Henry Ford Hospital Medical Journal

Volume 5 | Number 2 Article 5

6-1957

Beta-propiolactone For The Sterilization Of Biological Materials

Gerald A. LoGrippo

Follow this and additional works at: https://scholarlycommons.henryford.com/hfhmedjournal

Part of the Life Sciences Commons, Medical Specialties Commons, and the Public Health Commons

Recommended Citation

LoGrippo, Gerald A. (1957) "Beta-propiolactone For The Sterilization Of Biological Materials," *Henry Ford Hospital Medical Bulletin* : Vol. 5 : No. 2 , 94-99.
Available at: https://scholarlycommons.henryford.com/hfhmedjournal/vol5/iss2/5

This Article is brought to you for free and open access by Henry Ford Health System Scholarly Commons. It has been accepted for inclusion in Henry Ford Hospital Medical Journal by an authorized editor of Henry Ford Health System Scholarly Commons. For more information, please contact acabrer4@hfhs.org.

BETA-PROPIOLACTONE FOR THE STERILIZATION OF BIOLOGICAL MATERIALS

GERALD A. LOGRIPPO, M.D.*

INTRODUCTION

Although the sterilization of biological materials has been widely used for many years, the agents employed are still not entirely satisfactory. Moreover, the scope of biological materials which can be sterilized has been limited. Heat, ultraviolet irradiation, formalin and phenol have been the most commonly used agents for sterilization. However, with these agents there is a rather marked alteration of protein material with the loss of the biological characteristics necessary for clinical usefulness. In addition, in recent years the problem of virus contamination has been brought to the attention of the clinicians more forcibly than ever before. This has been further complicated by the fact that the bactericidal agents in common use destory the biological material before they effectively inactivate the viruses. This has been true in the sterilization of plasma where there has been no satisfactory means of destroying the hepatitis virus, an increasing hazard in the use of transfusions.[1,2] More recently Beutler and Dern[3] have demonstrated another virus agent that is transmitted from the blood of healthy donors to recipients.

It is known that at least 16 viruses, infectious to man, can produce a viremic phase. How many of these agents persist in the *"carrier stage"* in man is difficult to prove with our present laboratory technics. Although the technics are not sensitive enough to detect the trace quantities that may persist, these trace quantities are sufficient to transmit the disease to susceptible human hosts whenever blood, blood products or homologous tissue are used in man. With the increasing use of biological materials of human origin being used in man, sterilization of these products *must be* insured not only from the bacterial agents but also from all of the microbiological pathogens.

Beta-propiolactone (BPL) has been reported from this laboratory as a more satisfactory sterilizing agent[4,5,6,7,8,9] against viruses, bacteria, fungi, spore forms of bacteria and fungi and malignant cells. Moreover, sterilization can be obtained at drug concentrations which are not deleterious to tissue proteins. BPL has been successfully developed and employed in several different clinical problems involving the use of biological materials. These are (1) the sterilization of human plasma, (2) the establishment and operation of a human tissue bank and (3) the preparation of inactivated virus vaccines.

BETA-PROPIOLACTONE

The chemical agent, beta-propiolactone (BPL) $CH_2CH_2C = O$ (ring: |— O —|), is a colorless stable liquid in its concentrated state but is unstable in aqueous solutions. It can be satisfactorily stored in plastic containers or in neutraglass sealed ampules at -20 to -30 degrees centigrade. BPL has a specific gravity of 1.149 and is 37.5% (by volume)

*Division of Microbiology, Department of Laboratories, Henry Ford Hospital.

soluble in water at 25° C. It reacts readily with hydroxyl, amino, carboxyl sulfhydryl and phenolic groups,[10,11,12] all of which are associated with proteins.

