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Short Communication

In vitro interference by acetaminophen, aspirin, and metamizole in serum measurements of glucose, urea, and creatinine

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ABSTRACT

Objective: Here we aimed to investigate the in vitro effects of three analgesic–antipyretic drugs frequently used in clinical practice in Mexico — acetaminophen (AAP), aspirin (ASA) and metamizole (MMZ) — on serum measurements of glucose, urea, and creatinine.

Design and methods: Each analyte was measured in a base-serum pool spiked with the drugs at subtherapeutic, therapeutic, and toxic doses. Serum glucose and urea were measured using the hexokinase/G-6PDH and urease/GLDH kinetic assays, respectively. Serum creatinine (SCr) was measured with a Jaffe procedure based on the alkaline–picrate reaction and with an enzymatic dry-chemistry system. Measurements were carried out in IL-Monarch and Vitros DT60-II analyzers, respectively. Data were analyzed by the difference-paired interference test and by ANOVA.

Results: By the kinetic Jaffe/Monarch procedure, we found positive interference by the drugs on the SCr measurements and by only ASA for urea measurement. For creatinine measurements, the total errors (TEs) were 22–51%, 18–105%, and 15–26% for AAP, ASA, and MMZ respectively, while for urea measurement the TE was 16–21% for ASA. A negative interference by MMZ on SCr (TE = −47%), but no-interference for AAP or ASA, were found via the enzymatic/DT60-II system.

Conclusions: In vitro positive interference induced by AAP, ASA, and MMZ (via the alkaline–picrate reaction), or negative interference by MMZ (via a dry-chemistry system), on the SCr measurements highlights the importance of investigating all possible sources of variation that may alter the accuracy of the laboratory tests, in order to provide useful results for making medical decisions for optimal patient care.

Introduction

Interfering substances are a common problem for clinical laboratory analyses and affect accuracy of the measured results. These interfering substances arise from endogenous (e.g., hemoglobin, bilirubin, lipids, and paraproteins) and exogenous sources (e.g., drugs prescribed for the patient) [1], and their effects may be pharmacological or methodological. Spectrophotometric interferences affect measurement systems (nonspecific or analyte-independent). Chemical interferences (analyte-specific), so called because they react in the same manner as the analyte, produce a product similar to that measured (positive interference). Interferents can also compete for reagents with the analyte or inhibit indicator reactions (negative interference) [1]. However, this type of error may go undetected by physicians and laboratory professionals.

Although it is accepted that many drugs interfere with analytical methods, the extent to which the accuracy of the measurements is altered or at what concentrations of drugs these effects occur is generally unknown [2]. Clinical laboratories increasingly use more specific (enzymatic) methods for the measurement of many analytes (e.g., glucose and urea). However, creatinine measurements based on the alkaline–picrate Jaffe reaction are still widely used in some countries (due to its simplicity and low cost), despite the fact that non-specific reactions with many substances may cause interference [3–5]. The aim of our study was to investigate the effects of three drugs commonly used in medical practice in Mexico, on serum measurements of glucose, urea and creatinine.

Materials and methods

Estimation of most frequently used drugs

Six-hundred-eighty-three prescriptions were reviewed in one first-level health care center. We found that 88 drugs were prescribed.

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Among these, the anti-inflammatory analgesics most frequently used were acetaminophen (AAP), aspirin (ASA), and metamizole (MMZ). In addition, we found that glucose, urea, and creatinine are the three most frequently requested laboratory tests in clinical chemistry (Supplemental data). Therefore, the in vitro effects of these drugs on glucose, urea, and creatinine measurements were evaluated. MMZ was banned in Sweden, the USA, the UK, and other countries due to agranulocytosis risk. However, in Germany, Spain, Russia, and many other countries (including Mexico) its use is still considered suitable. Over the last 25 years, only one case of agranulocytosis due to metamizole was found in Mexico [6].

Current routine creatinine methods in Mexico

To gain further information concerning the routinely used methods to measure serum creatinine (SCr) in Mexico, we consulted two national proficiency testing (PT) providers (Supplemental Data).

Biological samples

The study conformed to the requirements of the Mexican legislation and the Helsinki Declaration. Blood (80–100 mL) without anticoagulant was drawn from 8 healthy volunteers who were not taking any medications. All volunteers provided informed consent. The serum was obtained via centrifugation, and similar volumes of serum were used to extend the base-serum pool (BSP), to which drugs were added to produce the test-serum pool (TSP).

Drugs

The drugs were donated by two pharmaceutical manufacturers, and their compositions were verified (purity >95–99%) as stated in the Mexican Pharmacopeia. Aqueous solutions (20×) of each drug were prepared, and one volume was mixed with 19 volumes of BSP (1:20 ratio) to obtain final concentrations in TSP of 40, 160 and 2097 μmol/L of AAP; 0.33, 1.0 and 3.6 mmol/L of ASA; and 15, 60 and 600 μmol/L of MMZ. These concentrations are equivalent to subtherapeutic, therapeutic, and toxic doses respectively [7].

