jnm/DIAGNOSTIC NUCLEAR MEDICINE

ENDOTOXIN AS A CAUSE OF ASEPTIC MENINGITIS AFTER RADIONUCLIDE CISTERNOGRAPHY

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The role of pyrogens in aseptic meningitis after radionuclide cisternography was studied by means of the Limulus test, a sensitive detector of endotoxin. During a 15-month period, 39 reactions associated with cisternography were reported. Ten samples of specific lots of the radioactive drugs implicated in 20 of these reactions were tested and all reacted strongly positive to the Limulus test. The less sensitive rabbit pyrogen test was negative for these preparations when tested on a dose-per-weight basis. Our findings apparently provide clinical evidence for the observation made in animals that endotoxin is at least 1,000 times more toxic intrathecally than intravenously. The data implicate endotoxin contamination as a cause of adverse reactions to radionuclide cisternography. We conclude that the USP pyrogen test is insufficiently sensitive for intrathecal injectables and should be supplemented by the Limulus test.

Radioiodinated human serum albumin (^{131}I-IHSA) and the DTPA chelates of ^{111}In and ^{169}Yb have become accepted as tracers for radionuclide cisternography because of their reliability in demonstrating cerebrospinal fluid dynamics. Nevertheless, apprehension attends the use of this procedure because of isolated reports of aseptic meningitis following intrathecal ^{131}I-IHSA injections (*1–4*). Alarmingly high reaction rates, up to 14% (Table 1), were reported in recent studies, and reactions to one of the newer agents were reported (*5–9*). Banerji and Spencer (*10*) reported that 27% of 60 patients had a febrile response of at least 1°F within 24 hr of ^{131}I-IHSA cisternography; when patients had accompanying pneumoencephalograms, the incidence was 41% of 88 patients. The survey of adverse reactions to radiopharmaceuticals sponsored by the Society of Nuclear Medicine presented 19 reports of aseptic meningitis following intrathecal injection of ^{131}I-IHSA (*7*). Adverse reactions after cisternography occurred at Georgetown Hospital in Washington, D.C., and led us to study their cause (*11*).

TABLE 1. ADVERSE REACTIONS FOLLOWING RADIONUCLIDE CISTERNOGRAPHY

Radioactive tracer*	Investigator(s) with reference	Symptomatic meningitis†
^{131}I-IHSA	Detmer and Blacker (1)	1
^{131}I-IHSA	Nicol (2)	1
^{131}I-IHSA	Oldham and Staab (3)	1
^{131}I-IHSA	Jonas and Braunstein (4)	1
^{99m}Tc-HSA and ^{131}I-IHSA	Barnes and Fish (5)	5/50 (10%)
^{131}I-IHSA	Messert and Rieder (6)	7/50 (14%)
^{131}I-IHSA	Atkins, et al (7)	19
^{111}In-DTPA	Alderson and Siegel (8)	2/130 (1.5%)
^{111}In-DTPA	Wagner, et al (9)	1/16 (6%)

* IHSA, radioiodinated (^{131}I) human serum albumin, USP; HSA, human serum albumin; DTPA, diethylenetriamine pentaacetic acid.

† Number of patients with symptomatic meningitis/total number of patients in study, if reported.

Although the etiology of these adverse reactions was unknown, the amount of albumin (*1–3,12*), chemical irritants (*1,5,8*), sensitization (*1,8*), and pyrogens (*5,8,10*) were advanced as possible causes. The advent of the Limulus test made available a rapid, sensitive method for detecting bacterial endotoxin (pyrogens) in radiopharmaceuticals and other parenterals (*13–15*). By means of the Limulus test, endotoxin was detected in radiopharmaceuticals, which caused aseptic meningitis after intrathecal injection, and this contaminant was implicated as the etiologic agent in the adverse reactions reported here.

Received Jan. 30, 1975; revision accepted April 10, 1975.

