (f2) is a logarithmic reciprocal square root transformation of the sum of squared error and is a measurement of the similarity in the percentage dissolution between the two curves.

Formula:

$$f1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} R_t} \times 100$$

$$f2 = 50 \log \left\{ \left[1 + \frac{1}{n} \sum_{t=1}^{n} (R_t - T_t)^2 \right]^{-0.5} \times 100 \right\}$$

where n is the number of time points, R_t is the % active pharmaceutical ingredient (API) dissolved of reference product at specific time point x, T_t is the % API dissolved of test product at specific time point.

Difference factor (f1) and similarity factor (f2) analyses were originally designed for assuring similarity between drug products of pre-change and post-change. We employed the concept and compared the fragment of clump with its corresponding amount of individual tabs of APAP.

For dissolution curves to be considered similar, f1 value should be close to 0 while f2 value should be close to 100. Generally, f1 value up to 15 (0–15) and f2 value greater than 50 signify equivalence of the two curves and vice versa [11].

Outcomes

The primary outcome was the presence of clump formation. Positive clump formation was defined as tablets sticking together and the ability to maintain its shape upon dissecting the pig stomach and lifting with fingers. The secondary

Figure 2. (a) Agilent 708-DS Dissolution Apparatus. (b) Agilent Cary Series UV-Vis spectrophotometer.

outcome was to describe the dissolution curve of APAP clumps and compare with that of corresponding individual APAP tablets.

Results

Formation of clumps was confirmed in the 37.5 g and 50 g APAP study groups at 4 h. For the 25 g APAP group, 83% (five out of six times) did not form clumps after 4 h in the *in vitro* environment. Photos of APAP bezoar are shown in Figure 3.

Clump formation was noted in the $FeSO_4$ group but not observed in the chlorpheniramine group. Photos of ferrous sulfate bezoar and chlorpheniramine are shown in Figure 4. The morphology of the APAP clumps and $FeSO_4$ clumps look quite different as shown in our experiment. Clumps of paracetamol (Figure 3) were soft mixtures of tablets, sticky, and coalesce without visualization of individual tablets, while the

