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Vascular permeability and Evans blue dye: a physiological and pharmacological approach

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ABSTRACT

The acute inflammatory response consists of three main vascular effects: vasodilatation and increased blood flow, increased vascular permeability, and leukocytosis into the injured tissues. All three events are induced relatively quickly, and, for all three, the pattern of response is complex but consistent. Evans blue dye is an alkaline stain, so there is an affinity for the alkali in the acidic nucleus. The use of Evans blue dye as an in vivo marker through vascular permeability, facilitates the investigation of the effect of pathological changes in various disorders mainly, immunological disorders, inflammatory disorders, cardiovascular diseases like atherosclerosis, myocardial infarction, cancers and others. Endothelials have pathophysiological roles in pulmonary hypertension, arterial hypertension, atherosclerosis, cerebral vasospasm and inflammatory processes. The present review discussed with role of evans blue in the assessment of vascular permeability for the various pharmacological activities which are helps for the future investigations.

INTRODUCTION

The acute inflammatory response consists of three main vascular effects: vasodilatation and increased blood flow, increased vascular permeability, and leukocytosis into the injured tissues. All three events are induced relatively quickly, and, for all three, the pattern of response is complex but consistent. In studies of paw edema, ear edema, and skin reaction, the cotton pellet implant inflammation model, rat air-pouch inflammation model, and rat pleurisy model are usually used for measuring vascular permeability as an index of inflammatory reaction (Kohji *et al.,* 2012). Most solid tumors are known to exhibit highly enhanced vascular permeability, similar to or more than the inflammatory tissues. Evans blue dye (EBD) was used to monitor vascular protein leakage. Florimetric measurement of EBD in formamide extracts of various tissues (Hiroshi *et al.,* 2003). The growth of new blood vessels is known as angiogenesis and it is an early event in inflammatory activity of arthritis and other disorders, dependent on endothelial cell (EC) activation, migration and survival (Koch, 1998). Angiogenesis is a complex, tightly-controlled process that is important in physiological and pathological situations. Angiogenesis enables the development of an inflammatory infiltrate (Tuyl *et al.,* 2005). The control of vascular growth and morphology is dependent on numerous factors including hypoxia, pro-inflammatory cytokines and mechanical factors such as shear stress. Angiogenesis requires the specific co-ordination of complex cellular and molecular signals. The initial process involves activation and proliferation of EC and this may include sprouting of cells from an existing vessel leading to vascular branch formation (Augustin, 2001). In parallel, the connective tissue matrix becomes disrupted by matrix metalloprotineases, notably the gelatinases MMP-2 and MMP-9, allowing for EC to migrate through the matrix and form primitve angiotubes (Hinsbergh *et al.,* 2006). It is from these angiotubes that the mature vessel develops recruitment of smooth muscle cells such as pericytes provide a more permanent structure to the vessel wall. Vascular endothelial growth factor (VEGF), a main angiogenic 'on' switch is produced in response to stimulation by cytokines (interleukin (IL)-1, tumor necrosis factor (TNF)-a, transforming growth factor (TGF)-b) from perivascular cells. It would appear that the effect of several other key growth factors such as hepatocyte growth factor, epidermal growth factor, prostaglandins,

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nitric oxide and hypoxia-inducible factor 1-a may all act predominantly via upregulation of VEGF (Ispanovic *et al.,* 2006). VEGF is particularly important early in vascular morphogenesis, stimulating EC proliferation and migration and withdrawl of VEGF leads to a loss of EC and blood vessel regression. Increasing evidence, however, suggests a complex interaction between VEGF and the angiopoietin (Ang)/Tie-2 family, which is critical to new vessel structure and function. Ang1/TIE-2 binding induces stabilisation and maintenance of maturing blood vessels. In contrast, Ang2 antagonises Ang1, stimulating vascular invasion and blocking maturation/stabilisation in the presence of abundant VEGF. These vessels remain in a 'plastic' state, responsive to VEGF resulting in increased capillary diameter, remodelling and sprouting of new blood vessels (Holash *et al.,* 1999; Suri *et al.,* 1996).

