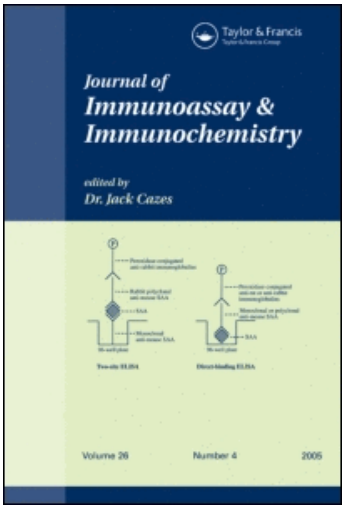


Journal of Immunoassay and Immunochemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597271

Radioimmunoassay of Trospiumchloride, A Quaternary Tropane Derivative

Timo Lehtola[a]; Olavi Lavikainen[a]; Aarre Huhtikangas[a]
[a] Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland

To cite this Article Lehtola, Timo , Lavikainen, Olavi and Huhtikangas, Aarre(1988) 'Radioimmunoassay of Trospiumchloride, A Quaternary Tropane Derivative', Journal of Immunoassay and Immunochemistry, 9: 3, 297 — 307
To link to this Article: DOI: 10.1080/01971528808053218
URL: http://dx.doi.org/10.1080/01971528808053218

JOURNAL OF IMMUNOASSAY, 9(3&4), 297-307 (1988)

RADIOIMMUNOASSAY OF TROSPIUMCHLORIDE, A QUATERNARY TROPANE DERIVATIVE

Timo Lehtola, Olavi Lavikainen and Aarre Huhtikangas
Department of Pharmaceutical Chemistry
University of Kuopio
POB 6, SF-70211 Kuopio, Finland

ABSTRACT

A specific radioimmunoassay (RIA) has been developed for the determination of picogram amounts of trospiumchloride, an anticholinergic agent, from unpurified serum and urine samples. Practically no interference was observed for various potential crossreacting compounds tested. The method is sensitive and excellent values for accuracy, precision and correlation were obtained.
(KEY WORDS: Trospiumchloride; radioimmunoassay; anticholinergic drug).

INTRODUCTION

Trospiumchloride [azoniaspiro-(3α-benziloyloxy-nortropane-8,1'-pyrrolidine)-chloride] , an anticholinergic agent (1,2,3) with quaternary amine structure is not easily quantitated by conventional methods of instrumental analysis. Immunoanalytical methods should offer the highest potential for successful analysis considering the analytical sensitivity required for pharmacokinetic

investigations of this type of compound as trospium-chloride (4,5,6).

MATERIALS AND METHODS

Chemicals

Trospiumchloride, azoniaspiro-(3α-hydroxynor-tropane-8,1'-pyrrolidine)-chloride and azoniaspiro-(3α-hydroxynortropane-8-spiro-1'-pyrroline)-chloride were obtained as a generous gift of Dr. Madaus GmbH & Co., Cologne, FRG. Tropenzilinbromide was obtained from Sandoz AG, Switzerland. Other compounds used in cross-reaction tests were obtained from Sigma Chemical Company, USA. [3',4'-^{3}H] -trospiumchloride (specific activity 37 Ci/mmol) and the ACS-scintillation fluid were obtained from Amersham, England. All other chemicals used were standard commercial products of analytical grade.

Preparation of biological samples

Serum and urine samples for RIA were obtained from healthy volunteers after oral and intravenous administration of 100 mg and 2 mg trospiumchloride, respectively. The samples were diluted with PBS-buffer solution (pH 7,2) for RIA-procedure.

Antiserum

The trospiumchloride-immunogen for antiserum production was prepared by diazo-coupling of one or both aromatic rings of the 2,2-diphenyl-2-hydroxyacetyl moiety with diazotized p-aminobenzoic acid (PABA), and subsequent coupling of bovine serum albumin (BSA) to carboxyl group of the PABA-moiety by using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) for the condensation (Fig. 1)(7,8).

