ETHIDIUM BROMIDE INDUCED HISTOLOGICAL CHANGES IN SPLEEN OF ALBINO MICE

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ABSTRACT

Adult Mice (BWt. 30 gms and 40gms) were treated at a 5mg\kgBWt. and 10 mg/kg BWt. of Ethidium Bromide for 10 days in drinking water. Control animals were given equal dose of deionised water. Quantitative and qualitative changes were studied in Spleen. The following features were evaluated: the overall cellularity of the spleen was noticed. The changes were characterised by, injury to spleen tissue; necrotic changes, cirrhosis and fibrosis. Also reduction in visceral epithelial cell number was noticed. The size of the spleen slightly decreased and interstitium shrinked. Also a Slight change in spleen weight was recorded. Body weight of the animal was slightly altered after challenge with Ethidium Bromide.

Key Words: Ethidium Bromide, Toxicity, Spleen, Histopathology, Albino Mice

INTRODUCTION

The spleen is an organ found in virtually all vertebrate animals. The spleen acts primarily as a blood filter. As such, it is a non-vital organ, with a healthy life possible after removal. The spleen plays important roles in regard to red blood cells (also referred to as erythrocytes) and the immune system. Spleen in humans is located in the left upper quadrant of the abdomen. It removes old red blood cells and holds a reserve of blood in case of hemorrhagic shock while also recycling iron. As a part of the mononuclear phagocyte system, it metabolizes haemoglobin removed from senescent erythrocytes. The globin portion of haemoglobin is degraded to its constitutive amino acids and the heme portion is metabolized to bilirubin, which is subsequently shuttled to the liver for removal Mebius and Kraal (2005). It synthesizes antibodies in its white pulp and removes antibody-coated bacteria along with antibody-coated blood cells by way of blood and lymph node circulation. The spleen is brownish. Mebius and Kraal (2005) Loscalzo et al., (2008) recently has found to contain in its reserve half of the body's monocytes within the red pulp. Swirski et al., (2009) reported that these monocytes, upon moving to injured tissue (such as the heart), turn into dendritic cells and macrophages while promoting tissue healing (Swirski et al., 2009; Jia and Pamer 2009). It is one of the centres of activity of the reticulo-endothelial system and can be considered analogous to a large lymph node, as its absence leads to a predisposition toward certain infections (Brender et al., 2005).

Ethidium bromide is commonly used to detect nucleic acids in molecular biology. Since ultraviolet light is harmful to eyes and skin, gels stained with ethidium bromide are usually viewed indirectly using an enclosed camera, with the fluorescent images recorded as photographs. In the laboratory the intercalating properties have long been utilized to minimize chromosomal condensation when a culture is exposed to mitotic arresting agents during harvest. The resulting slide preparations permit a higher degree of resolution and thus more confidence in determining structural integrity of chromosomes upon microscopic analysis. Anatomically the spleen, in healthy adult humans, is approximately 11 centimetres (4.3 in) in length. It usually weighs between 150 grams (5.3 oz) (Draper *et al.*, 2009) and 200 grams (7.1 oz) (Spielmann *et al.*, 2005). An easy way to remember the anatomy of the spleen is the 1x3x5x7x9x11 rules. The spleen is 1" by 3" by 5", weighs approximately 7 oz and lies between the 9th and 11th ribs on the left hand side. Lien Like the thymus, the spleen possesses only efferent lymphatic vessels. The spleen is part of the lymphatic system. Both the short gastric arteries and the splenic artery supply it with blood.

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Blackbourne and Lorne (2008) the germinal centres are supplied by arterioles called Penicilliary radicles (2011). The spleen is unique in respect to its development within the gut. While most of the gut viscera are endodermally derived (with the exception of the neural-crest derived suprarenal gland), the spleen is derived from mesenchymal tissue. Vellguth et al., (2009) reported specifically, the spleen forms within and from, the dorsal mesentery. However, it still shares the same blood supply — the celiac trunk — as the foregut organs. Other functions of the spleen are less prominent, especially in the healthy adult: Production of opsonins, properdin and tuftsin. While the bone marrow is the primary site of hematopoiesis in the adult, the spleen has important hematopoietic functions up until the fifth month of gestation. After birth, erythropoietic functions cease, except in some hematologic disorders. As a major lymphoid organ and a central player in the reticuloendothelial system, the spleen retains the ability to produce lymphocytes and, as such, remains an hematopoietic organ. Storage of red blood cells, lymphocytes and other formed elements. In horses, roughly 30% of the red blood cells are stored there. The red blood cells can be released when needed. Carey and Bjorn (2006) in humans, up to a cup (236.5ml) of red blood cells can be held in the spleen and released in cases of hypovolemia. It can store platelets in case of an emergency. Up to a quarter of lymphocytes can be stored in the spleen at any one time. In mice, the spleen stores half the body's monocytes so that upon injury they can migrate to the injured tissue and transform into dendritic cells and macrophages and so assist wound healing Swirski et al., (2009).