VASCULAR PERMEABILITY

Vascular permeability is the mechanism by which plasma and its solutes cross the vascular barrier. It is essential for the health of normal tissues and is also an important characteristic of many disease states in which it is greatly increased. Examples are acute inflammation and pathologies associated with angiogenesis such as tumors, wounds, and chronic inflammatory diseases. Permeability is an extremely complicated process that, however defined, is affected by many different variables. These include the intrinsic properties of the different types of microvessels involved (capillaries, venules, mother vessels); the size, shape, charge and type of extravasating molecules; the anatomic pathways molecules take in crossing the endothelial cell barrier; measurement of time course for permeability and nature of vascular beds (Dvorak, 2003; Nagy *et al.,*2003; Ramesh *et al.,* 2013).

The acute inflammatory response consists of three main vascular effects: vasodilatation and increased blood flow, increased vascular permeability, and leukocytosis into the injured tissues. All three events are induced relatively quickly, and, for all three, the pattern of response is complex but consistent. The characteristic increase of vascular permeability may be monophasic, diphasic or even triphasic. These various patterns of response may still divided into three main types of responses, namely, immediate, delayed, and early. Each of which may occur alone or in various combinations. The problem of mediation, therefore, becomes one of defining the mechanisms underlying each type of response. Immediate responses are usually mediated by histamine and/or 5-hydroxytryptamine. Delayed responses seem to result from pharmacological mediation although the corresponding factors remain unidentified. Early responses seem mainly due to disruption of endothelium, to which is added a small contribution by pharmacological mediation. Evidence will be presented to support the role of kinins as mediators of the delayed response. The evidence mainly arises from recent work illustrating that histamine induces refractoriness of susceptible blood vessels and so can evoke only a short-lived response, whereas kinins induce no such refractoriness and could, therefore, mediate the relatively prolonged responses obtained in delayed-type effects (Wilhelm, 1973).

Vascular permeability often in the form of capillary permeability or microvascular permeability, characterizes the capacity of a blood vessel wall to allow for the flow of small molecules (ions, water, nutrients) or even whole cells (lymphocytes on their way to the site of inflammation) in and out of the vessel. Blood vessel walls are lined by a single layer of endothelial cells. The gaps between endothelial cells (cell junctions) are strictly regulated depending on the type and physiological state of the tissue. If filtration greatly exceeds reabsorption, the result is edema (swelling), an abnormal increase in interstitial fluid volume. Edema is not usually detectable in tissues until interstitial fluid volume has risen to 30% above normal. Edema can result from either excess filtration or inadequate reabsorption.

Two situations may cause excess filtration, one is increased capillary blood pressure causes more fluid to be filtered from capillaries and other one is increased permeability of capillaries raises interstitial fluid osmotic pressure by allowing some plasma proteins to escape. Such leakiness may be caused by the destructive effects of chemical, bacterial, thermal, or mechanical agents on capillary walls.

One situation commonly causes inadequate reabsorption is decreased concentration of plasma proteins lowers the blood colloid osmotic pressure. Inadequate synthesis or dietary intake or loss of plasma proteins is associated with liver disease, burns, malnutrition and kidney disease. (Gerard and Bryan, 2009).

In cancer research, the study of permeability of the microvasculature that surrounds tumours is of great interest as the vascular wall is a barrier of large molecules into the tumours, the vessels control the micro-environment which affects tumor progression and changes to the permeability may indicate vascular damage with drugs.

