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Amino Acid Conjugation: Mechanism for The XL-I form of Bovine Liver Xenobiotic/Medium-Chain Fatty Acid

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Abstract

The XL-I form of xenobiotic/medium-chain fatty acid:CoA ligase was purified to apparent homogeneity from bovine liver mitochondria and used to determine the reaction mechanism. A tersubstrate kinetic analysis was conducted by varying the concentrations of ATP, benzoate and CoA in turn. Both ATP and benzoate gave parallel double-reciprocal plots against CoA, which indicates a Ping Pong mechanism, with either pyrophosphate or AMP leaving before the binding of CoA. Addition of pyrophosphate to the assays changed the plots from parallel to intersecting; addition of AMP did not. This indicates that pyrophosphate is the product that leaves before binding of CoA. Based on end-product inhibition studies, it was concluded that the reaction follows a Bi Uni Uni Bi Ping Pong mechanism, with ATP binding first, followed in order by benzoate binding, pyrophosphate release, CoA binding, benzoyl-CoA release and AMP release. A similar mechanism was obtained when the ligase was examined with butyrate as substrate. However, butyrate activation was characterized by a much higher affinity for CoA. This is attributed to steric factors resulting from the bulkier nature of the benzoate molecule. Also, with butyrate there is a bivalent cation activation distinct from that associated with binding to ATP. This activation by excess Mg(2+) results in non-linear plots of 1/v against 1/[ATP] for butyrate unless the concentrations of Mg(2+) and ATP are varied together.

INTRODUCTION

Antibiotics are frequently prescribed to older adults who reside in long-term care facilities. The median rate of systemic antibiotic use reported in this population is 7 courses per 1,000 resident-days, which is similar to the incidence of antibiotic use in acute care hospitals.1–5 Although the spread of multiresistant bacteria poses the greatest challenge to acute care hospitals,6 the detection of antibiotic-resistant bacteria in long-term care facilities, including multiresistant Enterobacteriaceae, enterococci resistant to aminoglycosides or vancomycin, and methicillin-resistant Staphylococcus aureus, has led to increasing concern about the use of antibiotics in this population.7–15 The potential for bacterial resistance, adverse drug reactions, and the financial costs associated with antibiotic use warrant that they be prescribed judiciously to older persons residing in long-term care facilities.16 Alt - prescriptions in residents of long-term care facilities have been described as inappropriate.2,3,19,20

For clinicians, the decision whether or not to prescribe an antibiotic to a long-term care facility resident can be difficult. Often, clinical signs are nonspecific, and the resident may not be capable of expressing symptoms due to cognitive, hearing, or speech impairment. Frequently, important microbiological diagnostic sources, such as sputum cultures, cannot be obtained, and diagnostic tools, such as x–rays, are not easily available. It is not surprising that the prescription of antibiotics for residents of long-term care facilities is most often empiric, i.e., without the benefit of Gram stain or culture results.16 Relatively few studies have evaluated the appropriateness of the initiation of empiric antibiotics in residents of long-term care facilities.2,3,19

We conducted a prospective study to determine the incidence and variability of antibiotic use

determined how often clinical criteria for infection were met when antibiotics were prescribed in these facilities.

METHODS

Study Facilities

The majority of patients who receive chronic care in Ontario are older than 65 years and require more than 3 hours of nursing care per day.22 This population is similar to residents of skilled nursing homes in the United States. In Ontario, chronic care is provided both by chronic care hospitals and by acute care hospitals with chronic care beds. Therefore, both types of facilities were enrolled in this study. Specialized chronic care hospitals (such as respiratory, spinal cord, or psychiatric facilities) and units providing primarily rehabilitation or palliative care were excluded. All 33 eligible facilities providing chronic care in south-central and southwestern Ontario were invited to participate in the study.

Each of the participating facilities and the institutional review board of the University of Toronto approved the study.

Data Collection

Patients who were being treated with systemic antibiotics were identified prospectively over the 12-month study period (November 1996 to October 1997) by each facility's infection control practitioner through daily review of pharmacy records. Data collected included the name of the antibiotic prescribed, route, start and stop date of administration, and site of infection for which the antibiotic was prescribed. Number of beds, staffing characteristics, and antibiotic-related policies were also recorded for each study facility.

To document the clinical features present in patients for whom antibiotics were prescribed, a random sample of 10 antibiotic courses, stratified by the presumed site of infection, was selected at 4-month intervals. Since smaller facilities generate fewer prescriptions, all antibiotic courses were reviewed for facilities with less than 75 beds. Data abstracted from patient charts included patient demographic, clinical, and laboratory information. Outcomes of antibiotic treatment, including adverse reactions and death, were also recorded. Since lower respiratory, urinary, and skin and soft tissue infections comprise the majority of bacterial infections in long-term care facilities,17,18 prescriptions for these indications were assessed to see if they fulfilled diagnostic criteria based on definitions of infection for long-term care facilities21(Table 1)In the absence of a validated reference standard for assessing antibiotic use, these clinical definitions, although intended for surveillance purposes, provide a conservative standard for assessing antibiotic-prescribing. To ensure accurate data abstraction, an audit of the chart abstraction at each facility for at least 5 of the first 10 prescriptions was performed. Data abstraction was considered acceptable if 80% or more of the items matched. Telephone help desk in case of any uncertainty. Each participating pharmacy had to collect all modified prescriptions (cases) during one predetermined day between February 25 and March 12, 1999. On the same day they had to collect at random an equal number of non-modified prescriptions (controls). After selection of cases and controls the pharmacists had to fill in a registration form for each case and each control.