The instability of the chemical structure of beta-propiolactone accounts for its unavailability in the past. Although the agent was known to exist in 1885, it was not until 1915 that Johannson[13] actually prepared the agent. In 1941, Kung[14] found a practical method for the manufacture of beta-propiolactone. After sitting on the shelves of the organic chemists for nine years with no practical use for the compound, it was tested for its virucidal action in plasma during a routine screening program conducted in this laboratory. The recent synthesis of the lactone form made possible an extensive study of reactions of the drug with biological materials for *sterilization of plasma, tissues* and *the preparation of inactivated virus vaccines.*

STERILIZATION OF HUMAN PLASMA

Previous publications give in more detail the superiority of BPL over nitrogen mustard, sulfur mustard, ethylene oxide, photodynamic action of dyes and ultraviolet irradiation for the inactivation of numerous viruses artificially seeded in plasma and those naturally occurring in plasma during the stage of viremia.[5,6] Extensive studies from this laboratory also show that a wide margin of safety exists between the virucidal concentrations[15] of beta-propiolactone and the toxicity concentrations[16,17] of beta-propiolactone. However, since the hepatitis virus is not an agent which can be yet studied in the experimental laboratory, the effectiveness of beta-propiolactone upon this agent remains to be answered in human volunteers. Although plasma treated with 0.6% beta-propiolactone concentration is not toxic to man upon intravenous administration, this concentration is 1.5 times that necessary to inactivate the most resistant laboratory virus (eastern equine encephalomyelitis) and 12 times that necessary to inactivate the most sensitive virus (lymphocytic choriomeningitis) when these agents are seeded in plasma. However, in order to maintain the integrity of the fibrinogen in plasma, a concentration of 0.45% beta-propiolactone should not be exceeded. Since this concentration is only slightly in excess of that necessary to inactivate the most sturdy laboratory viruses, it may not be sufficient to inactivate the trace quantities of hepatitis virus that may be theoretically present. For these reasons, the combination of beta-propiolactone and ultraviolet irradiation previously reported[5] was more extensively studied.[18,19,20,21,22]

Animal experimentations with the eastern equine encephalomyelitis virus (EEE) clearly show that there is always an additive and often a synergistic virucidal effect with beta-propiolactone and ultraviolet irradiation.* The same degree of virus inactivation (i.e., nondetectable quantities) can be obtained in combination with 1/3 to 1/4 the beta-propiolactone concentration and 1/3 to 1/4 the ultraviolet irradiation than when either agent is used alone. What is more important is the fact that the combination can be used in marked excess of that necessary for sterilization and still maintain the integrity of the fibrinogen and other plasma proteins as demonstrated by electrophoretic patterns. With a wider range between sterilization dosage and protein alteration, the combination here shown stands a greater chance of inactivating the hepatitis virus in human plasma.

Plasma treated with 0.35% beta-propiolactone and ultraviolet irradiation intensity recommended and used by the National Institute of Health Standards produces a

*(The Dill ultraviolet apparatus was used for these studies).

product which is clear and amber in appearance, stores well at refrigerator temperature, and is well tolerated by patients. Over 100 units have been administered to infants and adults without any clinical pathological manifestations.

ESTABLISHMENT AND OPERATION OF A HUMAN TISSUE BANK

With the ever increasing demand for sterile tissue transplants in the field of surgery and with human autopsy material being the most likely source for an adequate supply of homotransplants, a simple, inexpensive and safe procedure for sterilizing tissue is greatly needed. The usual practice of testing for bacterial and mycotic contamination overlooks the potential hazards of virus infection, malignant growths and parasites. It is possible with the use of beta-propiolactone to completely sterilize tissue supplied from autopsy material and thus establish a human tissue bank. Such a bank has been in operation at the Henry Ford Hospital for three and one-half years.

The human arterial bank was first established in October 1953, having been preceded by study of arterial transplants in dogs.[23] These experiments were initiated by Dr. C. Lam and associates in Surgery with the cooperation of the Department of Laboratories.[7,8] After two years work, the results in dogs were concluded to be highly satisfactory, and it was felt that trial of homologous grafts in man was justified. The clinical trial was carried out by Dr. D. E. Szilagyi[24,25,26,27] and associates in the Department of Surgery with the cooperation of the Department of Laboratories.

