Photo-irradiation effects on the activity of the Vesicular Stomatitis Virus (VSV)

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In the name of **Allalh, I** start my practical life as a junior biophysics researcher,Allah my guide in whole my life, I thank Allah and pray to him.

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Contents

Abstract

The aim of present work is to study the effect of ultraviolet irradiation on the activity of the Vesicular Stomatitis Virus (VSV) as hepatitis C virus (HCV) model, evaluating the inactivation parameters for the viral genes and calculation of the energy amount which required to complete destruction of the viral RNA and proteins.

By cell culture techniques, the Vesicular Stomatitism Virus was loaded on gamma globulin, and stock virus titer was detected. Samples of the Vesicular Stomatitis Virus and gamma globulin mix were exposed to ultraviolet- irradiation for different times up to 24 hours, and virus titer was detected for each sample. Ultraviolet source of wavelength = 253.7 nm, intensity: 2.84 W/cm^2 was used in this work.

Using the spectrophotometry and electrophoresis techniques the normal sample and irradiated samples showed maximum absorbance with different values at wavelength = 230 and 260 nm. Analysis at wavelength = 260 nm (the maximum absorbance of nucleic acid) proved that, after 24 hours of ultraviolet- irradiation, RNA of the Vesicular Stomatitis Virus denaturated and its activity is nearly stopped.

Samples of gamma globulin without the virus were exposed to ultraviolet – irradiation for 1,2,8,16,24 hours and it was found that, after all previous times of irradiation there was some changes in gamma globulin structure. Increasing time of exposure of the Vesicular Stomatitis Virus particles to ultraviolet radiation caused differential inhibition of the synthesis ***in vitro*** of the viral particles in relating to time.

From the obtained results from the gel electrophoresis technique it was noted that VSV and gamma globulin mix samples showed smear due to the protein overlapping while gamma globulin alone showed different integrated bands. It was not helpful in analysis for the samples of the Vesicular Stomatitis Virus and gamma globulin mix, but it was helpful with gamma globulin samples.

The inactivation parameters of the viral RNA were found to be dependent on time of irradiation and the synthesis of RNA strand showed single hit kinetics after each time of irradiation. Changes in the inactivation parameter were evaluated for each gene of the five proteins in the viral RNA; the results suggest that, the ultraviolet inactivation process is mainly dependent on the molecular weight of the virus and time of exposure.

Calculation of the lethal hit per incident hit proved that, as the time of irradiation increases, the value of the hit to be lethal increases. Energy values for complete destruction of peptide bonds of the five viral proteins and phosphodiester bonds of the viral RNA were calculated and

percentage of broken peptide bonds of each protein was determined, and it was found that, peptide bonds of N, G, and (M, P) proteins were completely destructed after 4, 8 and 16 hours respectively .

Bonds of L protein were not completely destructed even after 24 hours of exposure. The analysis of present data evoked some interesting features: (1) UV-C is an effective method in the (VSV) inactivation process, (2) UV-C (254 nm) for 24 hours exposure has some fractionation effect on the structure of gamma globulin, (3) Inactivation process of UV depends mainly on the target size and time of exposure even with small doses,(4) Exposure to UV-C for 24 hours with intensity 2.84 W/ cm^2 is enough to complete destruction of peptide bonds of G,M,N and P proteins.

It may be recommended that, detailed research is necessary about the effect of UV on the Vesicular Stomatitis Virus with changing some physical parameters as PH, temperature and pressure.

Inroduction

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Review of literature

Chapter 1

Introduction and review of literature

1.1 Introduction:-

Infectious complications of blood transfusion continue to be an important area of concern in transfusion medicine. In recent years numerous infectious agents (found worldwide) have been identified as potential threats to blood supply. These newly discovered agents namely transfusion transmitted virus (TTV) as, Human herpes virus-8 (HHV-8), hepatitis C virus (HCV),human immunodeficiency virus (HIV), West Nile virus and Prions present a unique challenge in assessing the possible risk they may pose to the safety of blood and plasma products and this make pathogen inactivation even more important **(Kaur and Basu 2005).**

Viral inactivation with the electromagnetic radiation (gamma ray, X- ray, UV, visible light and laser) was examined; and proved different degrees of success to each one. The principle of electromagnetic inactivation of viruses depends on the suitable amount of photons and so amount of energy to break the organic bonds of DNA or RNA which produce a genetic damage and leads to a defect in the viral protein production and affects the virus life cycle in the host cell ***(Takashi and Hajime,2006).***