PABA (0,45 mmol) was dissolved in 0,2 M HCl and the solution was cooled to 4°C. Then sodium nitrite (0,60 mmol) was dissolved in ice-cold water, and the solution was added dropwise to the solution of PABA. The reaction was allowed to stand for 45 minutes at 4°C. Sulfamic acid (0,15 mmol) was dissolved in ice-cold water and this solution was added dropwise to stop the diazotization reaction. Trospiumchloride (0,18 mmol) was dissolved in 1:1 N,N-dimethylformamide - 0,1 M sodium borate buffer (pH 9,0, 4°C). The diazotized PABA solution was added dropwise to the solution of trospiumchloride, maintaining the pH at 9,0 with 0,5 M NaOH. The reaction was allowed to proceed in the dark for 4 hours at 4°C, after which the pH of the reaction mixture was adjusted to 6,0 with 1,0 M HCl. Then BSA (0,0037 mmol) was added to the hapten solution (pH 6,0). Water-soluble EDC (0,16 mmol) was added and the reaction was allowed to

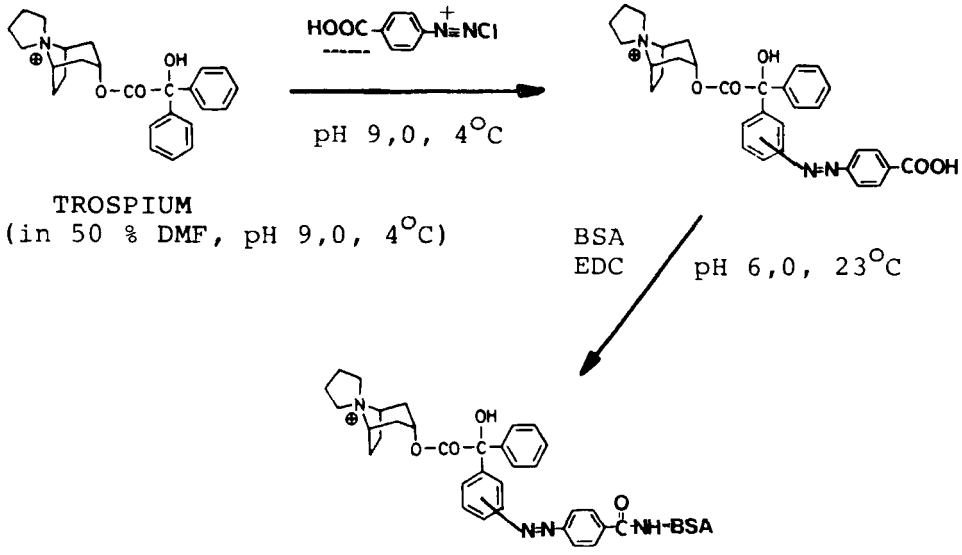


FIGURE 1. Synthesis of trospium-BSA immunogen. DMF = N,N-dimethylformamide; BSA = bovine serum albumin; EDC = 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide.

proceed overnight at 23°C. The BSA-trospiumchloride-immunogen was first dialyzed against water for four days at 4°C and then against physiological saline for 8 hours.

The purified and lyophilized immunogen was emulsified in Freund's complete adjuvant (9) and given in multisite subcutaneous injections (10) to six New Zealand albino rabbits at four-week intervals. The rabbits were bled after half an year from start of immunization. Antiserum was stored frozen at -20°C.

Radioimmunoassay

After addition of 100 µl of a standard solution, a sample dilution or a solution of a compound used in the crossreaction tests, 100 µl of a 1 % solution of human gammaglobulin in PBS-buffer, 50 µl tritiated trospiumchloride in EtOH-PBS-buffer and 50 µl of the PBS-buffer-diluted trospiumchloride antiserum were added to the tubes. Antiserum dilutions effecting 50 % binding of labelled antigen were used in the assay. The tubes were vortex-mixed and incubated 24 h at 4°C. Bound and free antigen were separated by polyethyleneglycol (PEG) precipitation (11) using 500 µl of a 25 % solution of PEG 6000 in PBS-buffer. After centrifugation (2000 g, 20 min) the supernatant was separated by decantation, mixed with 5 ml of ACS-scintillation solution and counted in a LKB-Wallac 1216 Rackbeta liquid scintillation counter.

RESULTS

Antibody production against trospiumchloride was detectable in five of the six rabbits. The titre (defined as the final dilution of the antiserum needed to bind 50 % of the added ^{3}H-trospiumchloride) of the best antiserum was 1:150.

The standard curve for trospiumchloride and the structures of the compounds used in crossreaction