The tissue bank operates in the following manner:

1. A special permit for the procurement of human arteries is necessary. This is easiest to obtain from the family at the time the autopsy permit is being signed. In obtaining the arteries, no exceptions are made regarding age and cause of death.
2. A surgical resident is charged with the responsibilities of obtaining, preparing, discarding poor segments, classifying and recording data pertaining to the arteries.
3. The arteries are obtained without aseptic precautions and sterilized within 24-36 hours from time of death, without elaborate procedures or inconvenience to any one concerned.
4. The sterilization and completion of the sterility tests are the responsibility of a bacteriology technician. The tissues are then returned to the respective surgical services for storage.

Two-hundred and sixty-eight operations have been performed with beta-propiolactone sterilized arteries by the Department of Arterial Surgery. In no case was there evidence of failure being due to inadequate sterilization, nor has there been any evidence of infection, either bacterial or viral. After successful sterilization of arteries and acceptance by the host, sterilization studies were extended to bone tissue with the cooperation of Dr. C. L. Mitchell and Dr. J. Fleming, Department of Orthopedic Surgery.

The bone bank was established in July 1955, again placing a resident in Orthopedic Surgery in charge of the preparation of the bone segments in the sizes and shapes desired by the surgeons. Usually ribs, iliac crest and vertebral bodies afford an ample supply of cancellous and cortical bone without special permits. To sterilize bone it

is important to remember that segments can be as long and as wide as the surgeon wishes, but they cannot be greater than 6 mm. thick. This maximum thickness was determined by experimental data obtained with bone pieces varying in thickness from 1-2-3-6-9 and 12 mm. sizes. These were contaminated in bacterial broth suspensions for 18 hours before sterilizing with the beta-propiolactone. In addition, varying thicknesses obtained from infected rabbits and guinea pigs during the stage of bacteremia and viremia were sterilized.[9] All specimens were consistently sterilized at 6 mm. thickness. Sterilization at 9 mm. thickness is inconsistent.

After sterilization the bone can be kept frozen at —20 to —30° C. in an ordinary deep freeze unit, or lyophilized (freeze-dried) and stored at room temperature in vacuum. The clinical results of beta-propiolactone sterilized bone have been very satisfactory.

As of March 1, 1957 specimens from the BPL treated bone bank have been used in 70 operations. The procedures have included filling of large cysts, spinal fusions for tuberculosis and scoliosis, grafting of fractures both fresh and old, and for various other fusions. Only one infection was noted in this series (open reduction of os calcis fracture with skin slough and infection on the 13th postoperative day). However, this case healed without losing the graft or the reduction.

More recently sterilized skin and cartilage have also been used with the cooperation of Dr. R. H. Clifford and Dr. W. D. Butt, Department of Plastic Surgery. However, this is still in the early trial stage. Sufficient evidence has been collected to say that beta-propiolactone sterilized, homologous, skin grafts are as serviceable as the non-treated, homologous, skin grafts, without the complication of infection. The rejection period is the same as that of the nontreated, homologous, skin grafts.

The results with cartilage, again few in number, indicate that sterilization is satisfactory. The cartilage transplants occasionally resorb as do fresh autogenous transplants or nonsterilized, refrigerated, homologous transplants. When resorption takes place the reaction is slow with no evidence of toxic or inflammatory reactions.

In conclusion, it can be stated that, with the use of beta-propiolactone as the sterilizing agent, a tissue bank can be established in a hospital of any size with a minimum of equipment and no need for highly skilled technical assistance. At the present time, establishment and operation of a tissue bank is most useful for the preparation and storage of sterile, homologous, arterial, bone, cartilage and skin transplants.

ANTIGENICITY OF BETA-PROPIOLACTONE INACTIVATED-VIRUS VACCINES

In the preparation of inactivated virus vaccines, the margin of safety between complete virus inactivation and the degree of antigenicity retained is so narrow that a search for a more satisfactory agent has not abated.