Gamma ray affects both the virus and protein of the host cell causing ionization to the protein content ***(Kempner, 2000).*** X- ray proved a success in dealing with some types of viruses and showed success only *in vivo* studies ***(Nokta et al., 1992)*** and it was found that, at 12 keV (1 Å wavelength), about ten absorbed photons are sufficient to "kill" a unit cell ***(Piotr et al., 2003).***

The effective region in the visible light was the UV region except this region, visible light was with limited inactivation efficiency ***(Tobias et al.,1989).***

Laser proved no evidence of herpes simplex virus ***(Korner et al.,1989)*** .The side effect from laser involve the potential for interaction with cellular and extracellular matrix molecules to generate reactive oxygen species and reactive nitrogen species which in turn can initiate lipid peroxidation, protein damage or DNA modification ***(Kim, 2003).***

More than one research proved that UV (UV-A, UV-B, UV-C) radiation is an effective way in dealing with both enveloped and non-enveloped viruses (***Martin et al., 1994; Poirot et al., 1985; Michael et al., 1982; Gordan et al., 1976).***UV radiation was an effective physical method even with small doses in the viral inactivation process of the vesicular Stomatitis Virus (VSV); the widely distributed virus as a model to hepatitis C virus (HCV). Many chemical compounds proved a success in dealing with the (VSV), the widely distributed is Beta –propapiolatone (BPL) and formline. The principle of UV effects on the virus is the dimerization of the thymine nucleotide after the exposure to UV which affect the replication and transcription of the viral RNA, and so viral protein production ***(Kundu et al., 2004).***

1.2 Review of literature:-

1.2.1 Photo-irradiation effect on viral inactivation :-

The use of photo-irradiation for the sterilization of viruses has been studied for more than 30 years ago. A quanta of energy of light possesses just the right amount of energy to break organic molecular bonds of the genetic materials (DNA or RNA), this bonds breakage translates into genetic damage for the viruses ***(Rajeshwar and Donat , 2000).***

Viral inactivation by gamma ray:

Several animal viruses were treated with gamma radiation from a Co^{60} source under conditions which might be found in effluent from an animal disease laboratory. Swine vesicular disease virus, vesicular stomatitis virus, and blue-tongue virus were irradiated in tissues from experimentally infected animals. Pseudorabies virus, fowl plague virus and swine vesicular disease virus were irradiated in liquid animal feces. All were tested in animals and in vitro. The D_{10} values, that is, the doses required to reduce infectivity by 1 log10, were not apparently different from those expected from predictions based on other data and theoretical considerations. The existence of the viruses in pieces of tissue or in liquid feces made no difference in the efficacy of the gamma radiation for inactivating them. Under the "worst case" conditions (most protective for virus) simulated in this study, no infectious agents would survive 4.0 M rads ***(Thomas et al., 1982)*.**

Gamma irradiation effectively inactivated gradient-purified rinderpest virus. Irradiated antigen and sera remained functional in

enzyme-linked immunosorbent assays, virus neutralization tests, and indirect fluorescent-antibody tests. Irradiation, however, led to a dose-dependent decrease in reactivity, particularly significant ($P < 0.05$) when both reagents were irradiated. To avoid false-positive reactions, only one reagent (serum or antigen) may be irradiated ***(Saliki, et al., 1993).***

The infectivity of Lassa, Marburg, and Ebola viruses was inactivated without altering the immunological activity after radiation with Co^{60} gamma rays. At 4 degrees ^{0} C, Lassa virus was the most difficult to inactivate with a rate of 5.3×10^{-6} log of 50% tissue culture infective dose per rad of CO^{60} radiation, as compared with 6.8×10^{-6} log 50% tissue culture infective dose per rad for Ebola virus and 8.4×10^{-6} log 50% tissue culture infective dose per rad for Marburg virus. Experimental inactivation curves, as well as curves giving the total radiation needed to inactivate a given concentration of any of the three viruses, showed that, this method of inactivation to be superior to UV light or beta-propiolactone inactivation and now routinely use it for preparation of material for protein-chemistry studies or for preparation of immunological reagents ***(Elliott et al.,1982).***

Whole-body gamma- irradiation to Semliki Forest virus (SFV) in the mouse up to 600 rad, showed distinct sensitivities in virus host interactions to gamma-radiation which are discussed in terms of the impairment and recovery of the lymphocyte compartments probably involved ***(Bradish et al., 1980).***

