APPLICATION OF CHEMILUMINESCENCE TO THE DIAGNOSIS OF SWINE DYSENTERY

E. M. Jenkins* and P. H. Klesius
School of Veterinary Medicine, Tuskegee Institute, Tuskegee Institute, AL 36088
Regional Parasite Research Laboratory, Auburn, AL 36830, U.S.A.

Polymorphonuclear (PMN) leukocytes are known to generate a chemiluminescence (CL), emission of light, subsequent to phagocytosis. The CL response is considered to result from relaxation of electronically-excreted carbonyl groups generated during singlet microbial oxidation of the ingested microbe (Yanai and Quie, 1981). This high energy oxygen molecule in turn is thought to excite other carbonyl compounds within the PMN (Easmon et. al., 1980). The phenomenon of CL generated by PMNs was reported originally by Allen et. al., (1972) and is associated with increased glucose oxidation via the hexose monophosphate shunt. Reactive oxygen radicals are formed by PMNs during phagocytosis. The sensitivity of CL assay is enhanced by luminol (5-amino-2,3dihydro-1,4-phthalazinedione) which reacts with singlet oxygen and other electronically excited compounds to increase the amount of light admitted (Trush et. al., 1978; Easmon et. al., 1980). When both leukocytes and bacteria are kept constant, the rate of CL reflects the opsonic capacity of sera used for opsonization, which may be indicative of prior exposure to that organism. The CL response has been used to diagnose chronic granulomatous disease (Stjernholm et. al., 1973). This assay has also been used to examine the opsonic activity of serum (Allen, R C, 1977; Stevens, P and Young, L S, 1977). In the present study we examined leukocytes isolated from the blood of swine affected with swine dysentery (SD) or convalescent to the disease to determine if CL could be useful as a diagnosis tool for the disease.

Blood was collected from the cranial vena cava of swines with preservative-free heparin (10 u/ml). The PMN leukocytes were collected from the same syringe on 5 ml of 6% solution of Dextran (mol. wt. 282,000) in 0.9% saline solution after the RBC were allowed to settle 1/2 hour at room temperature. Then with the same needle bent 45° the PMNs were transfered to 15 ml sterile polystyrene test tubes and centrifuged at 2000 RPM for 30 minutes. After centrifugation the plasma was discarded and the precipitant was treated with 5 ml buffered NH_4Cl for 5 minutes to lyse and RBC. An equal volume of citrate saline solution was then added to restore isotonicity and the cells were centrifuged at 2000 RPM for 10 minutes. The cells were then washed 2x in Earl's Balanced Salt Solution medium and counted with the Hycel Counter (Am. Scientific Co. Atlanta, GA). Finally the PMNs were adjusted to a centrifugation of 5×10^6 in complete Hank's Balanced Salt Solution (HBSS) with calcium and magnesium and without phenol red the PMNs were 62% of the cell pellet.

Luminol was converted to a soluble potassium salt by dissolving 0.0142 g luminol, 0.78 g potassium hydroxide and 0.618 g boric acid in 10 ml sterile water. This stock solution was wrapped in foil and allowed to settle for three days at 4°C. Two hours prior to use, an aliquot of stock solution was diluted to 1:80 in sterile water and dark adapted for use in the experiment. Treponema hyodysenteriae was grown in broth culture as previously described (Jenkins et. al., 1979).

The reaction mixture (a 1:1 ratio of spirochete to PMNs) consisted of 0.1 ml PMN suspended in complete, cold HBSS, 0.05 ml luminol diluted in complete HBSS and 0.1 ml suspension in 6x 50 mm glass reaction tubes. The final volume in the reaction tube remained constant throughout all experiments. The mixture was vortexed briefly and placed in the light-proof chamber of the Pico-Lite Luminometer from Packard Instuments (Downers Grove, IL). All constituents were kept at room temperature prior to assay, and the assay was run at 39°C. Counts were printed at 30 second intervals until peak emission was recorded. The CL response was run in triplicate and the results recorded as Counts Per Minutes (CPM).

The results of the experiments indicate that fresh PMNs (1-3 hrs) gave a significantly greater ($P < 0.05$) CL response than older cell (4-8 hrs). Likewise, there was no significant difference ($P > 0.05$) in the CL response of cells obtained from normal animals and those of infected ones. A 1:20 dilution of serum consistently gave the maximum CL response which was determined by titrating with hyperimmune anti-T. hyodysenteriae serum. Heat inactivated of sera from SD-infected swine resulted in a reduction of activity indicating opsonin-dependent CL. The mean and standard error of the mean CPM of 31 SD-infected pigs were significantly greater ($P < 0.03$), as determined by the Student's "T" Test than that of a comparable number of normal pigs ($2.11 \pm 0.430 \times 10^5$ vs $0.636 \pm 0.221 \times 10^5$, respectively). The CL response of the same sera against antigens of Salmonella cholerasuis (SC) and enteropathogenic Escherichia coli (EPEC) resulted in one half to one third the response of that of T. hyodysenteriae. However, when sera obtained before and after infection with T. hyodysenteriae were absorbed with SC and EPEC there was a significant reduction ($P < 0.05$) in CL response in both, indicating lack of specificity of the assay. Our results indicated that a significantly greater difference in the CL response of the sera of SD-infected swine compared to those of normal or uninfected pigs. However, absorption of these sera with heterologous organisms significantly reduced the CL response specific for T. hyodysenteriae. Therefore, the usefulness of this test as a diagnostic trial for field cases of SD is questionable.

Seclected references: Allen et al: Biochem. Biophys Res. Commun. 1972, 47:679-684; Allen, R. C., Infect. Immun. 1977, 15:828-833; Yanai, M. and Quie, P. G., Infect. Immun. 1981, 32: 1181-1186; Trush, M. A. et al: Method Eng. 1978, 57:462-494; Easmon, et al: Immunol. 1980. 41:67-74; Stjernholm, R. L. et al, Infect. Imm., 1973, 7:313-314; Steven, P., and Young, L. S. Infection and Immun. 1977, 16: 796-847; Jenkins, E. M., VM/SAC 1978, 73: 1931-1936.

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