In previous reports[28,29] and in studies in progress, beta-propiolactone seems superior to other chemical agents for the preparation of inactivated virus vaccines. It inactivates viruses completely and irreversibly in 10-15 minutes at 37° C. whereas formalin and phenol require days under the same conditions. The beta-propiolactone concentration can be added in excess of the minimal effective virucidal concentration with

a wide margin of safety between the complete inactivation of viruses and loss of antigenicity. Moreover, significantly higher degrees of antigenicity have been obtained with beta-propiolactone inactivated-virus vaccines than with phenol — or formalin — inactivated vaccines under parallel conditions. The vaccine preparations were made with rabies, eastern equine encephalomyelitis, murine (MM-strain) encephalomyocarditis and poliomyelitis viruses.

Upon demonstration by animal experimentation of the superiority of beta-propiolactone inactivated virus vaccines, human trials with such preparations have begun. Peck, Powell and Culbertson,[30,31] from the Lilly Laboratories for Clinical Research, Indianapolis General Hospital and the Lilly Research Laboratories, have recently prepared and clinically tested a beta-propiolactone killed rabies vaccine with good results in man. The rabies vaccine was prepared from embryonated duck eggs in place of the conventional rabbit brain material. The absence of brain tissue in this preparation and the appearance of detectable antibodies between 7 and 10 days after the first inoculation indicate marked improvement in the methods of immunization against rabies.

SUMMARY

Beta-propiolactone, a new chemical agent, has been demonstrated to be a useful substance in the sterilization of biological materials. Its unique properties, not available in other agents used for treating biological material, have made possible the treatment of human plasma for the inactivation of viruses, without destroying the usefulness of the product for clinical use. Beta-propiolactone has made possible the establishment of an inexpensive and practical tissue bank by successful sterilization of contaminated tissue from autopsy material without destroying the properties of the transplants for clinical use. In addition, beta-propiolactone is finding its place in the preparation of safer and more potent inactivated virus vaccines.

BIBLIOGRAPHY

1. Murray, R.: Viral hepatitis, Bull. New York Acad. Med. 31:341, 1955.

2. Sherman, I. L., and Eichenwald, H. F.: Viral hepatitis; descriptive epidemiology based on morbidity and mortality statistics, Ann. Int. Med. 44:1049, 1956.

3. Beutler, E., antd Dern, R. J.: Previously unrecognized transmissible agent in human blood; experimental and clinical studies, J. A. M. A. 159:989, 1955.

4. Hartman, F. W., Piepes, S. L., and Wallbank, A. M.: Virucidal and bactericidal properties of beta-propiolactone, Fed. Proc. 10:358, 1951.

5. Hartman, F. W., Kelly, A. R., and LoGrippo, G. A.: Four year study concerning the inactivation of viruses in blood and plasma, Gastroenterology 28:244, 1955.

6. Hartman, F. W., LoGrippo, G. A., and Kelly, A. R.: Preparation and sterilization of blood plasma, Am. J. Clin. Path. 24:339, 1954.

7. LoGrippo, G. A., Overhulse, P. R., and Szilagyi, D. E.: Procedure for the sterilization of contaminated arterial grafts with beta-propiolactone, Bact. Proc. 1954, p. 61.

8. LoGrippo, G. A., Overhulse, P. R., Szilagyi, D. E., and Hartman, F. W.: Procedure for sterilization of arterial homografts with beta-propiolactone, Lab. Invest. 4:217, 1955.

9. LoGrippo, G. A., Burgess, B., Teodora, R., and Fleming, J. L.: Procedure for bone sterilization with beta-propiolactone, J. Bone & Joint Surg. In Press.

10. Gresham, T. L., Jansen, J. E., and Shaver, F. W.: Beta-propiolactone. VIII. Reactions with organic and inorganic acids, acid chlorides and anhydrides, J. Am. Chem. Soc. 72:72, 1950.

11. Gresham, T. L., Jansen, J. E., Shaver, F. W., Bankert, R. A., Beears, W. L., and Pendergast, M. G.: Beta-propiolactone. VI. Reactions with phenols, thiophenols and their salts, J. Am. Chem. Soc. 71:661, 1949.

