Negligible Interference by Spironolactone and Prednisone in Digoxin Radioimmunoassay

Richard Ravel

Spironolactone and prednisone reportedly cause measurable values for apparent digoxin, even when none is present. Effects of these medications were evaluated with 125I-labeled digoxin kits from five different manufacturers. Values obtained for apparent digoxin were either negligible or not sufficiently great to be clinically significant, regardless of kit methodology or manufacturer.

Additional Keyphrase: "kit" methods

Serum digoxin assay is being requested more and more frequently. Reports indicate that chances of digitalis toxicity are considerably increased when digoxin concentrations in blood are not monitored to see how closely the quantity in blood after therapeutic dosage approaches a toxic concentration (1, 2). Other uses include assistance in diagnosis of toxicity and as an indication that a patient may be omitting medication. As is the case for most laboratory tests, results must be interpreted in the context of the clinical findings for the patient and with the knowledge that laboratory results can for various reasons be misleading, as, for example, when compounds are present that cross-react with antidigoxin antibody in radioimmunoassay systems to indicate measurable apparent digoxin when in fact there is none in the specimen. Most such compounds contain steroid configurations similar to those of the digitalis glycosides. A few reports (3, 6) and information in digoxin kits from several manufacturers (7-9) indicate that spironolactone and prednisone display the greatest potential for cross-reactivity among medications thus far investigated. Phillips (4) and Zeegers et al. (6) confirmed that these two drugs may in fact produce laboratory error in digoxin assay. Unfortunately, their studies were very limited and do not clearly indicate how seriously these compounds may interfere. Also, one would like to know whether the interference varies with different digoxin kits in which different antibodies and methods are used. Here, I attempt to provide this information.

Materials and Methods

Ten patients were selected who were receiving oral spironolactone (Alactone or Aldactazide, G. D. Searle and Co.), 25 mg twice a day. I also selected the following patients on oral prednisone: 10 who were receiving 10 mg three or four times a day, one who was receiving 15 mg of prednisone once a day, one receiving 20 mg once a day, two receiving 25 mg twice a day, and one receiving 50 mg twice a day. Most of these patients had never received digitalis compounds, and none had taken digitalis for at least three weeks. Specimens were drawn 2 h after the last therapeutic dose, on the assumption that absorption would then be maximum. Each patient's serum specimen was assayed by I-125 kits from each of the following five manufacturers: Clinical Assays ("Gammacoat"); Clinical Assays, Inc., Cambridge, Mass. 02142; Corning ("Immunophase"); Corning Co., Medfield, Mass. 02052; Kallestad ("Quantitope"); I-125 Digoxin; Kallestad Laboratories, Chaska, Minn. 55318; Mallinkrodt ("RIA-MAT" Digoxin I-125; Mallinkrodt Nuclear, Inc., Maryland Heights, Mo. 63043); and Schwarz-Mann ("Digoxin I-125"); Schwarz-Mann Co., Orangeburg, N. Y. 10962). These kits represent most of the current techniques for separating bound and free radioactive antigen (solid-phase, double antibody, resin strip, and dextran-coated charcoal). For some specimens, determinations were made in different laboratories with a kit supplied by the same manufacturer. Specimens that could not be tested immedi-

References


Nuclear Medicine Section, Department of Pathology, St. Francis Hospital, Miami Beach, Fla. 33141.
Received June 13, 1975; accepted Aug. 4, 1975.
In our laboratory, we used semiautomatic pipets for all pipetting and a Searle (Nuclear-Chicago) Model 4222 automatic well counter for counting radioactivity. All pipets had previously been checked for reproducibility by the isotope dilution technique; the well counter was checked for efficiency by the chi-square test.

Results

Tables 1 and 2 summarize the results. Values are rounded off to the nearest 0.1 µg/liter. Values apparently greater than zero but less than 0.2 µg/liter are reported as zero, because with current laboratory precision and kit sensitivity such values are not reliably distinct from zero. In most cases, values obtained were zero or so low as to be clinically insignificant. Values of 0.4 µg/liter or more were obtained from at least one kit in five of 10 patients taking spironolactone. However, this occurred in only one or two of the five kits used to test each specimen, with at least two of the remaining three kits yielding zero values. In only one instance was a value as high as 0.7 µg/liter obtained. In patients taking 10-mg doses of prednisone, even less reactivity was observed, with only one instance in which a value of 0.5 µg/liter was found. In more limited data on patients taking more than 10-mg doses of prednisone, slightly more reactivity was obtained in that three of five such patients had at least one kit assay value as high as 0.4 µg/liter (contrasted to two of 10 patients taking 10-mg doses). However, this difference is probably not significant, because in each case only one of the five kits produced the higher result. In no case were higher values obtained in patients receiving higher prednisone doses.

Discussion

These results indicate that spironolactone or prednisone therapy should not interfere significantly with digoxin assay. This is contrary to the experience of Zeegers et al. (6), who reported that four patients receiving 25 mg spironolactone daily and no digoxin produced false apparent digoxin values of 0.7, 1.7, 1.8, and 4.0 µg/liter. Burroughs-Wellcome tritiated (3H) kit was probably used, which admittedly is not included in this study. In addition, their work was performed at least two years before the current investigation, during which time significant changes may have been made in antibody specificity.