Ethidium bromide is commonly used in molecular biology laboratories to stain electrophoresis gels Huang and FU (2005). The compound forms fluorescent complexes with nucleic acids and these can be viewed under UV light. Ethidium bromide (EB) is described to be mutagenic and moderately toxic after an acute exposure National Toxicology Program (2005). EB can be absorbed through skin and therefore it is important to avoid direct contact with the chemical. Ethidium bromide as it is a known mutagen in certain animal and microorganism test systems Ohta *et al.*, (2001). Although the compound has not been thoroughly evaluated in humans, based on current toxicity data and its interaction with DNA it should be handled with considerable caution. Ethidium bromide is a large, flat basic molecule that resembles a DNA base pair. Because of its chemical structure, it can intercalate into a DNA strand.

Not enough evidences are available in mammals, therefore this study was planned. Therefore all individuals should regularly review their risk assessments and work practices for EtBr. Ethidium bromide may be a mutagen, carcinogen or teratogen although this depends on the organism and conditions. In the laboratory the intercalating properties have long been utilized to minimize chromosomal condensation when a culture is exposed to mitotic arresting agents during harvest. The resulting slide preparations permit a higher degree of resolution and thus more confidence in determining structural integrity of chromosomes upon microscopic analysis. Despite the performance advantage of using SYBR dyes instead of EtBr for staining purposes, many researchers still prefer EtBr since it is considerably less expensive. Ethidium bromide is thought to act as a mutagen because it intercalates double stranded DNA, thereby deforming the molecules. This can affect DNA biological processes, like DNA replication and transcription Huang and FU (2005). If the level is high enough, that exposure may interfere with replication of mitochondrial DNA in some human cell lines, although the implications of that are not clear. Testing in mice and humans and longer studies in any mammalian system is required. A low dose of ethidium bromide leads to an increase of total mitochondrial DNA while higher concentrations induce the mt-DNA 4997 deletion in a human neuronal cell line Wurmb-Schwark et al., (2006). It is used as a molecular probe for staining nucleic acids in fluorescent microscopy studies of multidrug resistance Nayfakh (1988). It is also used as a DNA probe for various studies including characterizing and quantifying DNA (Green, 1990). It is also used as a derivatizing analytical reagent in clinical settings for continuous monitoring of levels of anticancer drugs in biological fluids, including blood, serum and urine by measurement of dose-critical levels of DNA-binding. According to Lun and Sansone (1987) safe handling of EB in laboratories to avoid human exposures to mutagenic solutions containing EB has been

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addressed by Lun and Sansone (1987) and Quillardet and Hofnung (1988). They concluded that EB should be handled as a carcinogen in terms of identifying methods of safe waste disposal. EB is not known to occur naturally. No information was found in the available literature on detection of EB in environmental media. Several spill clean-up and disposal methods have been recommended in the available literature for EB. They are based on careful removal to achieve elimination of mutagenicity of solutions by decontamination and degradation. Published methods include treatment with potassium permanganate/hydrochloric acid or hypophorous acid/sodium nitrite, adsorption on activated charcoal and incineration at high temperatures Quillardet and Hofnung (1988).

The American Conference of Governmental Industrial Hygienists (ACGIH) has not adopted a timeweighted average/threshold limit value (TLV/TWA) for this compound. EB is categorized as an acute hazard under SARA sections 311/312 (40 CFR 370.21) Anon (1994b)

MATERIALS AND METHODS

Tissue Preparation

Young Mice of BWt. 30 gms and 40gms were used as a model in the present study treated at a 5mg\kgB Wt. and 10 mg \kg BWt. (each) of Ethidium Bromide for 10 days in drinking water. Control animals were given equal dose of deionised water. Total five groups of mice were set in the experiment. Each group had 6 mice. They were acclimatised to laboratory conditions for 15days prior to the commencement of the treatment. Mice were kept in open air cages at room temperature. Mice were fed standard rodent palate diet (Hindustan Lever ltd). Experimental animals were given Ethidium bromide orally through drinking water.

Animals of experimental and control group were sacrificed on tenth day of treatment by cervical dislocation. The spleen of experimental and control group of mice were fixed in formalin for 4 hrs. They were dehydrated, in graded EtOH series, cleared in xylene, in filterated with and embedded in pure filtered paraffin wax (M.P.58 degree centigrade). Deparaffinised sections (5-7 microns) were stained by haematoxylin and eosin to monitor the extent of changes in the spleen histoarchitechture. Every alternate section of the spleen was microscopically examined and appropriate areas were microphotographed and enlarged. Disintegration were also microphotographed to record the vulnarebility to Ethidium Bromide toxication. The behaviourable changes in mice were also observed. The degenerating cells were identified on the basis of desquamation of cells, nuclear pycnosis, chromatolysis and loss of shape. Necrotic and hyperplasia patches were seen and microphotographed. Serial sections were examined to assess glomerular structure.