12. Gresham, T. L., Jansen, J. E., Shaver, F. W., Gregory, J. T., and Beears, W. L.: Beta-propiolactone. V. Reactions with alcohols, J. Am. Chem. Soc. 70:1004, 1948.

13. Johannson, C.: Lund University, Annals 12, no. 8, 1915.

14. Kung, F. E.: Preparation of beta lactones, U. S. Patent 2,356,459, 1944.

15. LoGrippo, G. A., and Rupe, C. E.: Chemical sterilization of whole blood and plasma—beta-propiolactone, In Henry Ford Hospital: International Symposium on Hepatitis Frontiers, Boston, Little, Brown, 1957.

16. Hartman, F. W., and Kelly, A. R.: Tissue toxicity of beta-propiolactone and its degradation products, Fed. Proc. 12:390, 1953.

17. Kelly, A. R., Hartman, F. W., and Rupe, C. E.: Toxicology of beta-propiolactone, In Henry Ford Hospital: International Symposium on Hepatitis Frontiers, Boston, Little, Brown, 1957.

18. LoGrippo, G. A., Kelly, A. R., and Hartman, F. W.: Beta-propiolactone and ultraviolet combination for the sterilization of plasma, presented before the Panel on Sterilization of Blood and Plasma of the National Research Council, April 7, 1954.

19. Hartman, F. W., LoGrippo, G. A., and Kelly, A. R.: Combined procedures for virus inactivation in blood, Fed. Proc. 13:430, 1954.

20. Hartman, F. W., LoGrippo, G. A., and Kelly, A. R.: Procedure for sterilization of plasma using combinations of ultraviolet irradiation and beta-propiolactone, Fed. Proc. 15:518, 1956.

21. LoGrippo, G. A., Hartman, F. W.: Chemical and combined methods for plasma sterilization—beta-propiolactone and ultraviolet irradiation, Internat. Soc. of Blood Transfusion (6th Congress), Boston, Mass., Sept. 3, 1956.

22. Hartman, F. W., and LoGrippo, G. A.: Combined beta-propiolactone and ultraviolet irradiation for plasma sterilization, In Henry Ford Hospital: International Symposium on Hepatitis Frontiers, Boston, Little, Brown, 1957.

23. Trafas, P. C., Carlson, R. E., LoGrippo, G. A., and Lam, C. R.: Chemical sterilization of arterial homografts, A. M. A. Arch. Surg. 69:415, 1954.

24. Szilagyi, D. E., Overhulse, P. R., and LoGrippo, G. A.: Use of chemically sterilized arterial homografts; experimental clinical observations, Clin. Res. Proc. 2:108, 1954.

25. Szilagyi, D. E., Overhulse, P. R., Shonnard, C. P., and LoGrippo, G. A.: Sterilization of human arterial homografts with beta-propiolactone, S. Forum 5:244, 1954.

26. LoGrippo, G. A.: Sterilization of homostatic grafts with beta-propiolactone for human transplantation, Bact. Proc. 1956, p. 101.

27. Szilagyi, D. E., Whitecomb, J. G., and Smith, R. F.: Causes of late failures in grafting therapy of peripheral occlusive arterial disease, Ann. Surg. 144:611, 1956.

28. LoGrippo, G. A., and Hartman, F. W.: Antigenicity of beta-propiolactone inactivated virus vaccines, J. Immunol. 75:123, 1955.

29. LoGrippo, G. A., and Hartman, F. W.: Antigenicity of beta-propiolactone inactivated viruses, Fed. Proc. 13:503, 1954.

30. Powell, H. M., and Culbertson, C. G.: Antirabies vaccine prepared from fixed virus grown in embryonated duck eggs and killed with beta-propiolactone, Bact. Proc. 1956, p. 70.

31. Peck, F. B., Jr., Powell, M. H., and Culbertson, C. G.: Duck-embryo rabies vaccine; study of fixed virus vaccine grown in embryonated duck eggs and killed with beta-propiolactone (BPL), J. A. M. A. 162:1373, 1956.