EVANS BLUE DYE

Evans Blue is an alkaline stain, so there is an affinity for the alkali in the acidic nucleus. There is a lot of acid in the nucleus with all the structural and functional material nucleic acids namely DNA and RNA. This is a "basophilic staining reaction" - basophilic meaning base loving. Sometimes, though, you see this in the cytoplasm, probably from the ribosomes on the rough endoplasmic reticulum. The ribosomes are mainly RNA, so they react the way the (acidic) nucleus does. The use of EBD as an *in vivo* marker of myofibre permeability facilitates the investigation of the effect of pathology, muscle injury, and exercise on instantaneous and/or sustained permeability of skeletal myofibres. The EBD is widely used to study blood vessel and cellular membrane permeability as it is non-toxic, it can be administered as an intravital dye and it binds to serum albumin – using this as its transporter molecule. The EBD–albumin conjugate (EBA) can be: (i) identified macroscopically by the striking blue colour within tissue (Matsuda *et al.,* 1995); (ii) observed by red auto-

fluorescence in tissue sections examined by fluorescence microscopy (Matsuda *et al.,* 1995; Brussee *et al.,* 1997; Tidball *et al.,*1999); and (iii) assessed and quantified by spectrophotometry for serum samples (Brown *et al.,* 1992,), or homogenized tissue (Oztas *et al.,* 1992). Chemical nature and properties of EBD discussed in detail in table 1.

Handling of EBD (Alois and Jan,1983; Evans blue url; pubchem url)

Toxic Effects

Toxic effects are not available for Evans Blue Dye. However, it is considered a hazardous irritant and potential carcinogen.

Acute Effects

It causes respiratory tract irritation manifestations include, wheezing, coughing, and shortness of breath. When it swallowed it causes burning in the mouth and throat. When it is exposed to eye causes irritation with watering and burning sensations.

Chronic Effects

Evans blue dye contains a component that has been reported to potentially be carcinogenic. Prolonged exposure or overexposure may cause reproductive disorders based on tests with laboratory animals.

Carcinogenic Effects

Evans blue dye contains a component that has been reported to probably be carcinogenic.

Mutagenic/ Teratogenic Effects

Evans Blue Dye is not known to be mutagenic or teratogenic.

***Systemic effects*:** Not available

***Personal Protective Equipment* (PPE)**

Wear gloves, full-length lab coat, and safety glasses with side-shields or a face shield in addition to long pants and closed-toe shoes.

Always wash hands after removing gloves following handling Evans Blue Dye.

Engineering Controls

Always handle Evans Blue inside a certified chemical fume hood or ducted bio-safety cabinet.

First Aid Procedures for EBD toxicity

Skin & Eye Exposure: Skin: remove contaminated clothing and wash gently and thoroughly with running water and non-abrasive soap. Be particularly careful to clean folds, crevices, and creases. Cover irritated skin with an emollient. If irritation persists, seek medical attention. Wash contaminated clothing before reusing. Eye: check for and remove any contact lenses. Rinse immediately with copious amounts of running water for at least 15 minutes. Do not use any ophthalmic ointments. Consult a physician promptly.

Special Handling, Storage and accidental procedure

Keep away from heat sources and strong oxidizing agents. Needles used for EBD injection will be disposed of in approved sharps containers immediately following use. Needles used for EBD injection should never be bent, sheared, or recapped. In case of eye exposure, rinse eyes for 15 minutes with plenty of water, then seek medical attention. If inhaled, attain to fresh air, seek medical attention immediately. If absorbed, through skin, immediately flush with plenty of water, remove contaminated shoes and clothing, and seek medical attention immediately.

THE ENDOTHELIN SYSTEM

Endothelins (ETs) are a family of naturally occurring peptides with well-established growth-promoting, vasoactive, and nociceptive properties that affect the function of a number of tissues and systems (Iglarz and Clozel, 2010). ETs have pathophysiological roles in pulmonary hypertension, arterial hypertension, atherosclerosis, cerebral vasospasm and inflammatory processes (Masaki, 2004; Dhaun *et al.,* 2007).
Recently, new evidence has demonstrated that endogenous ETs also play a role in articular inflammation by regulating inflammatory pain, edema formation, leukocyte influx and the production of inflammatory mediators. The present chapter attempts to provide an overview of the evidence accumulated to date, which suggests that ETs play a pivotal role in articular inflammation, and the blockade of these endogenous peptides can represent a promising therapeutic tool for the treatment of RA and other articular inflammatory diseases.

