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Efficacy of Betaprone with Ultraviolet Irradiation on Hepatitis B Antigen in Human Plasma Pools

(A Retrospective Study)

Gerald A. LoGrippe, MD and Hajime Hayashi, PhD*

In a retrospective study, hepatitis B antigen (HB Ag) was found in 14 lots of human plasma pools (16-20 liters each) that had been treated by the combined Betaprone (BPL) with ultraviolet (UV) irradiation procedure. No evidence of clinical hepatitis was found during a six-month follow-up after 185.2 liters of HB Ag positive plasma were infused into 257 patients. Since HB Ag positive blood products treated by BPL with UV produced no clinical hepatitis, it is evident that our treatment is efficacious against hepatitis virus agents.

SINCE 1956, the "COLD STERILIZATION PROCESS" utilizing Betaprone (BPL) combined with ultraviolet (UV) irradiation has remained unchanged for the treatment of plasma or serum for clinical use.¹⁻³ More than 4,000 units of human plasma⁴⁻⁷ and 300,000 units of serum⁸⁻¹⁰ have been treated and safely transfused without clinical evidence of toxicity, allergenicity or clinical hepatitis.

This presentation comments on 257 recipients who received multiple units (varying from 50 ml to 5,500 ml) of HB Ag positive material intravenously from 14 lots of BPL with UV treated plasma pools (90-150 donors per lot). Results are compared with the incidence of overt clinical hepatitis with jaundice produced by various plasma pools reported by other investigators during and after World War II.

Materials and Methods

Plasma Pools, Source and Preparation for Treatment

From January 1956 to September 1966, 63 separate lots of human plasma pools were prepared every 8-10 weeks from outdated blood from our hospital blood bank. Each plasma pool was treated by the combined BPL with UV process and efficacy of the treatment was monitored by *Escherichia coli* T-3 bacteriophage.

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Plasma from single units of 21-day out-dated ACD-blood and plasma remaining after the use of packed erythrocyte transfusions were pooled in two-liter sterile glass bottles. The isoagglutinin titres of anti-A and anti-B were 1:8 or less. These pools were refrigerated until sufficient volume of plasma was accumulated for treatment. The plasma pools varied from 16-20 liters each, with 90-150 donors contributing to each pool. The day the plasma was to be treated, the two liter pools were transferred to a 20-liter sterile bottle. In this transfer, the plasma was passed through sterile millipore filters (1.2 microns) to clear the plasma of cellular elements, aggregates and chylous material. More donors were used in these pools than the total volume indicated, due to the loss of volume from fatty surface layer and sedimented red cell volume at the bottom of each two-liter bottle.

Methods of Treatment

The synergistic virucidal potency of combined Betaprone* (BPL) with ultraviolet (UV) irradiation has been elaborated upon in numerous publications.⁴⁻⁷ The procedure with the Dill UV apparatus and physical-chemical conditions described^{3,9} for BPL with UV have not changed since 1956. These procedures were rigidly followed in the preparation of treated plasma in this study.

Safety and Sterility Tests

Each lot of plasma processed was monitored, using at least 10^6 PFU of T-3 bacteriophage (*Escherichia coli*) per ml of plasma. A quantity of 1,000 ml of phage-seeded plasma was treated under conditions parallel to those under which the total volume of plasma was treated for clinical use. This method has been

reported.^{7,9,11} Standard tests for pyrogens and sterility were completed before plasma was released for clinical use.

Storage and Distribution of Treated Plasma

After the tests for safety and sterility were proven satisfactory, the packaged units (250 ml) of plasma were stored in the hospital blood bank and handled under standard blood bank procedures. The requisitioning and use of the treated plasma was left entirely to the discretion of the hospital staff.

Clinical Evaluation of Hepatitis among Plasma Recipients

The 1956-1966 study was evaluated on the basis of clinical hepatitis alone inasmuch as the 1951-1956 investigation included detailed procedures then available for investigation of liver disease. These included hematologic profile, urinalysis, serum thymol turbidity and cephalin flocculation tests, serum bilirubin values, and any evidence of allergic sensitization from altered plasma proteins.¹² The data for the present report does not include any laboratory evaluation. The hospital staff members using the plasma were aware of reporting any jaundice or suspected hepatitis cases to the investigators. A six-month follow-up by the authors consisted of examination of all hospital records from the laboratory and patients' histories and, when necessary, by communication with patients and their physicians.

Retrospective Assays of Hepatitis B Antigen

Of the 63 plasma lots treated and administered to man, aliquots from 30 lots were available in frozen storage (minus 20°C) for HB Ag radioimmunoassay. Agar gel diffusion¹³ and immunoelectrophoresis were employed for preliminary assay. In November 1972, the Abbott AusRIA-125 kit¹⁴ was made available for radioimmunoassay of HB Ag. The latter

*Betaprone - a specially purified grade of beta-propiolactone (BPL) available from Fellows Medical Mfg. Co., Inc., Oak Park, Michigan (U.S.A.), Zip 48237.

