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Detection of Endotoxin on Sterile Catheters Used for Cardiac Catheterization

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Pyrogen reactions during cardiac catheterization are an alarming complication that frightens patients and baffles many physicians. This report describes a simple, reproducible, precise technique for the measurement of endotoxin-like activity on the inner and outer surfaces of catheters intended for intravascular insertion. This technique is useful in documenting the cause of patient reactions. Quality control procedures should be instituted following the manufacture of angiographic catheters so that pyrogen-free products are available for patient use. Catheters processed in a hospital are easily contaminated with fever-producing lipopolysaccharides from tap water or from bacterial growth in residual moisture. Sterilization by steam or ethylene oxide does not destroy these lipopolysaccharides. Consequently, reprocessing reusable catheters must include concern for removing lipopolysaccharides as well as sterilization.

Chills, fever, and hypotension in patients undergoing cardiac catheterization have been suspected to be reactions to endotoxin. Lee et al. reported an incidence of reactions in 13% of patients undergoing cardiac catheterization over a 3-month period (4). Documentation of the presence of endotoxin on catheters has awaited the refinement of techniques for precise determination of endotoxin and for a simple, convenient, and reproducible method of eluting both the inner and outer surfaces of a catheter with a minimal amount of pyrogen-free water.

The technique for the detection of picogram quantities of endotoxin in fluids using *Limulus* amoebocyte lysate has been developed by Nandan and Brown (7). Mascoli and Weary have reported this *Limulus* amoebocyte lysate test to be more sensitive and reproducible for marginal values of endotoxin, such as 75 pg of *Escherichia coli* O55:B5 per ml, than the USP XIX three-rabbit test (6).

The technique for eluting the catheter was developed at the Specialty Microbiology Laboratory of the Peter Bent Brigham Hospital. The application of this technique to a clinical problem enabled the laboratory to demonstrate the presence of endotoxin on sterile catheters supplied for patient use.

In November 1976, two patients on successive days had severe chills, characterized clinically as pyrogen reactions, during cardiac catheterization. The reactions were described as "unexplained." One patient had a temperature rise to 38.9°C immediately after the procedure; the other patient experienced a drop in temperature. The patients stabilized and recovered within a few hours after the episodes. Blood cultures were negative.

In May 1978, a patient experienced what was called a severe "endotoxin reaction" after the administration of dye and normal saline during cardiac catheterization. He had shaking chills and became belligerent during the test. His blood cultures were negative.

Investigation revealed that catheters used on these patients had been reprocessed in the Cardiac Catheterization Laboratory. They had been washed in a small basin containing Edisonite (8.7% phosphates; Colgate-Palmolive Company, New York), rinsed in tap water, repackaged, and sterilized by ethylene oxide using the regular 5-h cycle with a 4-h ethylene oxide exposure. Each cycle had been monitored with *Bacillus subtilis* subsp. *niger* spores. The test strips were sterile.

Cultures of the detergent solution used for washing similar catheters on another day revealed gram-positive and gram-negative organisms. Although the catheters removed from the patients of interest had already been reprocessed, catheters similarly washed, packaged, and sterilized were available for pyrogen testing.

MATERIALS AND METHODS

We tested the catheters, using the new, improved, *Limulus* amoebocyte lysate-induced, protein precipitate test, to determine endotoxin-like activity (ELA). The endotoxin-precipitated protein was equated with picograms of *E. coli* O55:B5 endotoxin. Each angiographic catheter was threaded directly from the package into a pyrogen-free Pyrex glass tube sealed at one end. Each tube was long enough to contain the entire catheter. The outer diameter of the glass tube was 6 mm; the inner diameter was 4 mm. The glass tubing

was depyrogenated by heating in a bunsen burner flame for 15 min. This was accomplished by clamping the tubing horizontally to two support stands. It was allowed to cool before use. The threading was accomplished by maneuvering the glass tube around the catheter inside its original package in such a manner that the catheter itself was never touched but was manipulated through the packaging.

Ten milliliters of nonpyrogenic water (Sterile Water for Injection, USP, Travenol Laboratories, Inc., Deerfield, Ill.) was injected into the lumen of the catheter lying in the glass tube from a nonpyrogenic syringe attached to the hub of the catheter. One milliliter filled the catheter, and the remaining water filled the space between the exterior of the catheter and the glass tube. The water was aspirated back into the syringe. Three 0.1-ml samples of the water were reacted in depyrogenated test tubes with 0.1 ml of *Limulus* amoebocyte lysate (Hyland Laboratories, Inc., Costa Mesa, Calif.). A colorimetric determination of the precipitated protein was then done by the Oyama-Eagle modification (8) of the Lowry assay (5) as follows. After incubation in a 37°C water bath for 1 h, the test tubes were centrifuged, 12,500 × *g*, for 10 min. The supernatant in each tube was discarded, and the pellet was dissolved in 0.2 ml of 0.75 N NaOH. One milliliter of Lowry copper solution was added and incubated for 10 min before adding 0.1 ml of 1 N phenol reagent solution (Folin-Ciocalteu). After a 30-min incubation, absorbance at 660 nm was measured in a microsampling spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio).