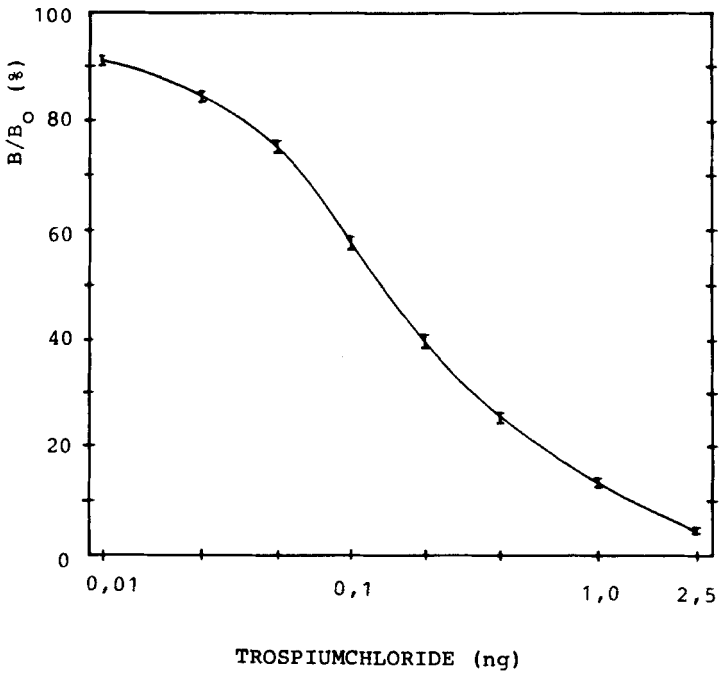
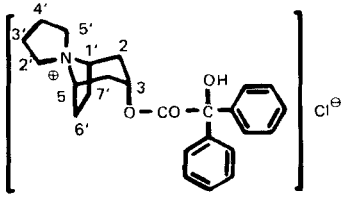


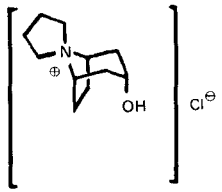
FIGURE 2. Standard curve for trospiumchloride radioimmunoassay. B and B_o are the binding percentages in presence and absence, respectively, of unlabelled trospiumchloride. Bars indicate the standard deviation.

tests with trospiumchloride as the reference are shown in Figs. 2 and 3, respectively. The crossreactivity of the trospiumchloride antiserum is presented in Table 1.

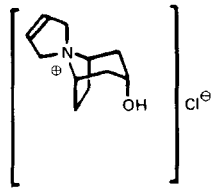
The assay detection limit, defined as the detectable concentration equivalent to twice the standard deviation of the zero-binding value, was approximately



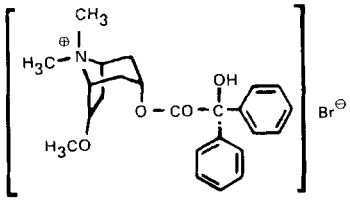
1



2

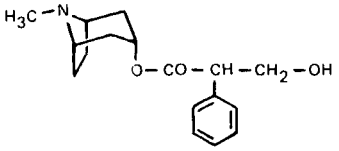


3

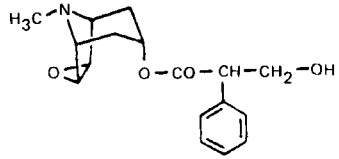


4

5



6



7

FIGURE 3. Structural features of the crossreacting compounds. 1. Trospiumchloride; 2. Azoniaspiro-(3α-hydroxynortropane-8,1'-pyrrolidine)-chloride; 3. Azoniaspiro-(3α-hydroxynortropane-8-spiro-1'-pyrroline)-chloride; 4. Tropenzilinbromide; 5. Nortropin; 6. Atropine; 7. Scopolamine.

TABLE 1

Crossreactivity of the trospiumchloride antiserum

Name of compound	Crossreaction (%)
Trospiumchloride	100
Azoniaspiro-(3α-hydroxynortropane-8,1'-pyrrolidine)-chloride	0,07
Azoniaspiro-(3α-hydroxynortropane-8-spiro-1'-pyrroline)-chloride	0,03
Atropine	0,03
Scopolamine	0,02
Tropenzilinbromide	0,02
Nortropin	0

The crossreactivities were determined at 50 % inhibition of the binding of labelled trospiumchloride.

10 pg in the 100 µl sample. The measuring range of the assay extends from 10 pg to 2,5 ng of trospiumchloride.

Linearity was investigated by the sample dilution method (12). The regression equation for the serum was y=2,10+0,99x, with a coefficient of correlation of r=0,999 (n=12). The corresponding values for the urine were y=0,12+1,00x and r=0,999 (n=12). Recovery of trospiumchloride added to the serum and urine pools was 103,81% ± 1,84% (n=12) for serum and 95,70% ± 3,10%

(n=12) for urine. Within-assay and between-assay variations for serum samples were 3,9% (n=30) and 6,7% (n=15), respectively. The corresponding values for urine samples were 4,8% (n=30) and 7,2% (n=15).

DISCUSSION

The present RIA is an excellent tool for the quantification of trospiumchloride from biological samples.