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was the most sensitive method available. All plasma lots found to be HB Ag positive were repeated and also verified by the adsorption tests, as recommended in the Progress Report of American Association of Blood Banks.¹⁵

Results

Six hundred and ninety-seven and five tenths (697.5) liters of plasma were administered to 977 recipients with 2,893 intravenous infusions. During the ten-year period, not a single case of homologous serum jaundice occurred among the recipients receiving plasma. No clinical manifestations of liver disease were encountered over a six-month period of study.

Table I gives the results on the 30 lots of pooled plasma evaluated. These are in the order they were pooled and treated during the respective years. The HB Ag positive pools are indicated by the (+) sign preceding the radioactive count per minute (CPM) shown for the AusRIA test. The third column gives the number of standard deviations (SD) calculated above the negative mean (349 CPM with SD 32) of the negative controls furnished with the Abbott kit. Fourteen of 30 plasma lots available for radioimmunoassay were found positive for HB Ag. Repeat assays and adsorption tests confirmed all positive findings for HB Ag in the 14 lots. All 30 lots of plasma were negative for HB Ag by the less sensitive techniques, including double diffusion and counter immunoelectrophoresis.

Table II gives the year, plasma lot number and liters of plasma administered intravenously. These were all HB Ag positive and were potentially contaminated with hepatitis B virus which was treated by the BPL-UV method. It also gives the number of recipients transfused with the number of units of plasma from the respective lots. One hundred and eighty-five and two tenths (185.2) liters of treated plasma containing HB Ag were

TABLE I
RETROSPECTIVE STUDY FOR
HEPATITIS B ANTIGEN IN
30 LOTS OF HUMAN PLASMA POOLS
TREATED WITH BETAPRONE AND
ULTRAVIOLET IRRADIATION

Lot No. (Year)	Hepatitis B antigen* by AusRIA (CPM)	S.D. above negative mean**
(1961)		
28	+ 3767	106.8
29	506	4.9
30	435	2.7
31	+ 2585	69.9
32	357	0.3
(1962)		
33	360	0.3
34	+ 1542	37.3
35	339	-0.3
37	376	0.8
38	+ 2673	72.6
39	461	3.5
(1963)		
40	+ 2054	53.3
42	+ 2070	53.8
43	+ 3206	89.3
44	+ 1664	41.1
45	300	-1.5
46	+ 1372	32.0
(1964)		
47	550	6.3
48	+ 8825	264.9
49	324	-0.8
50	+ 4203	120.4
51	371	0.7
52	513	5.1
53	+ 6592	195.1
(1965)		
54	+ 3965	113.0
55	513	5.1
56	472	3.8
60	380	1.0
61	528	5.6
(1966)		
63	+ 1738	43.4

14 lots with Hepatitis B antigen positive/ 30 lots were tested.

*All pools negative for HB Ag by immunoprecipitation techniques (double immunodiffusion and counter electrophoresis).

**Negative mean = 349 CPM with S.D. 32 of Abbott-AusRIA negative controls. Positive cutoff = 733 CPM (12.0 S.D. from negative mean).

transfused into 257 patients with 739 transfusions. Eighty-five (85) patients received single units (50 ml/pediatric unit and 250 ml/adult unit) of treated plasma and 18 patients received 9 units or more (ranging from 2,250 to 5,500 ml). There was no evidence of clinical hepatitis to

TABLE II
NO OVERT HEPATITIS WITH JAUNDICE AMONG RECIPIENTS OF 14 PLASMA LOTS WITH HEPATITIS B ANTIGEN AFTER COMBINED BETAPRONE WITH ULTRAVIOLET IRRADIATION TREATMENT

Lot No.* (Year)	Liters Administered	Number of Recipients				Total	Overt Hepatitis
		(1 u.)**	(2-5 u.)	(6-9 u.)	(≥10 u.)		
(1961)							
28	7.1	10	7		1	18	0
31	9.4	3	7	1		11	0
(1962)							
34	13.0	9	4	2	1	16	0
38	15.6	4	12	5		21	0
(1963)							
40	15.5	5	6	4	1	16	0
42	16.0	6	6	5		17	0
43	15.9	4	10	3	1	18	0
44	12.6	9	9	4		22	0
46	18.0	4	6	3	2	15	0
(1964)							
48	7.4	4	7	1		12	0
50	12.7	7	9	3		19	0
53	14.1	9	19			28	0
(1965)							
54	16.4	7	16	2	1	26	0
(1966)							
63	11.5	4	13		1	18	0
14 lots	185.2 liters		(739 infusions)			257	0

* Each lot varied from 16-20 liters and consisted of from 90-120 donors per pool.

