Inspections, Compliance, Enforcement, and Criminal Investigations

Pyrogens, Still a Danger

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Introduction

The advent of the hypodermic needle provided a new route to administer drugs. \1\ But by today's reference, the early parenteral \a)\ drugs were crude and unsafe in that the increased use of these early injection solutions brought about their attendant infections, adverse drug reactions, fevers of unknown etiology, and even deaths from shock. It was perplexing to the early workers in the field of microbiology that sporadic fevers resulted from the injection of even the sterile solutions.

Modern injection solutions are safer, yet the literature \2\ continues to report adverse reactions. In this issue we share the concerns for the patient who cannot take fluids by mouth, due to surgery or certain kinds of illness, who could suffer the consequences from unwanted toxins known to be fever producing substances, if present in the parenteral product. We hope to show benefit from what has been learned in this area.

Historical - Pyrogens

It was known in the latter part of the 19th century that some parenteral solutions caused a marked rise in body temperature. The fever producing agents were not known. Maladies from this "fever substance" were variously described as "injection fever," "distilled water fever," and "saline fever," among other terms. Today, bacterial pyrogens \b)\ are recognized as the causative agent responsible for many of those early fevers and for many of the other biological effects described incidental to parenteral therapy. From this we can understand why the utmost care must be taken during preparation and use of injection solutions to exclude the surreptitious pyrogen.

Natural Defense and Pyrogens

As healthy humans subjected to a universal distribution of microbes in the environment, we coexist with the microbial world. Ordinarily the body's natural defenses restrict the microbial and their metabolites (toxins, etc.) to areas where they can be tolerated, such as on the skin and in the alimentary tract. The parentera route of administration of a drug allows a pyrogen, if present, to bypass the normal body defenses. The host's response is mediated through the leukocytes (white blood corpuscles) which in turn release their own kind of pyrogen (endogenous pyrogen) and this in turn initiates the febrile response and a multitude of othe biological reactions.

Biological Effects of Endotoxins

The wide variety of biological effects attributed to bacterial pyrogens have aroused considerable interest. \3\, \4\ The host's reactions to pyrogens are expected to be in the following categories: 1) fever production, 2) shock, and 3) changes in physiological functions.

Fever is a well-known effect, hence the term "pyrogen." About one hour after the injection of pyrogens into rabbits (or man), there is a rise in body temperature. When bacterial pyrogens are injected in sufficient amounts, perhaps in microgram quantities, the fever produced is accompanied by chills, body aches, a rise in blood pressure, and possibly a state of shock and death. From smaller injection quantities, the body shown increased capillary permeability and a-wide variety of other circulatory changes. Examples of these changes are shown by a reduction followed by an increase in the number of white cells, tumor hemorrhages and changes in venous pressures.

In special cases, pyrogens can demonstrate the Shwartzman's phenomenon. This is a severe hemorrhagic reaction with localized necrosis. It can be demonstrated in a rabbit which is first injected subcutaneously with a bacterial pyrogen, and the rabbit is then injected intravenously 24 hours later with the same pyrogen. The site of the later injection turns blue at the center and red at the periphery.

Pyrogen tolerance is another important reaction that develops when the animals are given repeated injections of a pyrogen. A reduced sensitivity to the same and other pyrogens develops which nullifies the febrile response and requires that the tolerant animal be withdrawn from further pyrogen testing. "Sensitivity" means the animal reacts to a minimal amount of pyrogenic material. Although the rabbit is the most often used test animal, man is considered to be the most sensitive to pyrogens.

Bacterial Toxins

There are two general kinds of bacterial toxins. \5\ Exotoxins are produced during the growth phase of certain kinds of bacteria and are liberated into the medium or tissue. Exotoxins are protein in nature and their reactions are specific. For example, Clostridium botulinum produces an exotoxin of unusual potency which affects only neurological tissue. Other well-known examples of exotoxins are tetanus toxin, Shiga toxin, and diphtheria toxin.

Endotoxins are another kind of toxin that can be extracted from a wide variety of gram-negative bacteria. The term "endotoxin" is usually interchangeable with the term "pyrogen," although not all pyrogens are endotoxins and pyrogen testing alone cannot be used entirely for detection and characterization of microbial endotoxins. Higher doses of endotoxin are required to produce a lethal effect in the experimental animal than are required for exotoxins. The effects produced by endotoxins on the host are systemic such as fever and general body reactions, rather than strictly neurological effects, as is the case with most exotoxins. Endotoxins are found in the gram-negative bacteria mostly, and are obtained subsequent to the death and autolysis of the cells. The endotoxins are extracted from and associated with the cell structure (cell wall). Good examples of pyrogen producing bacteria are S. typhosa, E. coli, and Ps. aeruginosa.