Measurement procedures

Serum glucose and urea were measured by hexokinase/G-6PDH (IL-Test™ Glucose) and urease/GLDH (IL-Test™ Urea) kinetic assays respectively. SCr was measured by an alkaline–picrate reaction-based compensated kinetic assay (IL Test™ Creatinine). An instrument correction of −13 μmol/L was set by the manufacturer due to unspecific interactions. All reagents, standards, and commercial quality controls were obtained from the Instrumentation Laboratory Company (IL Co, Milan, Italy). The measurements were carried out in an IL-Monarch analyzer (IL Co, Milan, Italy). An enzymatic dry-chemistry system was also used for creatinine measurements in a Vitros DT60-II analyzer (Ortho Clinical Diagnostics Inc., NY, USA). A preliminary evaluation of the repeatability of each measurement procedure was conducted by assaying 20 measurements in sequence using BSP. Reproducibility was estimated using one run per day with four replicates of BSP for 5 days. The results were validated using commercial control material for quality assessment according to the manufacturer's instructions.

Interference testing

Glucose, urea, and creatinine concentrations were measured in the BSP for the baseline concentration and in the TSP for interference tests. A 3 × 4 design with five or six replicates was followed, and interferences (d_{max}) of ±0.14 mmol/L, ±0.28 mmol/L, and ±17.7 μmol/L were detected for glucose, urea, and creatinine respectively [7].

Data analysis

Data were analyzed for the difference-paired interference test [7] and for ANOVA; the Shapiro–Wilk test was used for normality (SPSS v.17.0, Chicago, IL, USA). p-Values < 0.05 were considered statistically significant. The cut-off value of the differences (d_c) was 0.06 mmol/L, 0.25 mmol/L, and 7.3 μmol/L for glucose, urea, and creatinine respectively [7]. If $(\overline{X_{test}} - \overline{X_{baseline}} > d_c)$, the substance interferes. If $TE_{test} > TE_A$, the difference was clinically significant [8].

Results

Current routine creatinine methods in Mexico

Eight-one percent (1899/2350) of the Mexican laboratories enrolled with the PACAL PT provider measured creatinine via autoanalyzers, with the remaining (19%) 451/2350 using manual techniques. The alkaline–picrate reaction assay was used in 934/1899 (49%) and 308/451 (68%) of automated and manual laboratories, respectively, followed by dry-chemistry in 205/1899 (11%) and enzymatic in 111/1899 (6%) (Supplementary Table S1). Similar results were obtained from the Qualitat PT provider (Personal communication, December 12th, 2014).

Precision

The CV_W (coefficient of variation within-subject) were 1.3, 1.4 and 8.2% for repeatability and the CV_G (coefficient of variation between-subject) were 1.5, 5.4 and 8.8% for reproducibility in glucose, urea, and creatinine respectively, using the Monarch analyzer. The CV_W and CV_G were 1.6 and 2.9% respectively, for creatinine in the Vitros DT60-II.

In vitro interference

A positive interference by AAP and ASA (at therapeutic and toxic doses), and MMZ (at all doses), was found for creatinine. In the case of urea measured by the Monarch analyzer, only ASA was found to interfere. Differences in glucose measurements were non-significant (Table 1). A negative interference was detected at therapeutic doses of MMZ for creatinine but not by AAP and ASA, via a Vitros DT60-II analyzer. Fig. 1 shows the in vitro drug effect on the serum measurements expressed as % of interference, using the Jaffe and enzymatic procedures.

Table 1
Mean values of glucose, urea and creatinine in BSP and TSP with subtherapeutic, therapeutic and toxic concentrations of drugs.

Analyte	Drug	BSP	Subtherapeutic	Therapeutic	Toxic
Glucose	AAP	4.89	5.01 (2.5%)	4.94 (0.9%)	5.00 (2.1%)
(n = 6,	ASA	4.97	5.03 (1.2%)	4.85 (−2.4%)	4.79 (−3.6%)
Monarch)	MMZ	5.00	4.98 (−0.4%)	5.13 (2.2%)	5.00 (0.0%)
Urea	AAP	6.25	5.56 (−11%)	6.37 (2.0%)	6.08 (−2.6%)
(n = 6,	ASA	5.40	6.28 (16%)	6.30 (16%)	6.54 (21%)*
Monarch)	MMZ	5.76	5.34 (−7.2)	5.70 (−1.1)	5.86 (1.7)
Creatinine	AAP	113.5	138.5 (22%)	159.1 (40%)**	170.9 (51%)&
(n = 6,	ASA	110.5	90.6 (−18%)	159.1 (44%)**	226.9 (105%)&
Monarch)	MMZ	107.6	129.7 (21%)**	135.6 (26%)&	123.8 (15%)*
Creatinine	AAP	67.0	65.3 (−1.8%)	67.2 (0.6%)	68.6 (2.4%)
(n = 5,	ASA	66.8	68.7 (3%)	69.3 (4%)	71.5 (7%)
DT60-II)	MMZ	419.3	419.7 (0.08%)	392.7 (−6.4%)	220.7 (−47%)&

Mean values of five/six replicates expressed in mmol/L for glucose and urea, and in μmol/L for creatinine. In parentheses percentage of total error of the test (TE_{test}, %) compared to baseline value. BSP, base-serum pool; TSP, test-serum pool; AAP, acetaminophen; ASA, aspirin; MMZ, metamizole.
* $p < 0.05$ by ANOVA.
** $p < 0.005$ by ANOVA.
& $p < 0.000$ by ANOVA.