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MATERIALS AND METHODS

Glassware was cleaned, sterilized, and rendered pyrogen-free by heating in a dry-air oven at 200°C for at least 2 hr. Sterile, pyrogen-free solutions were used for all procedures. Specimens of Limulus polyphemus (horseshoe crab) were collected near Chincoteague, Va., and Limulus amebocyte lysate was prepared by a modification of the Levin method (*14,15*). The amebocytes were lysed by adding Sterile Water for Injection, USP, to cells in a 2:1 ratio by volume while agitating vigorously. The supernate was removed and stored at 40°C.

Purified endotoxins of Escherichia coli (E. coli 026:B6, Difco No. 574514) and Klebsiella pneumoniae, the FDA reference pyrogen (*16*), were prepared in stock solutions of 100 μg/ml. The sensitivity of lysate preparations was determined with freshly prepared twofold dilutions of these endotoxins, 2 through 0.125 ng/ml concentrations. Acceptable lysate detected 1 ng/ml of these endotoxins within 1 hr by forming a firm gel. Sodium Chloride for Injection, USP (Abbott Laboratories), or Sterile Water for Injection, USP, was used for endotoxin stock solutions and dilutions and served as the negative control when mixed with Limulus amebocyte lysate.

Test units of lyophilized lysate were prepared by lyophilizing 0.1 ml of tris-buffered lysate (pH 7) in 2-ml funnel-top ampuls (Wheaton Glass Co.) and sealing with rubber closures under vacuum. The lyophilized lysate was stored below 0°C or at 4°C and assayed as described above. A slight increase in sensitivity was often observed for the lyophilized units. This was attributed to the fact that the relative endotoxin concentration was higher in the test ampuls when reconstituted with the test solution than when mixed in equal parts with liquid lysate. A commercial Limulus amebocyte lysate (Pyrogent, by Mallinckrodt, Inc.) was used as directed by the package insert in a comparative study.

The Limulus test was conducted by injecting 0.25–0.3 ml of the radiopharmaceutical directly into a test ampul of lyophilized lysate. The assay mixture was thoroughly and quickly mixed and then incubated immediately in a water bath at 37°C. Test ampuls were observed periodically without further agitation for at least 60 min for the formation of an opaque gel. The time required for gel formation was recorded in minutes as the time of gelation. The results were graded in our laboratory as follows: G, formation of a firm gel that remains intact when inverted slowly to 180°; V, increase in viscosity, opacity, and observance of gelatinous granules; and N, negative, no formation of opaque gelatinous granules.

G and V grades are positive for bacterial endotoxin. It has been our experience with endotoxin solutions and small-volume parenteral products that a positive Limulus test with a gel time greater than 25 min will not produce a pyrogenic response in rabbits when the same solution is injected intravenously at a dose of 1 ml/kg (*13,14*). On this assumption, positive reactions for radiopharmaceuticals are categorized as follows by the gel time (the time in minutes required for a firm gel to form after prompt mixing and incubation of the assay mixture): strong positive, gel time less than 30 min; positive, gel time 30–60 min; and trace, gel time greater than 60 min or a V grade test result.

Preparations of ^{131}I-IHSA, radioiodinated (^{131}I) human serum albumin, USP, were the products of Abbott Laboratories (Risa-131-H) and E. R. Squibb & Sons (Albumotope-^{131}I). Preparations of ^{111}In-diethylenetriamine pentaacetic acid (^{111}In-DTPA) were obtained from one supplier.

RESULTS

Limulus tests were conducted on over 100 different commercial lots of ^{131}I-IHSA and ^{111}In-DTPA preparations during a 15-month period beginning in March 1972. Test samples and notifications of adverse reactions came to us from investigators and industry or by response to a published request for participation in this study (*17*). Of 39 cases of aseptic meningitis, we obtained 10 samples of radiopharmaceuticals for assay implicated in 20 adverse patient reactions. Every lot of ^{131}I-IHSA and ^{111}In-DTPA tested associated with an adverse reaction yielded a strongly positive result by the Limulus test.