Additional Properties of Pyrogens

In listing additional properties, it can be said that pyrogens: (1) are known to consist biochemically of a lipid-polysaccharide-peptide substance; (2) are heat stable at the temperature of boiling water; (3) are relatively low in acute toxicity for man; (4) demonstrate a low order of immune response; and (5) may be produced from persistent gram-negative bacteremias which could have a 50% mortality rate. \6\

Physicians are instructed to search for the cause of persistent bacteremias immediately. Possible sources could be phelbitis at the catheter site, infusion equipment, or the parenteral solution. It is interesting to note that the management of patients in pyrogen shock includes the administration of parenteral fluids (hopefully nonpyrogenic).

Make It Pyrogen Free

Bactericidal procedures such as heating, filtration, or adsorption techniques do not eliminate pyrogens from parenteral solutions. All ingredients must be kept pyrogen free in the first place. For this assurance the manufacturer carries out comprehensive pyrogen screening tests on all parenteral drug ingredients and sees to their proper storage prior to use. Ideally, the manufacturer recognizes the critical steps in the manufacturing operations that could allow growth of pyrogen producing bacteria, and he monitors these areas routinely. For example, the water in the holding tanks would be tested for pyrogens and the manufacturer would insist on minimum holding times so that only pyrogen-free water is used. Pyrogen-free water, as "water for injection" outlined in the USP, is the heart of the parenterals industry.

Pyrogen Assay - USP

The current USP clearly outlines the pyrogen assay. USP XIX considers a solution to be pyrogenic when 10 m1/kg is injected into a rabbit and there is a rise of temperature of 0.6 C or more for any rabbit, or a total rise of more than 1.4 C for three rabbits in a three rabbit test group. The official rabbit method requires considerable time, expense, training, and experience to master. There are few shortcuts. The consequence of not testing for pyrogens could be even more costly in terms of patient reactions and drug recalls.

Pyrogen Assay - Limulus Amoebocyte Lysate

Many laboratories conduct pyrogen assays by means of the limulus amoebocyte lysate (LAL) test method. \7\ The LAL method is useful especially for screening products that are impractical to test by the rabbit method. Products best tested for endotoxins by LAL techniques are: radiopharmaceuticals, anesthetics, and many biologicals. Essentially, the LAL method reacts hemolymph (blood) from a horseshoe crab (limulus polyphemus) with an endotoxin to form a gel. The quantity of endotoxin that gels is determined from dilution techniques comparing gel formation of a test sample to that of a reference pyrogen, or from spectrophotometric methods comparing the opacity of gel formation of a test sample to that opacity of a reference pyrogen. The LAL test is considered to be specific for the presence of endotoxins and is at least a hundred times more sensitive than the rabbit test. \8\, \9\ Even picogram quantities of endotoxins can be shown by the LAL method. Although LAL is a relatively new pyrogen testing method, there has been shown a wide variety of polysaccharide derivatives that give positive limulus test results and also show fever activity. It is also a fact that some substances interfere with the LAL test even when pyrogens are present.

Some firms use the LAL test for screening pyrogens in raw materials, and follow up with pyrogen testing on the final product by means of the USP rabbit assay. The LAL test for pyrogens in drugs requires an amendment to the NDA on an individual product basis. LAL test reagents are licensed by the Bureau of Biologics. For devices, a firm must have its protocol approved by the Director, Bureau of Medical Devices, before it can substitute the LAL assay for the rabbit. \10\ The future of LAL testing appears promising in that it is being considered for inclusion in the USP, but it is not an official method at this time.

What is certain is that pyrogens remain a potential source of danger with use of parenteral therapy. Total exclusion of pyrogens requires our continued surveillance relative to parenteral drug manufacturing. \a)\Parenteral (para = beyond; enteron = intestine) Not through the alimentary tract but by some other route, such as subcutaneous, intramuscular, intravenous, intraspinal, etc.

\b)\Pyrogen - a fever producing agent of bacterial origin; endotoxin. Dorland's Illustrated Medical Dictionary 25th E.W.B. Saunders, Philadelphia.

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