RESULTS AND DISCUSSIONS

The spleen of control mice weighed 0.536 mgs (mean value). The weight of both control and experimental mice were recorded before and after the experiment. The weight of spleen in all the groups of mice before and after the experiment were observed according to TABLE-I. The overall shape of the spleen was not altered nor there do any significant change in organ weight as compare with control. But still slight changes in the weight were noticed.

Behavioural Observations

(a) Control group- All animals showed normal behaviour and there was no mortality or lingering of animals.

(b)Treated Group (30gms mice) - Administration of Ethidium Bromide to rats resulted in marked alterations in bevahiour revealing nervous manifestations (abnormal neurobevahiour) in the treated groups as increased landing of the limbs, weakness of the muscles, general emasciation .The severity of clinical science was dose and time dependent as these manifestations appeared on the 3rd day of EtBr treatment in 30gms mice given with a dose of 10mgs\kg BWt. two out of six died. But just lingering was observed in mice with 30gms weight with a dose of 5mgs\kg BWt. in 3 out of 6 mice.

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(C)Treated Group(40gms mice)- Animals weighing 40 gms administered 5 milligram per kg body weight showed normal bevahiour and their appetite was normal but the animals weighing 40 gms administered with 10 milligram/kg body weight of the dose showed drousiness and their apetite was reduced. No mortality and lingering was observed in the mice.

Histological observations of spleen

Histopathological changes of Ethidium Bromide treated rats of 30gms BWt with a dose of 5mgs/kg body wt showed little degenerative changes characterized by injury, Lymphoid Necrosis/Apoptosis. Ethidium Bromide treated mice with a body weight 30 gms, and a dose of 10mgs\kg BWt showed the necrosis of the white pulp. When evaluating a splenic specimen by light microscopy on an HandE stained section. In slide 1 normal structure of spleen was seen. In slide 2 diffuse (non-neoplastic) hyperplasia of the red pulp was seen. The erythroid component was seen predominating (erythroid hyperplasia) secondary to hemorrhage or erythrocyte destruction (hemolytic or autoimmune anemia), the myeloid component was predominating (myeloid or granulocyte hyperplasia) secondary to inflammatory conditions. Myeloid hyperplasia shares some histological similarities to granulocytic leukemia (Long *et al.*, 1986). Some degree of extramedullary hematopoiesis is present in mice, increased extramedullary hematopoiesis resulted from hematotoxic insult, systemic anemia and infections elsewhere in the body.Irregular and often eccentric nuclei and abundant pale staining cytoplasm was seen in Figure 3.

Slight change in spleen weight was recorded. Body weight of the animal was slightly altered after challenge with Ethidium Bromide. No study has been done on the histopathological changes in spleen of Albino mice due to Ethidium Bromide toxicity. Hence the present study has been done. As seen in (Figure 2) the changes were charecterised by disorganization of Splenic cells, Necrotic changes, hyperplasia and erythroid hemorrhage were noticed. Quantitative changes were also characterised by significant decrease in the number of splenic cells. The spleen cells began to degenerate along with the haemorrhage in blood capillaries (Figure 2) but most of the glomerulus reached the maturity in mice with a BWt of 30gms treated with 5mgs\Kg BWt. The percentage of degenerating splenic cells was hence found to be still high as compared with control in mice with a BWt of 30gms treated with 10mgs\KgBwt (Figure 3) and (Figure 4). In mice with 40gms BWt treated with 5mgs\kgBWt (Figure 5) dose showed lesser and still lesser disintegration respectively. The overall shape of the spleen was not altered nor there do any significant change in organ weight as compare with control. Acute ethidium bromide lead to necrosis and caused a notable reduction in splenic cell number. However, widespread spleen injury is characterized by hyperplasia.



Figure 1: Normal spleen seen in control mice



Figure 2: Abnormal spleen seen in30gms mice treated with 5mgs/kg Bwt



Figure 3: Abnormal spleen seen in30gms mice treated with



Figure 4: Extensive generalised paranchymal fibrosis seen in spleen of 10mgs/kg Bwt 40gms mice treated with 10mgs/kgBwt



Figure 5: Necrosis and infilteration in mice of 40gms treated with 5mgs\kgBwt

GROUPS	snleen Weight Before Experiment(av)	snleen weight After Evneriment(av)
Control		0.20gmg
$\frac{20}{20} \frac{1}{20} $	0.20gms	0.20gms
Sugrins(Sings()	0.20gins	0.19gms
30gms(10mgs\)	0.20gms	0.18gms
40gms(5mgs)	0.22gms	0.21gms
40gms(10mgs)	0.20gms	0.18gms

Table 2: Percent Degenerative changes in splenic cells		
GROUPS	%Degenerative changes in splenic cells	
Control	0%	
30grms(5mgs\)	30%	
30gms(10mgs\)	55%	
40gms(5mgs\)	20%	
40gms(10mgs\)	30%	

Percent Degenerative Changes in Splenic Cells



Figure 6: Graph showing percent degenerative changes in kidney cells

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