One reaction occurred after Risa-131-H intrathecal injection but the specific lot was unavailable for a Limulus test. However, 35 of the lots of this drug tested by the Limulus test during this period were either negative or contained trace levels of endotoxin.

Of 10 lots of Albumotope-^{131}I associated with 24 reactions, 5 lots were obtained that were implicated in 8 adverse reactions. All five reacted strongly positive with the Limulus test (Table 2). Another lot tested during this time was also strongly positive.

One lot of Albumotope-^{131}I (assay date of July 21, 1972, Table 2), which was both Limulus-test positive and associated with untoward effects, was assayed after serial dilutions to approximate the endotoxin concentration. The undiluted solution and 1:10 and 1:100 dilutions (in Sodium Chloride for Injection, USP) gave 12-, 15-, and 25-min gel times, respectively, by the Limulus test. These results indicated a high level of endotoxin contamination in the

TABLE 2. ADVERSE REACTIONS TO RADIONUCLIDE CISTERNOGRAPHY AND LIMULUS TEST RESULTS FOR ALBUMOTOPE-^{131}I*

Assay date for lot	Adverse reactions		Limulus tests†
	Cases	Hospitals	
April 28, 1972	2	2	NA
June 2, 1972	2	2	NA
June 9, 1972	2	1	G-12
July 14, 1972	1	1	G-12
July 21, 1972	2	2	G-12
July 28, 1972	7	2	NA
August 4, 1972	4	2	NA
September 1, 1972	1	1	G-18
September 22, 1972	2	1	G-20
October 18, 1972	None reported		G-20
October 25, 1972	1	1	NA

* Radioiodinated (^{131}I) human serum albumin USP.

† G, positive result with time (min) required for formation of a firm gel of Limulus amebocyte lysate after prompt mixing and incubation of the assay mixture. NA, not available for Limulus test.

original sample, i.e., greater than 0.1 μg/ml when compared to purified endotoxins (see MATERIALS AND METHODS).

A second lot of Albumotope-^{131}I (assay date of September 1, 1972, Table 2) yielded a strongly positive Limulus test: a gel time of 18 min. The rabbit pyrogen test (0.14 ml/kg), conducted at the time of release by the supplier, was negative with a 0.32°C mean temperature increase; a 0.5°C mean temperature increase was defined as a positive pyrogen test for a biologic at the time of this study. Two months later this lot was retested at a greater dose (1 ml/kg) and produced a positive result, a 0.83°C mean temperature increase in three rabbits.

The Albumotope-^{131}I lots discussed above (1% albumin) were prepared by radioiodination of a 25% human serum albumin, Hyland Lot 0490R015A. A Limulus test of the parent lot of human serum albumin (25%) was positive with a gel time of 40 min, but a tenfold dilution of the same lot was negative. Also, the rabbit pyrogen test (3 ml/kg) of the 25% human serum albumin lot was negative. The parent lot of concentrated human serum albumin was not a significant source of contamination. Low-level endotoxin-like contamination is not uncommon in these albumin products (*14*).

During preparation, ^{131}I-IHSA is passed through a washed anion-exchange resin column to reduce the amount of nonprotein-bound radioiodine. Since the resin was suspected as a source of contamination, an initial resin column wash was supplied by one manufacturer for Limulus testing. Serial dilutions to 1:10,000 gave positive results, which indicated that the initial wash contained endotoxin greater than 10 μg/ml. Therefore, inadequate column washing could have contributed significant amounts of endotoxin to these radioiodinated drugs.

We learned of 14 reactions to the ^{111}In-DTPA preparations. Five were isolated events, but nine reactions followed the use of two specific lots in May 1973. The manufacturer supplied samples of these two lots and all solutions associated with their formulation as well as three other implicated lots. All five lots of ^{111}In-DTPA were found positive by the Limulus test. By testing the solutions used in formulation, the source of pyrogenic contamination was traced to a phosphate buffer. Eleven other lots of ^{111}In-DTPA were negative by the Limulus test.