The endothelin system comprises a family of three highly conserved vasoactive peptides, which bind to two endothelin receptors (endothelin receptor types A [ETA] and B [ETB]), with differing affinities that are determined by the N-terminal domain of the peptide. ET-1 has a higher affinity than ET-2, which, in turn, has a higher affinity than ET-3. In humans, the affinity of ET-1 for the ETA receptor is 1,000-fold higher than that of ET-3 (Wagner *et al.,* 1992).

ET-1, the most prominent representative of the ET family, was first identified as a potent vasoconstrictor secreted by vascular endothelial cells. Since the initial description of ET-1, it has become evident that in addition to modulating vascular tone, ET peptides are also involved in numerous other pathophysiological processes and are produced not only by endothelial cells but by a wide variety of cells in virtually all organs (Iglarz and Clozel, 2010).

ET-1 has been demonstrated to participate in the pathogenesis of a number of diseases, such as sepsis, bronchial asthma and pulmonary hypertension (Shah, 2007). In addition to their well recognized vasoconstrictive properties, ETs play an

important role in inflammatory reactions modulating hyperalgesia, edema formation and cell migration. Considering their pro-inflammatory properties and the presence of ETs in the plasma and synovial flu from RA patients, the participation of ETs in RA is strongly indicated (Sampaio *et al.*, 2000; Zouki *et al.*, 1999).

EVALUATION OF VASCULAR PERMEABILITY TEST

Generally EBD is used to monitor vascular protein leakage. In general, to perform vascular permeability test, at the end of experiment, EBD 50 mg/kg of body weight administered via the jugular vein into the anaesthetized animal. After 4hr of EBD administration, the rats were sacrify by anesthetic ether. The specified tissue can be dissected (in case of Freund's complete adjuvant (FCA) induced rat arthritis model, anterior and posterior synovial capsules and fat pad were dissected from each knee joint). The tissues obtained were weighed and made into smaller pieces, mixing them with acetone in 1% sodium sulphite in the ratio of 7:3. The samples were shaken gently and continuously for 24 h at room temperature. Each preparation was centrifuged for 10 min at 2000 rpm and 2 ml of the supernatant was separated for measurement of absorbance at 620 nm using UV-spectrophotometer or flourimeter. The amount of dye recovered content of vascular permeability was calculated by extrapolating with standard curve prepared with different concentrations of EBD solution (Franchis *et al.*, 2004).

ROLE OF EVANS BLUE IN PHARMACOLOGICAL SCREENINGS

Here we discussed some of methods to evaluate the various biomarkers using EBD by vascular permeability test.

Performed fluorometric measurement of Evans blue in formamide extracts of rat tracheal tissue after induction of protein leakage by electrical vagus nerve stimulation and compared with the widely used colorimetric detection. In this study fluorimetric method was used because, the fluorescence method was approximately 100 × more sensitive than the colorimetric method. Evans blue fluorescence (excitation at 620 nm, emission at 680 nm) was used for microscopic investigation of cryostate sections of tracheal tissue. Extravasated Evans blue after electrical nerve stimulation was mainly found in the sub-epithelial layer of the trachea obviously bound to tissue constituents. It is suggested that Evans blue fluorescence can be applied for quantification of protein leakage as well as for tissue localization of protein leakage at the microscopic level (Alois and Jan, 1983).