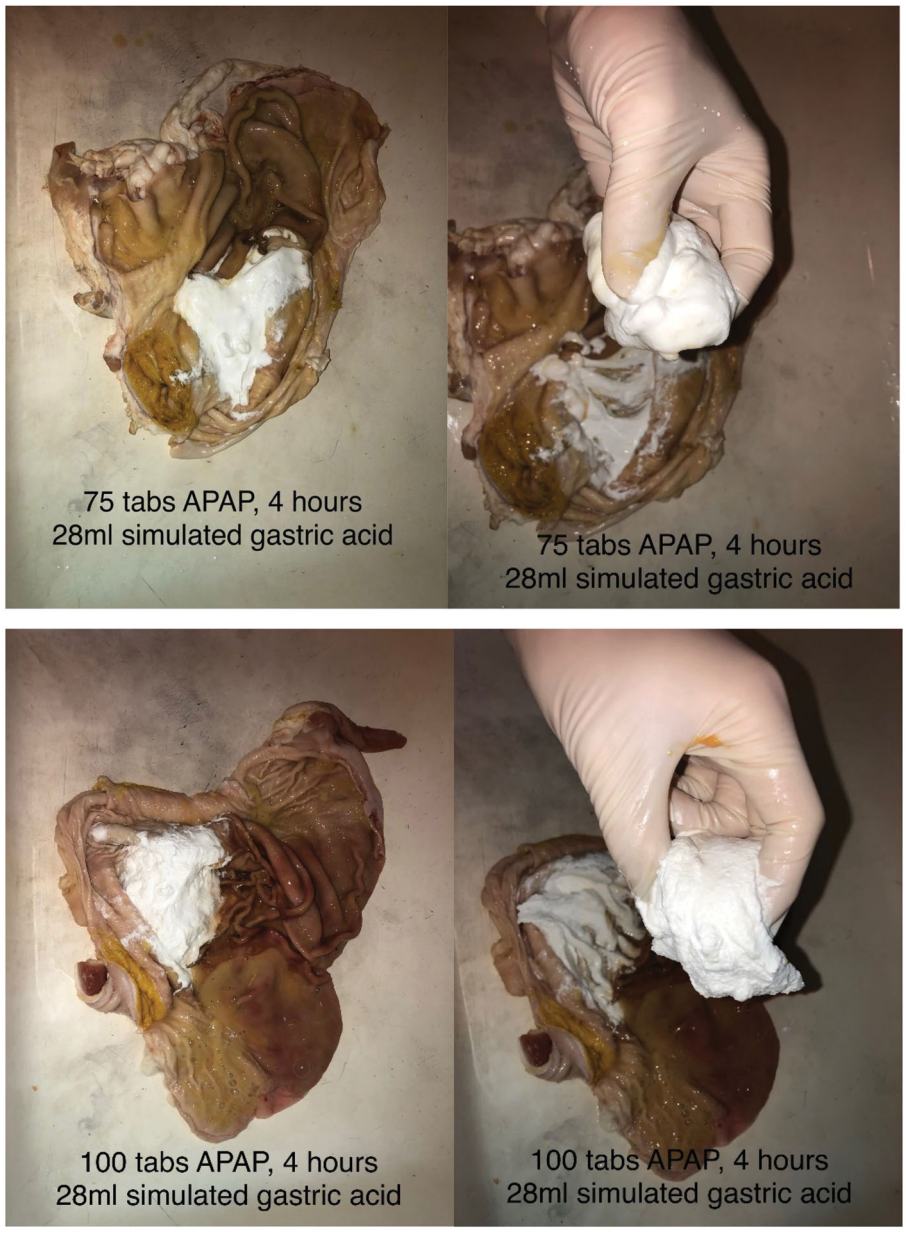

Figure 3. Photos of acetaminophen bezoar formation.

clumps of $FeSO_4$ (Figure 4) were individual tablets sticking together by the external coating with the loss of some of the content, i.e., partial ghost tablet formation.

The dissolution profiles of fragment of clump with equivalent amount of 10 tabs APAP and corresponding 10 individual tabs of APAP are shown in Figure 5. Dissolution testing was completed for each group three times.

The dissolution profiles of each clump group exhibited slower rate of dissolution and a smaller percentage of dissolved APAP at all time points. It also demonstrated steady release without reaching plateau at 60 min. On the other hand, individual tablets showed faster dissolution and attained plateau concentrations after 20 min.

f1 and f2 were calculated with raw data presenting in Table 1. The mean percentage of dissolved APAP represented the mean of the three curves within its respective group at specific time point. The f1 value was 35.7 and f2 value was 29.0, both of which demonstrated the clump profile was different compared to the referenced dissolution profiles.

Discussion

The etiology for altered pharmacokinetics of APAP overdose is multifactorial. These factors may include modified release preparations, large ingestions, delayed gastric emptying (e.g., opioid co-ingestants), decreased solubility of tablets, enterohepatic circulation, and pharmacobezoar formation [12,13].

In the majority of acute immediate release APAP overdose, absorption and peak concentrations are reached within 4 h of ingestion [14]. However in large or modified release

Figure 4. Chlorpheniramine (top picture) and ferrous sulfate (bottom pictures).

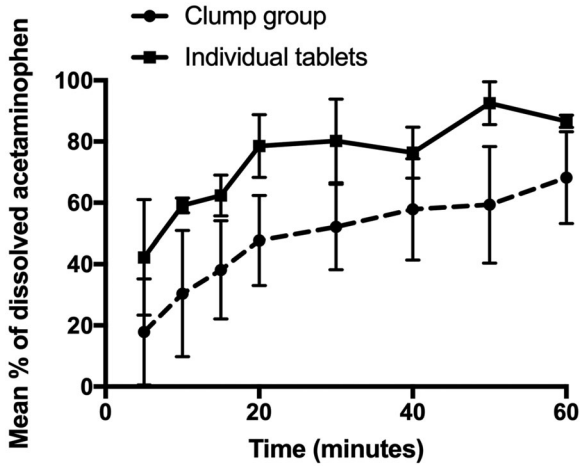

Figure 5. Dissolution profiles of clumps (equivalent to 10 tabs APAP) and corresponding 10 individual tabs APAP.