Cultures of the ^{131}I-IHSA and ^{111}In-DTPA lots were negative. Also, when sample volume permitted, the commercial Limulus amebocyte lysate was also used to test these radiopharmaceuticals; test results were essentially the same.

DISCUSSION

Adverse reactions to radionuclide cisternography, usually described as aseptic or chemical meningitis, have been reported as mild to serious (*1–9*). Recovery was usually rapid with no residual sequelae. All cultures of cerebrospinal fluid and the implicated radiopharmaceutical were negative.

In the first two reported reactions, 28 mg albumin (^{131}I-IHSA) was administered intrathecally to one patient and 100–130 mg to the second (*1,2*). DiChiro, et al (*12*) suggested that the total amount of albumin administered be limited to 4 mg because their experience with high specific activity ^{131}I-IHSA (over 200 patients) and ^{99m}Tc-HSA (40 patients) was without untoward reaction. Subsequently, reactions were reported with the high specific activity ^{131}I-IHSA and ^{111}In-DTPA, when it became available, with similar clinical and laboratory findings (*8*). Therefore, albumin apparently was not the single causative factor.

The two commercial preparations of ^{131}I-IHSA used in cisternography (not an FDA-approved use) contain 1% albumin, benzyl alcohol as a preservative, and a buffer system to maintain neutrality. The intrathecal toxicity of benzyl alcohol in dogs was studied by DeLand; he found no evidence of meningeal reaction following cisterna magna injections in concentrations up to ten times that found in this product (*18*). Although the product formulations were similar, the adverse reactions to ^{131}I-IHSA reported in this study followed the use of one supplier's product in 24 of 25 cases. The 24 adverse reactions to Albumotope-^{131}I (Table 2) occurred during a 7-month period. Since all six samples of the radio-

active drug that were produced during this period reacted similarly to the Limulus test (gel times of 20 min or less), there may have been endotoxin contamination in all the lots prepared by the manufacturer during the 7-month period.

A likely source of pyrogenic contamination for the ^{131}I-IHSA preparations was the anion-exchange resin used in production for radiochemical purification. Resin washes provided by one manufacturer were highly contaminated according to Limulus test results. This reinforces the necessity to wash ion-exchange resins copiously with pyrogen-free solutions.

Barnes, et al (*5*) and DiChiro (*12*) have demonstrated that stringent quality-control measures for preparing intrathecal radiotracers are effective in reducing aseptic meningitis following radionuclide cisternography. After the manufacturers employed the Limulus test in their quality-control program, no reactions to intrathecal ^{131}I-IHSA were reported to the Society of Nuclear Medicine Registry of Adverse Reactions in 1973 (*19*).

We suggest that endotoxin or an endotoxin-like contaminant was the cause of most, if not all, of the adverse reactions reported here. The evidence supporting this contention may be summarized as follows: (A) the Limulus test detected endotoxin in all ten available lots of radiotracers that were implicated in meningeal reactions; (B) a single source of ^{131}I-IHSA was associated with 24 of 25 reactions although both formulations were similar; (C) meningeal reactions followed the use of two different radiotracers (^{131}I-IHSA and ^{111}In-DTPA); (D) for one Limulus-positive ^{131}I-IHSA lot, a repeat pyrogen test, at a higher dose, was pyrogenic in rabbits; (E) endotoxin contamination was traced to production components; and (F) to date quality-assurance measures have reduced the incidence of this adverse reaction.

The preparations that were positive for endotoxin by the Limulus test had passed the supplier's pyrogen and sterility tests. Endotoxin administered intravenously induces host cells (granulocytes, Kupffer cells, and others) to release endogenous pyrogen that acts on the thermoregulatory center in the hypothalamus to increase body temperature (*20*). However, the fate of intrathecally administered pyrogen and its mechanism of meningeal irritation is unknown. Bennett, et al (*21*) demonstrated that endotoxin in the subarachnoid space of dogs and rabbits was at least 1,000 times more potent than the intravenous route in producing febrile response. These findings were reproduced in cats (*22*). Our data may be the first clinical evidence of the increased potency of intrathecally administered bacterial endotoxin. These data also demonstrate that the USP pyrogen test is not sufficiently sensitive to serve as a screening test for intrathecal drugs.