The participation of histamine H_3 receptors in the regulation of skin vascular permeability changes in mast cell-deficient mice was studied. Although intradermal injection of histamine H_3 antagonists, iodophenpropit and clobenpropit, at a dose of 100 nmol/site caused significant increases in skin vascular permeability in both mast cell-deficient (WBB6F1 W/W^v) and wild-type (WBB6F1 +/+) mice, this response was significantly lower in mast cell-deficient mice than in the wild-type controls. Histamine also caused dose-related increases in skin vascular permeability in both wild-type and mast cell-deficient mice. Significant effects were observed at doses of 10 and 100 nmol/site, and no significant difference in skin vascular permeability was observed between mast cell-deficient and wild-type mice. However, histamine contents of dorsal skin in mast cell-deficient mice were significantly lower than in wild-type mice. In addition, the H_1 antagonists diphenhydramine and chlorpheniramine and the NK_1 antagonists, L-732,138 and L-733,060, were able to antagonize H_3 antagonist-induced skin vascular permeability. These results indicated that blockade of H_3 receptors by H_3 antagonists induce skin vascular permeability through mast cell-dependent mechanisms. In addition, histamine and, to a lesser extent substance P are involved in the reaction (Maria *et al.*, 2003). The intradermal injection histamine H_3 antagonists in rat skin increase in vascular permeability. Pretreatment of the recipient animals with serotonergic antagonists, including the specific 5-HT_2 receptor blocker ketanserin, potently inhibits the platelet-mediated and the 5-HT-induced vascular defect. Amine depletion of platelets or skin tissues with reserpine reduces the response to platelets. Platelet prostanoid and lipoxygenase derivatives play no major role in the vascular response to platelet. The permeability increase induced by exogenous 5-HT and by activated platelets is reduced by α_1-adrenergic stimulation with noradrenallne or phenylephrine and by β_2-stimulation with terbutaline or isoprenaline, and is potentiated by adenosine; this points to a modulation of permeability by blood flow changes and to a direct β-adrenergic effect at the endothelial cell membrane. This study demonstrates a predominant role for 5-HT in the platelet-mediated vascular permeability increase in a sensitive species like the rat (De *et al.*, 1985). Bradykinin (BK) is involved in tumor angiogenesis. To elucidate the mechanism underlying BK-induced angiogenesis, it was evaluated the roles of BK in tumor-associated vascular permeability and angiogenesis in the different phases of tumor development in mice bearing sarcoma 180 cells. The vascular permeability was significantly enhanced in the early growth phase (which peaked at day 5), and was thereafter markedly reduced. By contrast, tumor angiogenesis increased gradually over a 20-day experimental period. Oral administration of a B2 receptor antagonist, FR173657 (30 mg/kg/day), significantly suppressed the vascular permeability, but a B1 antagonist, $desArg^{10}$-Hoe140 (1mg/kg/day) did not. An immunohistochemical study revealed the presence of immunoreactive B2 receptor in the endothelial cells in the early phase, whereas B2 receptors were also observed in the stromal fibroblasts in the late phase. Furthermore, VEGF immunoreactivity was attenuated by the treatment with FR173657. Tumor angiogenesis was significantly reduced by treating the tumor tissues with FR173657 both in the early phase (days 1–6, 30 mg/kg/day, oral administration) and in the late phase (days 7-12, 30 mg/kg/day, oral administration), whereas it was inhibited by neutralization with anti-VEGF antibody (1 μg/site/day, local injection) only in the late phase. These results suggest that BK would promote angiogenesis by increasing vascular permeability in the early phase via B2 receptor in the endothelial cells and by

promoting up-regulation of VEGF via B2 receptor in the stromal fibroblasts in the late phase (Keiko *et al.,* 2002). Activation of nociceptors causes them to secrete neuropeptides.