Viral inactivation by X- ray :-

The effect of X-irradiation on the replication of human immunodeficiency virus (HIV) in vitro in CD4+ cells was examined. Infected MT-4 cells were irradiated at the time of infection or following infection with X-ray doses of 25-300 cGy. Doses of 50, 150, and 300 cGy enhanced HIV replication by 1.6, 2, and 4.8-fold, respectively. Irradiating the cells prior to infection also resulted in similar enhancement of HIV replication. This phenomenon was also observed with wild-type HIV isolates grown in peripheral blood mononuclear and in HIV chronically infected cells. In addition, the enhancement was associated with a radiation-induced increase in intracellular levels of cAMP. The use of the cAMP-dependent protein kinase A inhibitor, H-8, inhibited HIV replication by 65%. These data suggest that in vitro exposure to low doses of X-ray enhances HIV replication partially via a cAMP-dependent pathway ***(Nokta et al., 1992).***

Viral inactivation by ultraviolet light:

The ability of ultraviolet (UV) light to inactivate viruses is well established. Effective processing of large-scale batches of UV –opaque protein solutions has been achieved using a continuous- flow device. The operation of this device has been modeled and a design equation derived to relate the processing conditions and product characteristics the degree of virus inactivation obtained. Variables include in the model are UV absorbance at 254 nm (A (254)), hydrodynamic properties of the protein solution, residence time, intensity of UV light and diameter and length of irradiation tube. With this information a specific constant was calculated for each virus which denotes its relative sensitivity to UV and from which the degree of virus inactivation expected can be estimated ***(Li et al., 2005).***

Dynamic continuous- flow UV-C irradiation is a new way to inactivate a large range of pathogens without adding any photosentizers. The efficacy of different methods was evaluated against the following viruses: murine parvovirus MVMp, human B19,the encephalomyocarditis virus(EMC, a picornavirus used as a model for hepatitis A virus),and bovine herpes virus type 1 (BHV, a model for enveloped viruses such as hepatitis B virus).The results showed that continuous-flow UV-C irradiation is very effective, particularly against resistant pathogens (e.g. parvoviruses and bacteria) at UV-C doses preserving protein activity ***(Caillet et al., 2004).***

Viral inactivation by visible light:-

Light in the violet part of the visible spectrum (wavelength 420-430 nm) is responsible for virus inactivation. Reduced reverse transcriptase-dependent cDNA generation post-entry accounts for the loss in infectivity and is likely due to a polymerase processivity defect. The virion- associated reverse transcription complex is thus photolabile ***(Tobias et al., 2005).***

Viral inactivation by laser:-

In vitro investigations were performed to study the effect of infrared Nd:YAG laser irradiation on herpes simplex virus and the viral replication in herpes – infected Vero cell microcultures. In addition, the influence of laser irradiation on human immunocompetent cells was investigated by irradiation and incubation of herpes –infected cell cultures overlaid with leucocytes and observation of leucocyte migration under agarose after laser irradiation. There was no evidence of herpes simplex virus inactivation by laser irradiation. Irradiation of Vero cell

microcultures infected with virus did not influence the development of the viral cytopathic effect. However, irradiated cultures showed an increase of about 50 % in the virus yield. Only a slight indication of laser influence on immunocompetent cells was found ***(Korner et al., 1989).***

It was evaluated that, the influence of low-intensity laser therapy (wavelength 690 nm, intensity: 80 mW per cm^2, dose: 48 J per cm^2) in 50 patients with recurrent perioral herpes simplex infection (at least once per month for more than 6 months) in a randomized . In conclusion, a total of 10 irradiations with low-intensity laser therapy significantly lowers the incidence of local recurrence of herpes simplex infection ***(Andreas et al., 1999).***

It has been reported that excimer laser irradiation might elicit herpes simplex virus (HSV) genome activation. A clinical case was described in which HSV DNA sequences were detected quantitatively after phototherapeutic keratectomy (PTK). It was concluded that, excimer laser photokeratectomy stimulated viral shedding in the tear film. Ophthalmologists should be aware that laser irradiation can reactivate latent HSV ***(Tatsunori et al., 2004).***

For most of the patients with chronic hepatitis B the immunologic function is deficient. Immunopotentiation and immunoregulation can be used as effective treatments. Laser irradiation can potentiate the cellular immune function of the human body and has good effects on improving clinical symptoms, cutting short the process of diseases, and promoting HBsAg negative change ***(liu-Da et al., 1993).***

1.2.2 UV inactivation of the (VSV):-

The effect of ultraviolet radiation on the expression of the genes of vesicular stomatitis virus (Indiana type) was studied, three of the five viral proteins (N, NS and M) were synthesized accurately and efficiently; a putative precursor to the viral glycoprotein (G) was also made, but synthesis of the L protein was not detected ***(Andrew et al., 1975).***

Increasing exposure of the Vesicular Stomatitis Virus particles to ultraviolet radiation caused differential inhibition of the synthesis in vitro of individual mRNA species which code for the viral structural proteins L, G, M, NS and N. The synthesis of each mRNA species showed single hit kinetics ***(Jordan et al., 1976).***