** 50-250 ml/unit (pediatric and adult volumes respectively)

warrant liver function tests by the physician. Neither did the physician record any cases of jaundice in the history of the plasma recipients.

The BPL with UV treatment did not significantly alter the serologic specificity of the HB Ag as measured by radioimmunoassay, hemagglutination, agar-gel diffusion and immunoelectrophoresis techniques.

Discussion

A number of studies during World War II estimated the jaundice attack rate from *unirradiated pooled human plasma* to be from 4.5% to 11.9%.¹⁶⁻¹⁸ Brightman and Korn¹⁹ found the overall incidence from all lots of plasma released by the Ameri-

can National Red Cross to be 4.5% and the attack rate from one lot to be 22% and that at least 18 of 385 lots of plasma were icterogenic.²⁰ Homologous serum jaundice associated with UV irradiated pooled human plasma²¹⁻²⁶ varied from 3% to 60%. The 60% attack rate reported by James and Associates¹⁷ consists of 12 clinically typical icteric cases among 20 persons who received irradiated human pooled plasma from a single commercial lot.

In the human volunteer studies conducted from 1951-1954, Murray²⁷⁻²⁸ reported that the parenteral administration of 1-4 ml of the icterogenic plasma was sufficient to produce 40% hepatitis with jaundice among the volunteer subjects. The number of hepatitis cases with jaun-

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dice was high among the volunteers receiving plasma treated by three types of UV equipment. In these UV irradiation studies, evaluated for inactivation of the hepatitis agent, 11 of 30 volunteers developed *hepatitis with jaundice* from plasma treated by the Dill irradiator. Four of 30 volunteers developed *hepatitis with jaundice* from plasma treated by the Habel-Sochrider irradiator and five of 16 volunteers developed *hepatitis with jaundice* from plasma treated by the Oppenheimer-Levinson Centrifugal Filter. In the control group, 12 of 25 cases showed *hepatitis with jaundice*. Ineffectivity with the icterogenic plasma pool used in these volunteers showed a hepatitis rate of 52.7% (40% with jaundice and 12.7% without jaundice).

During a 12-year period (1956-1967), plasma treated with BPL combined with UV in this hospital should have produced an increase in hepatitis such as was reported in Michigan and throughout the U.S.A. in the epidemic years of 1960-62.²⁹ However, no hepatitis was reported in patients receiving treated pooled plasma in this hospital during this period.

The association between HB Ag and hepatitis B virus(es) in blood and blood products is generally accepted to carry a high risk of clinical hepatitis with

jaundice.³⁰⁻³⁴ The 14 plasma pools that were HB Ag positive and treated with combined BPL with UV irradiation should have produced from 3% to 60% jaundiced cases among the 257 recipients. Of the 14 HB Ag positive plasma pools treated, no cases of jaundice were reported.

Since November 1966, Biotest Serum Institute, Frankfurt, Germany, has been treating pooled human serum (1,000 donors per pool) by our BPL with UV process. The treated serum has been distributed and used throughout the German hospitals. More than 300,000 units were administered intravenously without reported hepatitis. In contrast, the use of their untreated serum product (1960-1966) brought 49 reports of hepatitis to the Biotest Serum Institute.⁸⁻¹⁰

From the data presented, it seems logical to conclude that human plasma and serum pools treated by the BPL with UV process is safe from clinical hepatitis. Human plasma and sera have been administered in adequate volumes to man and evaluated by a sufficient number of physicians in numerous hospitals to warrant acceptance of the BPL with UV process as an effective method of sterilizing plasma and plasma products against hepatitis B and hepatitis A virus infections.

References

1. Hartman FW, Kelly AR and LoGrippe GA: Four years' study on inactivation of viruses in plasma and whole blood. *Amer Assoc Gastro-Ent* **28**:244, 1954
2. Hartman FW, LoGrippe GA and Kelly AR: Sterilization of blood plasma. *Amer J Clin Path* **24**:339-48, 1954
3. LoGrippe GA and Hartman FW: Chemical and combined methods for plasma sterilization. *Proc 6th Congress of Int Soc of Blood Transfusion*, Boston Sep 1956. S Karger/Basel:**225**, 1958
4. LoGrippe GA, Wolfram BR and Rupe CE: Human plasma treated with ultraviolet and propiolactone: six-year clinical evaluation. *JAMA* **187**:722-26, 1964
5. LoGrippe GA: A ten-year clinical study of plasma treated with Betaprone and combined Betaprone plus ultraviolet irradiation. *Pac Med Surg* **72**:298-302, 1964
6. LoGrippe GA: Status of Betaprone for cold sterilization of biologic products. *Vox Sang* **17**:52-60, 1969
7. LoGrippe GA: Present status of sterilizing blood and blood products against the hepatitis agent(s). *Proc IVth Int'l Congress for Infectious Diseases*, Munich, Germany, Apr 1966. FK Schattauer-Vorlag, Stuttgart: **65**, 1967