The binding of these peptides to receptors on blood vessels causes vasodilation and increased vascular permeability that allows loss of proteins and fluid (plasma extravasation, PE); this contributes to inflammation. This study defines the relationship between electrical activation of nociceptors and PE and evaluates the time course of this response in the skin of rats. We measured the time course and extent of PE by digital imaging of changes in skin reflectance caused by leakage of EBD infused in the circulatory system before stimulation. Stimulation of the exclusively sensory saphenous nerve caused the skin to become dark blue within 2 min due to accumulation of EBD. While PE is usually measured after 5-15 min of electrical stimulation, we found that stimulation for only 1 min at 4 Hz produced maximum PE. This response was dependent on the number of electrical stimuli at least for 4 Hz and 8 Hz stimulation rates. Since accumulation of EBD in the skin is only slowly reversible, to determine the duration of enhanced vascular permeability we administered EBD at various times after electrical stimulation of the saphenous nerve. PE was only observed when EBD was infused within 5 min of electrical stimulation but could still be observed 50 min after capsaicin (1%) injection into the hind paw. These findings indicate that enhanced vascular permeability evoked by electrical stimulation persists only briefly after release of neuropeptides from nociceptors in the skin. Therefore, treatment of inflammation by blockade of neuropeptide release and receptors may be more effective than treatments aimed at epithelial gaps. We propose, in models of stimulation-induced inflammation, the use of a short stimulus train (Carmichael *et al.,* 2008). Many pathological conditions associated with inflammation are exacerbated by the peripheral release of vasoactive agents that enhance vascular permeability. The endothelium, which forms the interface between blood and tissues, controls the extravasation of macromolecules and prevents the loss of blood constituents, such as albumin and other plasma proteins.

The neuropeptide, substance P (SP), plays a significant role in altering endothelial permeability. Following activation of nociceptors, SP is released and binds to the neurokinin-1 (NK-1) receptor on blood vessels, eventually causing the formation of small gaps that allow plasma extravasation (PE) (McDonald *et al.,* 1999; Baluk *et al.,* 1997). SP also acts on mast cells and leukocytes, inducing the release of inflammatory substances such as histamine, serotonin and prostaglandins, which play a role in prolonging leakage (Jorizzo *et al.,*1983; Hartung *et al.,*1998; Filliatre *et al.,* 2001; Schmelz and Peterson, 2001).

Primary afferent terminals also contain calcitonin gene-related peptide (CGRP) (Gamse and Saria, 1985; Bartfai *et al.,* 1998), which, when released following activation, causes vasodilation and potentiates the effects of SP. CGRP does not produce PE by itself at physiological concentrations (Gamse and Saria, 1985; Brain *et al.,* 1985). Together, the actions of SP and CGRP provoke an inflammatory response known as neurogenic inflammation (NI).

A noxious stimulus activates the nociceptive afferents thereby causing depolarization and release of SP and CGRP (Bayliss, 1901; Jancso *et al.,* 1967). Although, a variety of substances are stored in primary afferent terminals that can contribute to NI, SP and CGRP are the principal mediators of NI (Garret *et al.,*1991; Xu *et al.,* 1992; Gonzalez *et al.,* 2005). Experimentally, NI can be elicited by electrical stimulation of primary afferent nerves at intensities sufficient to activate nociceptive fibers (Szolcsanyi, 1998) or by application of a variety of inflammatory irritants, such as capsaicin or mustard oil (Xu *et al.,* 1992; Gonzalez *et al.,* 2005) which activates nociceptors.

Vascular permeability was assessed following induction of NI. Spectrophotometric analysis of EBD content in tissues and staining of endothelial gaps at various time points after inducing PE have been used to describe the duration of vascular permeability increases in rat trachea due to systemic injection of SP or stimulation of the vagus nerve (McDonald *et al.,* 1999). List of some of pharmacological activities by assessment of vascular permeability are discussed in table 2.

Table 1: Physical & Chemical Properties of EBD.