Published ultraviolet inactivation data and in vitro transcription studies have suggested that Vesicular Stomatitis Virus the (VSV) leader RNA was slowly responsible for the inhibition of host cell RNA synthesis by this virus. Since no protein product is encoded in leader RNA, this

conclusion implied that no protein synthesis should be required for this effect. Therefore, the inhibitory activity of the (VSV) was examined in the presence of the protein synthesis inhibitors, cycloheximide, pactamycin, and emetine. It is concluded that protein synthesis is required for the inhibitory activity of the (VSV), presumably because the active inhibitory complex is a nucleoprotein containing leader RNA and either a cellular protein or the viral N protein. The cellular protein would have to be in limiting supply since de novo protein synthesis was required for the inhibition to take place ***(Poirot et al., 1985).***

There are three different inactivation procedures to inactivate the (VSV) there are formaldehyde, Beta-propiolactone or UV-light using Vesicular Stomatitis Virus the (VSV) as a model antigen. All three inactivation procedures astically impaired induction of neutralizing IgG responses, both formaldehyde and Beta-propiolactone completely abrogated the induction of the (VSV) -specific cytotoxic T cells (CTLs), whereas UV-inactivated virus was able to induce significant and long-lasting CTL responses ***(Martin et al., 1994).***

Ultraviolet irradiation of infectious vesicular stomatitis virus was employed to study the relationship between the expression of the viral gene functions, viral infectivity, protein synthesis, and viral mRNA synthesis were all highly susceptible to inactivation by UV radiation; however, low levels of viral transcriptase activity were detected in vitro in virus preparation subjected to large doses of UV radiation ***(Phillip et al., 1995).***

1.2.3 Irradiation effects on the structural proteins:-

Gamma- ray effects:-

Gamma rays ionize molecules at random along their trajectories. In each event, chemical bonds are ruptured, releasing radiolytic products that diffuse away. A solution of macromolecules is mostly water whose principal radiation products are H (+) and OH (-). These can diffuse to and react with macromolecules; this indirect action of radiation is responsible for 99.9% of the damage to proteins ***(Kempner, 2001).***

X- ray effects:-

For overall damage to several different kinds of protein crystals at flux densities up to 10^{15} ph/sec/mm^2 it was found that, at 12 keV (1 Å wavelength), about ten absorbed photons are sufficient to “kill” a unit cell. As this corresponds to about one elastically scattered photon, each

unit cell can contribute only about one photon to total Bragg diffraction. The smallest protein crystal that can yield a full data set to 3.5 Å resolution has a diameter of about 20 μm (100 Å unit cell) ***(Piotr et al., 2003).***

Ultraviolet effects:-

The radiation damage of several proteins has been investigated in aqueous solution, after preceding UV-irradiation. In order to differentiate between different radiation effects, a series of physico-chemical and biochemical methods as well as novel evaluation and normalization procedures have been applied. Numerous changes of several molecular parameters characterizing functional ability and primary, secondary,tertiary and quaternary structure of proteins could be found. ***(Durchschlag et al., 1996).***

The thermal stability of UV-B irradiated collagen in bovine lens capsules and in bovine cornea UV irradiation induced changes in collagen which caused both stabilization and destabilization of the collagen structure. After irradiating for longer times (20–96 h) the helix–coil transition peak occurred at much lower temperatures. The peak was very broad and suggested that collagen was reduced by UV to different polypeptides of different molecular weight. The irradiation of lens capsules with UV-B light in vitro resulted in changes in the thermal properties of type-IV collagen consistent with lower thermal stabilities ***(Alina, 2005).***

The formation of the thioester in the reaction between Cr (VI) and cysteine in aqueous media has been studied by monitoring the decrease of Cr (VI) at 370 nm. It was observed that the reaction is catalyzed by the UV irradiation of the Cr (VI) and cysteine mixture ***(Semiha et al., 2005).***

Several proteins have been crosslinked to DNA by low dose iradiation.The principle of the method is based on an efficient and fast radiation induced reaction of amino acid residues with DNA at low pH. The method seems to be of general applicability for cross linking proteins to DNA in a very simple one step procedure. Some of such DNA-protein conjugates have been used as probes for hybridization experiments (***Czichos et al., 1989).***

Laser irradiation effects:-

The possible molecular mechanisms of laser actions remain unclear and the damaging effects of laser irradiation are still controversial. The side effects of laser therapy involve the generation of reactive oxygen and nitrogen species which in turn initiate lipid peroxidation, protein damage