The technique for the detection of picograms of endotoxin contamination was based on the formation of a specific protein precipitate. The measurement of precipitated protein is a more sensitive method for the detection of endotoxin than simple gelation of the *Limulus* lysate, a method in common use (7). Levels of *E. coli* endotoxin as low as 6 pg/ml are detectable. Optical density readings were interpreted by reference to a standard curve of *E. coli* O55:B5 endotoxin (Difco Laboratories, Detroit, Mich.) determined on the day tests were done.

Water flushed through the catheters was assayed for endotoxin levels in picograms per milliliter, and the determination was corrected for the total volume of water used to elute the interior and exterior surfaces of the catheter.

To determine whether the Pyrex glass tube was depyrogenated by direct heating, we snapped off the sealed end of one tube. The tube was heated in a bunsen burner flame for 15 min. After cooling, the bore was flushed with 10 ml of sterile, nonpyrogenic water (Sterile Water for Injection, Travenol Laboratories, Inc.) using a syringe and needle. Seven picograms of ELA per ml was recovered. The tube was heated a second time, and a solution containing 49 pg of *E. coli* endotoxin O55:B5 per ml was flushed through, and 49 pg of ELA per ml was recovered from the rinse. Without heating, the tube was immediately flushed with 10 ml of pyrogen-free water. Six picograms of ELA per ml was recovered. Heating of the tube, followed by flushing with 10 ml of pyrogen-free water, resulted in no detectable ELA, demonstrating a reduction in ELA by heating the tube and also indicating that *E. coli* endotoxin O55:B5 introduced in the tube could be recovered and did not adhere to the tube itself. This demonstrated that endotoxin eluted from the catheter could be recovered by flushing with pyrogen-free water.

RESULTS

To establish a base line for the ELA of commercially prepared angiographic catheters, we purchased from three manufacturers 106 catheters, packaged, sterile, and ready for insertion. An additional 25 catheters which were described as "unfinished" and not sterilized were also obtained from one manufacturer. All were tested for ELA. Table 1 shows the ELA in picograms per catheter. Levels of 50 pg or higher per catheter were defined as pyrogenic. Good manufacturing practices can easily maintain endotoxin levels below this level as shown in Table 1. Endotoxin levels starting at 100 pg/ml are detectable in rabbits when a 10-ml/kg dose is injected intravenously (3). Although 50 pg/ml is readily detectable and can be precisely assayed by the endotoxin-induced protein precipitation test, that quantity of endotoxin does not produce a positive reaction in the rabbit.

Table 2 shows the results of tests on the angiographic catheters retrieved from the shelf in the Cardiac Catheterization Laboratory where they were stored until used. Because no pediatric patients are catheterized at the Peter Bent Brigham Hospital, all catheters tested were adult size. All catheters that had been processed in the laboratory for reuse had ELA in excess of 50 pg/catheter. One test exceeded the sensitivity of the assay and was higher than 7.8 ng/catheter. Three catheters retrieved from the shelf in the original manufacturer's package had ELA values

TABLE 1. *ELA[a] of 131 angiographic catheters*

Manufacturer[b]	No. of catheters tested	ELA (pg/catheter)	Pyrogenicity (>50 pg/catheter)
A (sterile)	20	21.5 ± 12.2	Nonpyrogenic
B (sterile)	7	6.9 ± 3.4	Nonpyrogenic
C (sterile)	30	7.3 ± 3.2	Nonpyrogenic
D (sterile)	24	11.5 ± 13.1	Nonpyrogenic
E (no finish process)	25	23.1 ± 14.4	Nonpyrogenic
F (sterile)	25	55.6 ± 25.1	Pyrogenic[c]

[a] In *E. coli* O55:B5 endotoxin equivalents.

[b] A, Cooke Engineering Co., Alexandria, Va.; B, C, D, and E, Cordis Laboratories, Inc., Miami, Fla.; F, USCI-Positrol II, Billerica, Mass.

[c] Of 25 assayed, 14 were pyrogenic, 11 were nonpyrogenic.

of 6, 15, and 25 pg/catheter and were, therefore, considered nonpyrogenic.

Repeated flushings of a catheter with high initial levels demonstrated high sequential levels of ELA. Table 3 shows the recovery of ELA from three randomly selected catheters which had been reprocessed, packaged, and sterilized, ready for patient use. Five sequential flushes of each catheter resulted in readily detectable ELA, indicating a persistent elution of endotoxin from the catheter. This implies that when catheters are introduced into a patient, ELA is eluted from the catheter by liquids passing through the lumen, as well as by direct contact of the outside of the catheter with blood. Precisely what dose of endotoxin causes patient reactions is unknown. Whether these reactions are a response to the incremental endotoxin levels, i.e., the total amount eluted over the period of use, or to individual, transitory endotoxin levels due to boluses of infusates is also unknown. For example, the rabbit test detects a concentration of purified *E. coli* endotoxin of 1 ng/ml when a 1-ml/kg dose is used, but it will also detect a concentration as low as 0.1 ng/ml when a 10-ml/kg dose is used (3).