All prescriptions for medicines and other health care products (e.g. dressings, incontinence materials, syringes and needles) that were offered on the predetermined day to the community pharmacy by the patient, or by fax or telephone had to be included. Cases were all prescriptions that were modified by the pharmacy on that particular day (even if actual dispensing took place on another day). Reasons for including a prescription modification as a case were defined in the protocol and in the registration form for cases. If there were two or more reasons for modifying a prescription the pharmacist had to select the one he/she considered most relevant. The protocol excluded the following modifications because of their lack of potential impact on patient care: address incorrect or absent, no or incorrect insurance data, incorrect package size, product not in stock, unit of dosage or package specified incorrectly (e.g. ml instead of g), generic substitution and legal requirements (e.g. for narcotic drugs). During the data management process we divided the nature of prescription modifications into three groups. In the first group a clarification was needed to carry out the prescription order. In most cases an essential administrative feature of the prescription was missing or obviously incorrect. In fact the pharmacy could not have dispensed the drug without clarification. In the second group for items identified as 'Correction prescription error' the prescription was administratively correct, but could potentially have had clinical consequences if not altered. Those identified as 'wrong dose' is an important example, for which there are several reasons, like too high/low dose according to standard references or in conflict with the patient's own records. The third group included reasons for modification not covered by the first two categories.

Selection of controls

The pharmacists had to provide an equal number of nonmodified prescriptions (controls) by selecting this number at random from a box containing all prescriptions of the same day.

data pharmacists were asked to send in the registration forms as well as relevant copies of the prescriptions and 6 month medication records of the patients concerned. This information was stripped of personal data. Incorrect data in the registration form when compared with the copies of the prescription and/or medication record could lead to an alteration in the final form registered by the research team. For these reasons various cases were excluded from the study. Where double or triple reasons for modification were given, the one considered most relevant was selected so that only one modification per prescription was counted.

Classification of prescriptions

Following Dutch reimbursement regulations items prescribed were classified as prescription only medicines (POM), prescribed OTC medicines (such as paracetamol and miconazole), and nonmedicines (such as dressings, incontinence materials, syringes and needles). The number of prescribed OTC medicines were too small to be worth analysing. All medicines were classified into therapeutic groups using the Anatomical Therapeutic Chemical (ATC) classification of the WHO Collaborating Centre for Drug Statistics Methodology [4].

Analysis

After inspection, data from the registration forms were entered in a Microsoft Access database and statistically analysed using SPSS version 9.0. Logistic regression analysis was used to estimate the association between characteristics and modification of a prescription.

Results

The characteristics of the enrolled pharmacies were comparable with the characteristics of all Dutch community pharmacies in the study period. However, the number of pharmacy assistants in the participating pharmacies was somewhat lower than that in the average Dutch pharmacy, leading to a slightly increased workload per individual.

There was a large variation in the total number of prescriptions per pharmacy, which probably reflects the fact that both small and very large pharmacies were involved in our study.

On the study day, the overall incidence of modifications by the community pharmacies was 4.3% (2014 cases of 47 374 prescriptions) (Table 2). The number of modifications per pharmacy varied from 0 to 100 with a mean of 14.3 prescription modifications per pharmacy. The incidence of modifications for prescription only medicines was 4.9% compared to only 1.4% of the prescriptions for nonmedicines. Modifications of POM prescriptions were most frequently found in the following therapeutic domains: nervous system (ATC group N), respiratory system (R), alimentary tract and metabolism (A), and cardiovascular system (C) (Table 3a).

In 219 cases (12.2%), the modification of a POM prescription was triggered by a signal of the computerized medication surveillance system of the pharmacy concerning a change in therapeutic regimen (e.g. different strength or dose), a potential drug–drug interaction, contraindication or double medication (combination of two medicines with the same or similar ingredient). More than half of the problems concerning POM prescriptions (51.2%) were solved by communication with the patient or his representative, and the same was found for nonmedicines (52.7%). In 282 cases (15.6%), the pharmacy consulted the prescriber about a POM prescription, but the prescriber was contacted less often for nonmedicines (7.5%). Contacts with the prescriber's assistant were similar for POM prescriptions (4.9%) and for prescription modifications of nonmedicines (5.5%) (Table 3b).

In Table 4 the nature of the prescription modifications is summarized. The majority (1294; 71.8%) of the reasons for the 1802 POM modifications concerned the clarification of an insufficiently specified prescription (e.g. dose not specified, insufficient patient data, wrong strength or strength not specified), whereas in 400 cases (22.2%) a prescription error was corrected that might have had clinical consequences ('Correction Prescription Error'). Dose corrections were more prevalent in this latter group (13.7%) than other interventions, such as for a drug–drug interaction, contraindication or double medication (8.5%). In Table 5 we present some individual examples of modifications of POM.