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8. Stephen W: Individuelle unterschiede bei der behandlung von plasmen und seren mit B-propiolacton. *Z. klin, Chem u klin Biochem* **6**:481-3, 1968
9. Stephen W: Hepatitis-free and stable human serum for intravenous therapy. Proc Blood Research Institute, Boston, Nov 1970. *Vox Sang* **20**:442-57, 1971
10. Stephen W.: Hepatitis-free reagents in tests for Australia antigen. *Vox Sang* Vol **24**, supplement 1, 1973
11. LoGrippe GA and Hayashi H: Human plasma treated with combined beta-propiolactone (BPL) and ultraviolet (UV): monitoring with E. coli T-3 phage. *Fed Proc* **21**:466, 1963
12. Kelly AR, Hartman FW and Rupe CE: The toxicology of betapropiolactone. In *Hepatitis Frontiers*, Chap 32, Little, Brown, Boston, Mass, 1957
13. Hayashi H and LoGrippe GA: Persistence of "Australia antigenemia" in mentally retarded children seven years after virus hepatitis. *Henry Ford Hosp Med J* **19**:27-34, 1971
14. Ling CM and Overby LR: Prevalence of hepatitis B virus antigen as revealed by direct radioimmune assay with ¹²⁵I-antibody. *J Immunol* **109**:834-41, 1972
15. American Assoc of Blood Banks. *Hepatitis testing*. The State of the Art, Progress Report II, 1973
16. Lehand E, Kwantes CMS, Upward MG and Thomson DR: Homologous serum jaundice. *Brit Med J* **2**:572-4, 1949
17. James G, Kornrs RF and Wright AW: Homologous serum jaundice associated with use of irradiated plasma. *JAMA* **144**:228-9, 1950
18. Sborov VM, Giger B, and Mann JO: Incidence of hepatitis following use of pooled plasma. *Arch Intern Med* **92**:678-83, 1953
19. Brightman IJ and Kornrs RF: Homologous serum jaundice in recipients of pooled plasma. *JAMA* **135**:268-72, 1947
20. Hamit F: Status of human plasma as a plasma volume expander. *JAMA* **174**:1617-24, 1960
21. Blanchard MC, Stokes J Jr, Hampil B, Wade GR and Spizizen J: Methods of protection against homologous serum hepatitis: inactivation of hepatitis virus SH with ultraviolet rays. *JAMA* **138**:341-43, 1948
22. MacCallum FO: Homologous serum hepatitis. *Proc Roy Soc Med* **39**:655-57, 1946
23. Murphy WP and Workman WG: Serum hepatitis from pooled irradiated dried plasma. *JAMA* **152**:421-3, 1953
24. Oliphant JW: Jaundice following administration of human serum. *Bull NY Acad Med* **20**:429, 1944
25. Oliphant JW and Hollaender A: Homologous serum jaundice: experimental inactivation of etiologic agent in serum by ultraviolet irradiation. *Pub Health Rep* **61**:598-602, 1946
26. Aach D and Aronson DG: Association of serum hepatitis with one lot of dried irradiated pooled plasma. *JAMA* **193**:105-7, 1965
27. Murray R, Oliphant JW, Tripp JT, Hampil B, Ratner R and Diefenbach WCL: Effect of ultraviolet radiation on infectivity of icterogenic plasma. *JAMA* **157**:8-14, 1955
28. Murray R: Viral hepatitis. *Bull NY Acad Med* **31**:341-58, 1955
29. *Hepatitis Surveillance Report No 27*, Sep 1967, National Communicable Disease Center
30. Dane DS, Cameron CH and Briggs M: Virus-like particles in serum of patients with Australia-antigen-associated hepatitis. *Lancet* **1**:695-700, 1970
31. Gocke DJ, Greenberg HB and Kavey NB: Correlation of Australia antigen with post-transfusion hepatitis. *JAMA* **212**:877-9, 1970
32. Okochi K, Murakami S, Ninomiya K and Kaneko M: Australia antigen transfusion and hepatitis. *Vox Sang* **18**:289-300, 1970
33. Holland PV, Alter HJ and Purcell RH: Development of post-transfusion hepatitis in recipients of blood containing hepatitis-associated antigen. Presented at *13th meeting of Int'l Soc of Blood Transfusion*, Munich, Germany, Aug 6, 1970
34. Barker LF, Shulman NR, Murray R, Hirschman RJ, Ratner F, Diefenbach WCL and Geller HM: Transmission of serum hepatitis. *JAMA* **211**:1509-12, 1970