Initial studies of the relative sensitivities of the rabbit and Limulus tests for purified pyrogen demonstrated that the Limulus test was five to ten times more sensitive when its endpoint was formation of a firm gel within 1 hr and the rabbit pyrogen test dose of 1 ml/kg produced a minimal average increase in rectal temperature of 0.5°C in three or more rabbits (*13*). Elin, et al (*23*) confirmed this observation in a study of three bacterial endotoxins using similar criteria.

The supplier's intravenous pyrogen test dose to rabbits was 0.14 ml/kg for ^{131}I-IHSA and 1 ml/rabbit for the ^{111}In-DTPA preparation. The total human dose was usually less than 1 ml administered intrathecally. Pyrogen testing of parenteral products on a dose-per-weight basis has been validated by Greisman, et al (*24*) but only for intravenous administration. Since the pyrogen test (*25*) allows small volume parenterals to be tested relative to the human dose, it is common practice in the drug industry to build a safety factor into the rabbit pyrogen test dose. For example, the human dose for this procedure was assumed to be 1 ml ^{131}I-IHSA/70-kg man (0.014 ml/kg); therefore, the 0.14-ml/kg rabbit pyrogen test dose described above provided a safety factor of 10. By the same reasoning, a 1-ml/kg pyrogen test dose would have a safety factor of 70. The Limulus test would have a safety factor of 350 for a 1-ml dose of ^{131}I-IHSA in adults. Since endotoxin is apparently more potent by the intrathecal route, we believe that a Limulus test should be required for intrathecal drugs to serve as a more sensitive test and provide a higher safety factor.

Since the gelation of lysate by endotoxin in the Limulus test is dependent on the concentration of bacterial endotoxin (*13,15*), more than qualitative information may be gained from a Limulus test. An estimate of the pyrogenic level may be made by comparing the gel time or serial dilution assays of the test sample with purified endotoxins, i.e., lipopolysaccharides from the cell wall of gram-negative bacteria.

Several substances other than endotoxin have been reported to induce gelation of Limulus amebocyte lysate. Elin, et al (*23*) found that synthetic polynucleotides gelled lysate; however, we were unable to reproduce these results using commercial Poly (I)-poly (C). They also reported that certain thromboactive materials gelled lysate but the presence of endotoxin contamination in these drugs was not ruled

out (*23*). Microgram levels of isolated peptidoglycans from gram-positive bacteria produced positive Limulus tests but intact gram-positive organisms failed to induce gelation (*26*). Only endotoxin has been reported to induce gelation at nanogram concentrations; therefore, it is unlikely that any other contaminant could have produced the biologic responses and positive Limulus tests reported here (*13,15,23,26*).

When the Limulus test was used clinically to detect endotoxin in other bodily fluids, these test results correlated well with clinical and bacteriologic findings (*27,28*). This study demonstrates the usefulness of the Limulus test to detect endotoxin in drugs for which the rabbit pyrogen test is either insufficiently sensitive or otherwise unsuitable. Although the Limulus test is unofficial at this time, we conclude that it should supplement the rabbit test in screening intrathecal drugs for pyrogen.

ACKNOWLEDGMENTS

We are grateful to the physicians who provided samples for Limulus testing and clinical information on adverse reactions, and to Hal Atkins, who supplied data from the Society of Nuclear Medicine Registry of Adverse Reactions. We are also grateful to individuals in the radiopharmaceutical industry and the FDA's Bureau of Biologics who supplied information regarding the radiopharmaceuticals discussed here.

This work was presented in part at the 20th Annual Meeting of the Society of Nuclear Medicine in June 1973. We thank Ben Friedman and Jack Levin for reviewing this manuscript.

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