With the crossreaction percentages 0,07% and 0,03%, azoniaspiro-(3α-hydroxynortropane-8,1'-pyrrolidine)-chloride and azoniaspiro-(3α-hydroxynortropane-8-spiro-1'-pyrroline)-chloride, respectively, did not interfere in the assay. The former compound originates as the main metabolite of trospiumchloride through hydrolysis of the ester bond. Tropenzilinbromide, a spasmolytic agent structurally related to trospiumchloride, crossreacted only 0,02% in this RIA. The crossreaction percentages of atropine and scopolamine, two naturally occuring anticholinergic alkaloids, were 0,03% and 0,02%, respectively. Furthermore, nortropin did not crossreact in this assay. The high specificity of the method obviously results from the position to which the protein component has been attached on trospiumchloride in the immunogen conjugate (13). The specificity of binding of free hapten to antibody is largely determined by the hapten substituent groups which are most distant from protein moiety in the conjugate (14).

The sensitivity of the assay allows extended monitoring of serum and urine levels of this compound which, as a quaternary amine is quite rapidly excreted.

The results concerning the pharmacokinetics of trospiumchloride will be published later.

ACKNOWLEDGEMENTS

This study was supported by Dr. Madaus GmbH & Co., Cologne, FRG. The authors wish to thank Dr. Madaus & Co. also for providing the serum and urine samples.

Address for reprint requests: Dr. T. Lehtola, Dept. Pharm. Chem., Univ. Kuopio, POB 6, SF-70211 Kuopio, Finland.

REFERENCES

1. Lembeck, F., List, W. and Neumann, K. Untersuchung über die Verwendbarkeit von Spasmex [Azoniaspiro-(3α-benziloyloxy-nortropan-8,1-pyrrolidin)-chlorid] zur Anaesthesie-Prämedikation am Menschen. Anaesthesist. 1974;23: 345-350.

2. Lux, G. and Frühmorgen, P. Hemmung der gastralen Säuresekretion und Motilität durch Trospiumchlorid. Fortschr. Med. 1978;41: 2113-2116.

3. Albrecht, H., Bruhn, R., Lorenz, D., Lücker, P.W and Schumacher, M. Pupillometrie:Eine nicht-invasive pharmakokinetische und pharmakodynamische Untersuchungsmethode zur Wirkung von Trospiumchlorid (Spasmolyt) an der glatten Muskulatur. Meth. Find. Exp. Clin. Pharmacol. 1983;5: 585-587.

4. Lehtola, T., Huhtikangas, A., Hiltunen, R. and von Schantz, M. Radioimmunoassay of digoxigenin glycosides in Digitalis lanata. Planta Medica 1981;42: 250-254.

5. Lehtola, T., Huhtikangas, A. and Virtanen, R. Application of radioimmunoassay to the quantitation of hyoscyamine in plant material. Planta Medica 1982;45: 237-239.

6. Huhtikangas, A., Lehtola, T., Lapinjoki, S. and Lounasmaa, M. Specific radioimmunoassay for vincristine. Planta Medica 1987;53: 85-87.

7. Wurzburger, R.J., Miller, R.L., Boxenbaum, H.G. and Spector, S. Radioimmunoassay of atropine in plasma. J. Pharmacol. Exp. Ther. 1977;203: 435-441.

8. Virtanen, R., Kanto, J. and Iisalo, E. Radioimmunoassay for atropine and l-hyoscyamine. Acta Pharmacol. Toxicol. 1980;47: 208-212.

9. Freund, J. The effect of paraffin oil and mycobacteria on antibody formation and sensitization. Amer. J. Clin. Pathol. 1951;21: 645-657.

10. Collins, W.P., Mansfield, M.D., Alladina, N.S. and Sommerville, I.F. Radioimmunoassay of plasma testosterone. J. Ster. Biochem. 1972;3: 333-348.

11. Shaw, W., Schulman, L. and Spierto, F.W. The carrier effect of gamma globulin when a polyethylene glycol separation technique is used in digoxin radioimmunoassay. Clin. Chim. Acta 1975;60: 385-389.

12. Nickel, S. and Staba, E. Suitability of antidigoxin antiserum for digoxin in plant extracts. Lloydia 1977;40: 230-235.

13. Nieschlag, E., Loriaux, D.L. and Lipsett, M.B. Radioligand assay for Δ^5-3β-hydroxysteroids. Steroids 1972;19: 669-679.

14. Gross, S.J., Campbell, D.H. and Weetall, H.H. Production of antisera to steroids coupled to proteins directly through the phenolic A-ring. Immunochemistry 1968;5: 55-65.