In our analysis of determinants, we focused on modifications of POM prescriptions, since these form the most important group (Table 6). Of the patient-related factors, gender was not significant, but patients of 40–65 years had a lower rate of modifications than younger people (OR = 0.74 [0.64–0.86]). With respect to drug-related factors, we found a higher frequency of POM modifications in the respiratory domain (OR = 1.48 [1.23-1.79]), while a decreased frequency was observed for nervous system POMs (OR = 0.71 [0.61–0.83]). There was no difference between initial and refill prescriptions for POMs, but when a nonmedicine was prescribed for the first time the chance of a modification was much higher than when it was refilled (OR = 3.75 [2.07-6.80]).

With regard to prescriber-related determinants modifications were found three times more often in hand written prescriptions.

with methanol. The resulting solution was centrifuged at 3000 rpm for 5 min and the drug content of the supernatant was determined (1000 and 100 µg/mL for Metoprolol and Ramipril respectively). 2µL of this solution (2000 and 200ng/spot for Metoprolol and Ramipril respectively) was applied to a TLC plate which was developed in an optimized mobile phase. The analysis was repeated in triplicate. The possibility of excipient interference with the analysis was examined.

RESULTS AND DISCUSSION

The results of validation studies on the simultaneous estimation method developed for Metoprolol and Ramipril in the current study involving Methanol: toluene: ethyl acetate: ammonia (2.5: 3.0: 5.0: 0.7 v/v/v/v) as the mobile phase for TLC is given below.

Linearity

The drug response was linear (r2 = 0.997 for Metoprolol and 0.999 for Ramipril) over the concentration range between 2000-12000 ng/spot for Metoprolol and 200-1200 ng/spot for Ramipril. The slope and intercept for Metoprolol and Ramipril were 1.284 (± 0.982), 1979(± 1.25) and 2.947 (± 0.862) and 658 (± 1.06), respectively.

Precision

The results of the repeatability and intermediate precision experiments are shown in Table 1. The developed method was found to be precise as the RSD values for repeatability and intermediate precision studies were < 2 %, respectively as recommended by ICH guidelines.

Table 1: Precision study for Metoprolol and Ramipril

Drug	Concentration ng per band	Intra-day(n=3)		Inter-day(n=3)	
		SD	RSD%	SD	RSD%
Metoprolol	60	14.28	1.040	18.15	1.326
	120	6.96	0.317	5.91	0.269
	180	24.83	0.865	32.73	1.141
Ramipril	60	56.65	1.904	49.86	1.708
	120	33.97	0.671	31.33	0.618
	180	40.51	0.627	41.03	0.635

LOD and LOQ

Signal-to-noise ratios of 3: 1 and 10: 1 were obtained for the LOD and LOQ respectively. The LOD and LOQ were found to be 50 ng/spot and 100 ng/spot for Metoprolol and 50 ng/spot and 150 ng/spot for Ramipril, respectively. The standard deviation of peak areas was calculated for each parameter and the % RSD was found to be less than 2 %. The low values of the % RSD, as shown in Table 2 indicated the robustness of the method.

Table 2: Robustness Testing of Metoprol and Ramipril

Parameters	Metoprolol		Ramipril	
	SD	%RSD*	SD	%RSD*
Mobile phase composition (± 0.1 ml)	10.42	1.235	10.42	1.235
Amount of mobile phase (± 0.5 %)	20.14	1.018	20.14	1.018
Time from spotting to chromatography (± 20 min)	15.36	0.942	15.36	0.942
Time from chromatography to scanning (± 20 min)	20.10	1.085	20.10	1.085

Specificity

The peak purity of Metoprolol and Ramipril was assessed by comparing their respective spectra at the peak start, apex, and peak end positions of the spot,i.e., r (S, M)=0.998 and r (M, E)=0.999. A good correlation(r=0.9997) was also obtained between the standard and sample spectra of Metoprolol and Ramipril, respectively. Also, excipients from formulation were not interfering with the assay.

Recovery Studies

As shown from the data in Table 3 good recoveries of the Thiocolchicoside and Aceclofenac in the range from 98.32 to 99.45 % were obtained at various added concentrations. The average recovery of three levels (nine determinations) for Metoprolol and Ramipril was 98.95 % and 98.98 % respectively.

Table 3: Recovery studies of Metoprolol and Ramipril

Label claim (mg/tablet)	Amount Added (%)	Total amount (mg)	Amount* recovered (mg ± % RSD)	Recovery (%)
Metoprolol 25	80 (20mg)	45	44.65 ± 0.222	99.22
	100 (25mg)	50	49.16 ± 0.154	98.32
	120 (30mg)	55	54.63 ± 0.130	99.32
Ramipril 2.5	80 (2mg)	4.5	4.46 ± 0.526	99.11
	100 (2.5mg)	5.0	4.92 ± 0.360	98.4
	120 (3mg)	5.5	5.47 ± 0.344	99.45

Analysis of a formulation

Experimental results of the amount of Metopr-

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