DISCUSSION

The cell wall lipopolysaccharides, or endotoxins, of gram-negative bacteria are biologically active substances. They survive sterilization by steam or ethylene oxide and are destroyed by exposure to dry heat at 250°C for over 1 h (9). Residues of gram-negative bacteria in sterile solutions prepared for intravenous infusions have caused patient reactions ranging from mild chills and fever to severe circulatory collapse and irreversible shock and death. This observation has led to testing parenteral solutions and biological products for bacterial endotoxins. Not only the solutions, but the devices by which they are administered, as well as devices introduced intravenously or intraarterially, should be sterile and free of lipopolysaccharides.

The testing of angiographic catheters to document absence of detectable endotoxin has awaited the development of an appropriate, convenient, sensitive, and reproducible technique for detecting ELA on the external surface, as well as the luminal wall, of the catheters.

The official USP XIX test for pyrogenicity or ELA of solutions is currently based on the febrile response of three rabbits as evidence of the presence of endotoxin. The rabbit test can detect 3 ng of endotoxin using a 3-kg rabbit. However, the rabbit test can also lead to false-negative results because of the variation in the response of individual rabbits to the same quantity of endotoxin (3).

Cooper et al. (2) reported the results of comparison of the USP rabbit pyrogen test and the *Limulus* assay for detection of endotoxin. They demonstrated that the *Limulus* assay was 10-fold more sensitive than the rabbit pyrogen test using purified endotoxins of *E. coli* and *Klebsiella* species.

Mascoli and Weary (6) reported test results comparing the detection of marginal pyrogenic doses of endotoxin using the USP rabbit test and the *Limulus* amoebocyte lysate test. Ten solutions of 5% dextrose were spiked with 75 pg of *E. coli* endotoxin O55:B5 per ml. Only one of the 10 USP rabbit tests showed a temperature rise indicative of pyrogens in these solutions.

Recommendations were made to the Cardiac Catheterization Laboratory for processing catheters to eliminate pyrogens. Processing involved disassembly, cleansing of the lumen, as well as

TABLE 2. *ELA[a] of reusable angiographic catheters reprocessed in Cardiac Catheterization Laboratory*

Catheter no.	ELA (pg/catheter)	Pyrogenicity (>50 pg/catheter)
1	>1,860	Pyrogenic
2	91	Pyrogenic
3	639	Pyrogenic
4	883	Pyrogenic
5	787	Pyrogenic
6	1,533	Pyrogenic
7	1,760	Pyrogenic
8	97	Pyrogenic
9	618	Pyrogenic
10	175	Pyrogenic
11	202	Pyrogenic
12	735	Pyrogenic
13	>7,800	Pyrogenic

[a] In *E. coli* O55:B5 endotoxin equivalents.

TABLE 3. *ELA recovered from five sequential flushes[a] of three reusable angiographic catheters*

Catheter	ELA (pg/catheter) in order of flush					Cumulative ELA (pg/catheter)
	1st	2nd	3rd	4th	5th	
1	>2,000[b]	>2,000	>2,000	>2,000	>2,000	>10,000
2	2,940	5,720	3,780	470	310	13,220
3	630	190	130	130	20	1,100

[a] Flushes of interior and exterior of catheters.
[b] Exceeds sensitivity of test.

the outer surfaces, with a detergent containing sodium hypochlorite followed by a copious rinsing with hot (maintained at 60°C), freshly distilled water or, if unavailable, bottled, sterile distilled water (10). Contact with tap water, skin, and wet cleaning tools was avoided. No further reactions have occurred.

Cognizance of the problem and awareness of timely and proper cleaning procedures should eliminate the necessity for retesting of catheters for endotoxins unless patient reactions reoccur.

We have served hospitals in Atlanta, Boston (1), East Chicago, Philadelphia, Sacramento, and Ann Arbor as consultants concerning febrile reactions during angiography. In addition to catheters discussed here, guide wires, stopcocks, manifolds, glass syringes, cannulas, injectors, and disposable gloves have been implicated as sources of pyrogen. Pyrogen is eluted from any surface by water and can be imprinted from pyrogen-contaminated surfaces onto surfaces that are inserted intravascularly. Hence, items that are reused must be processed, packaged, and sterilized promptly to limit the time for bacterial growth.

Purchase orders for disposable devices through which fluid is to be injected, devices that are inserted intravascularly, or items that are likely to come into contact with such devices should specify "sterile and nonpyrogenic" labeling.

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