It is very common for digitalis to be administered to patients on spironolactone, and spironolactone is prescribed with a significant degree of frequency in patients taking digitalis. This fact is the basis for undertaking the present investigation. It also provides an example of certain problems in studies of this type. Several patients initially had significantly elevated and presumably false results for digoxin assay, as assayed with any of the kits, some between 2 and 3 µg/liter. On retrospective inquiry, all of these patients had to be excluded because their values probably represented true digoxin. In two instances, digoxin therapy was begun during the interval between the time a blood sample was ordered and actual specimen collection next day. In the other cases, digoxin had been administered before admission, but was not mentioned in the physician’s admitting note.

Although oral prednisone therapy does not seem to interfere with digoxin assay, I was not able to evaluate the effect of intravenous hydrocortisone, because all patients undergoing this type of therapy during the study were also receiving digitalis.

I thank the following persons for performing part of this study in their laboratories: Mr. Arturo Espinola, NMT (ASCP, ARRT), St. Francis Hospital (all five methods); Ms. Marian Mers, Sayet Associated Laboratories, Dr. Maxwell Sayet and Mr. Peter Sayet, Directors (Kallestad Kit); Dr. Aida Soto, Dade Division of American Hospital Supplies, Miami, Dr. K. Wayne Chambiliss, Director (Kallestad Kit); Dr. Albert Heal, Jackson Memorial Hospital, Drs. August Miale and Fuad Ashkar, Directors (Corning Kit); Ms. Karen Carr, Parkway General Hospital, Dr. Harold Garber, Director (Schwarz-Mann Kit). Special thanks are due the St. Francis Hospital Pharmacy Department, Dr. Alfred A. Rinehardt, Director, for assistance in locating the patients.

References

Screening for Drug Abuse: Use of NaCl to Increase Drug Recovery from Papers Coated with Ion-Exchange Resin

George J. Alexander

Use of papers loaded with ion-exchange resins to adsorb drugs from urine specimens resulted in large losses during the procedure. The first step, removal of drugs from urine specimens, was 25–85% efficient. The second step, elution of drugs from paper for further processing, was approximately 40–70% complete. The efficiency of the first step was decreased and the efficiency of the second step was increased by addition of NaCl, except in the case of barbiturates. Presence of salt during elution increased the yield of dihydromorphine by 20%, of methadone by 16%, of amphetamine by 34%, and of chlorpromazine by 40%, but did not enhance the yield of pentobarbital. Overall recovery rates were: 51% for the opiates, 57% for methadone, 72% for a phenothiazine tranquilizer, but only 35% for amphetamine and 15% for a barbiturate.

Additional Keyphrases: urinalysis • drug assay • toxicology

Uptake of drugs by and their subsequent elution from papers loaded with ion-exchange resin has played a key role in a technique commonly used for routine screening of urine specimens from persons suspected of drug abuse (1). Use of such papers offered obvious advantages in terms of shipping and storing specimens but came under justifiable criticism because of large losses of drugs during the process (2). Because the procedure is so convenient, I have re-investigated the losses at various steps in the procedure and sought to improve the efficiency of drug recoveries.

Materials and Methods

Recoveries of drugs during all stages of the procedure have been followed, either by means of radioisotope-labeled compounds or by fluorometric analysis.


CLIN. CHEM. 21/12, 1803–1804 (1975)

Drugs. [7,8-3H]Dihydromorphine, [1-3H]methadone hydrobromide and [2,14C]pentobarbital were purchased from New England Nuclear, Boston, Mass. 02118. [3H]-Amphetamine sulfate was purchased from the Radiochemical Centre, Amersham, England. Homogeneity of these compounds was tested on thin-layer chromatographic plates with ethyl acetate/methanol/ammonia (17/2/1 by vol) as the developing system (3), and 95% of the radioactivity was present at the correct position (Rf 0.32 ± .04 for dihydromorphine, 0.63 ± .11 for amphetamine, 0.92 ± .07 for methadone, and 0.65 ± .05 for pentobarbital).

The labeled compounds were diluted with appropriate nonradioactive material and aliquots of 20 to 150 nCi (10–40 μg) were individually added directly to scintillation vials for radioassay, and to control urine specimens for extraction on paper loaded with ion-exchange resin. All tests were performed in quadruplicate. Because no radio labeled phenothiazine tranquilizer was available to us, pure crystalline chlorpromazine (kindly provided by Smith, Kline and French, Philadelphia, Pa. 19101) was used. During extraction, the amounts present were assayed fluorometrically (4).

Extraction. Urine specimens (20 ml) were diluted with an equal volume of tap water and drugs adsorbed from them onto paper squares loaded with ion-exchange resin (6 x 6 cm, SA-2; Reeve-Angel Co., Clifton, N. J. 07014), as recommended by Dole et al. (1). The papers were washed briefly with tap water and dried. The treated (“spent”) urines were retained for assay of residual radioactivity. The adsorbed drugs were then eluted from the papers with chloroform/isopropanol (3/1 by vol) at the appropriate pH (2.2 for barbiturate, 9.3 for morphine, methadone, and phenothiazine, and 11.0 for amphetamine). Portions of all ion-exchange squares, urine, buffers, and solvent layers were transferred to scintillation vials, when necessary evaporated to dryness or to a small volume, treated with solubilizer (Scintisol GP; Isolab, Elkhart, Ind. 46514) and ethanol, and counted after addition of the counting cocktail [2,5-diphenyloxazole and 1,4-bis-2-(5-phenyloxazolyl)-benzene phosphors in toluene] in a Tricarb Scintillation counter.

Neurotoxicology Research Unit, New York State Department of Mental Hygiene, 1500 Waters Place, Bronx, N.Y. 10461.
Received June 26, 1975; accepted Aug. 7, 1975.