preparation APAP overdose, absorption may be prolonged [15–17]. It is possible that tablet clump or pharmacobezoar formation could contribute to these findings [5]. Delayed peak and "double hump" pharmacokinetics in APAP overdose were also reported in previous case studies [7,18]. This may lead to an increased risk of developing liver injury or hepatotoxicity [19]. It also opens the possibility for intervention, e.g., use of activated charcoal after 1 h or increased acetylcysteine dosing [16,20].

For the primary outcome, our study demonstrated the formation of clumps with larger quantities of APAP. Repeated tests within subgroups minimized confounding factors and improved accuracy. Our study suggests that clump formed at 37.5 g (75 tabs) and above. However, clump formation was unlikely at 25 g (50 tabs). This has implications in that larger APAP ingestions are more likely to have altered toxicokinetics which may include ongoing absorption. It is unclear

Table 1. Data and calculations in f1/f2 analysis.

	5 min	10 min	15 min	20 min	30 min	40 min	50 min	60 min
Clump group								
Mean percentage of dissolved APAP (%)	17.9	30.4	38.1	47.7	52.1	57.9	59.4	68.2
Relative standard deviation (%)	17.4	20.6	16.0	14.7	13.9	16.5	19.0	15.0
Individual tab group								
Mean percentage of dissolved APAP (%)	42.2	59.1	62.4	78.5	80.2	76.4	92.5	86.6
Relative standard deviation (%)	18.8	2.4	6.7	10.3	13.7	8.4	7.0	2.0

whether traditional acetylcysteine dosing regimens are suitable in these settings [21].

The slower rate of dissolution within the clump groups studied compared to individual tablets and the steady release of APAP without reaching plateau could explain the delayed peak concentrations in large overdose. Only a portion of the clump was studied, however we postulate the dissolution profile of a larger clump may have had an even slower rate of dissolution. In addition, factors such as smaller percentages of dissolved APAP could result from decreased surface area to volume ratio.

The apparent ability of larger amounts of APAP to form clumps *in vitro* contributes to the understanding of pharmacobezoar formation. Persistently high concentrations of APAP following large overdose and multiple peak concentrations should raise the possibility of pharmacobezoar formation in the clinical setting.

Limitations

There are several limitations to this study. Human research on pharmacobezoars is challenging due to ethical concerns. Therefore, we performed an *in vitro* experimental study. Experimental conditions differ to *in vivo* circumstances which include continuous secretion of gastric juice, hormonal release, absorption, digestion, and gastric emptying. Although potentially difficult to model *in vitro*, set ups that mimic these conditions are necessary to validate and confirm the findings of our experiment. Future experiments might consider use of animal models to further investigate pharmacobezoar formation. In addition, experimentation only lasted for 4 h. Longer periods of observation may have resulted in greater dissolution of the clumps formed.

Only fragment of clumps underwent dissolution testing and this was compared to equivalent individual tabs of APAP. This was based on the assumption that APAP was distributed evenly within the clump. However, this might also represent the delayed peak concentrations that can occur in massive overdose, e.g., more absorption of non-active tablet ingredients followed by a spike in active ingredient absorption. The whole clump was not directly studied for its dissolution profile because the apparatus would only allow 5 g of dissolution testing at a time.

There were also drops in percentage of dissolved APAP at later time intervals with higher concentration of APAP, especially among individual tab curves. This could be related to deviations of the Beer–Lambert law [22].

Conclusions

Acetaminophen clump formation was present in doses greater than 37.5 g and also resulted in delayed dissolution in this *in vitro* study. This has implications in the understanding of the mechanism of prolonged and delayed peak concentrations of APAP in massive overdose.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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