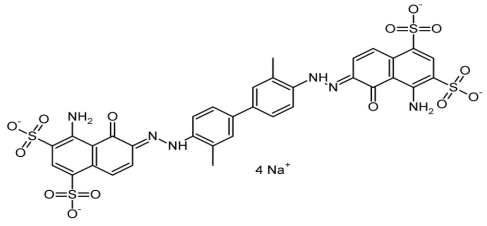
Properties	**Description**
Class	Chemical dye, Irritant and Carcinogen
Structural Formula	
Molecular Formula	$C_{34}H_{24}N_6O_{14}S_4Na_4$
Molecular weight	960.82 gm
IUPAC name	6,6-[(3,3'-Dimethyl[1,1'-biphenyl]-4,4'-diyl)bis[4-amino-5-hydroxy-1,3-naphthalenedisulfonic acid] tetra sodium salt
Synonyms	Direct Blue 53, tetrasodium salt, Diamine Sky Blue FF, Diazobleu, Diazol Pure Blue, Evan's Blue sodium, Geigy Blue 536, T-1824, Azovan Blue, Chlorazol Sky Blue FF.
Physical form	Solid, powder
Appearance	Dark brown powder
Solubility	Aqueous
Volatility	Stable under recommended storage conditions. May be combustible at high temperatures
Storage	Store in a cool and dry place

Table 2: List of some of pharmacological activities by assessment of vascular permeability.

Pharmacological activity	Animal/ Tissue	Experimental Method	Assessment by the study
Identification of permeable skeletal myofibres as damaged as results in muscular dystrophy	*mdx* mice	Three mouse models of experimental injury and repair -cut injury, whole muscle grafts, and exercise-induced muscle damage	Compared intravenous to intraperitoneal injection; tissue fixation; volume of EBD; time of availability in tissue; and persistence after injection in *mdx* mice (with endogenous muscle damage) and control mice (Kohji *et al.*, 2012).
Screening of anti-inflammatory drugs and food components	Rodent animals	Studies of paw edema, ear edema, and skin reaction, the cotton pellet implant, rat air-pouch inflammation model, and rat pleurisy model	Measuring VP as an index of inflammatory reactions (Kohji *et al.*, 2012).
Tracheal endothelial activity	Rat/Trachea	Electrical vagus nerve stimulation	Quantification of protein leakage as well as for tissue localization of protein leakage at the microscopic level (Evans blue url).
Action of histamine on histamine receptors of mast cells	Mice/skin	Intradermal injection of histamine H_3 antagonists	Participation of histamine H_3 receptors in the regulation of skin vascular permeability changes in mast cell-deficient mice (Maria *et al.*, 2003).
Role of 5-HT in platelet aggregation	Rat/skin	Intradermal injection of serotonin and serotonergic antagonists	Role for 5-HT in the platelet-mediated vascular permeability (De *et al.*, 1985).
Role of bradykinin involved in tumour angiogenesis	mice	Tumor development in mice bearing sarcoma 180 cells	Bradykinin would promote angiogenesis by increasing vascular permeability in the early phase via B2 receptor in the endothelial cells and by promoting up-regulation of VEGF via B2 receptor in the stromal fibroblasts in the late phase (Keiko *et al.*, 2002).
Activation of nociceptors causes them to secrete neuropeptides	Rat/skin	Electrical stimulation of about 1 min at 4 Hz produced maximum plasma extravasation	This study defines the relationship between electrical activation of nociceptors and plasma extravasation & evaluates the time course of this response in the skin of rats (Carmichael *et al.*,2008).
Study of cellular & molecular basis of angiogenesis in wound healing by non-invasive strategy to assess VP of the wound capillary bed.	Mice	Excisional wound model in mice	To quantify the kinetics of re-vascularization. To assess changes in VP of wound bed compared to normal adjacent skin. To establish a non-invasive & quantitative assay for measurement of VP to facilitate the rapid & reproducible characterization of vascular integrity (Ashkaun *et al.*, 2009).
To study tissue drug distribution kinetics with minimal disturbance of the animal physiology	Sprague- Dawley rats	Single-pass in situ rat hindlimb preparation	Characterization of events occurring in the tissue beds immediately and soon after drug administration, with minimal disturbance of the animal physiology (Raquel *et al.*, 2000)
Akt signaling is both necessary and sufficient for VP in an *in vivo* model	Hairless albino guinea pigs	Miles assay of VP in guinea pig skin was modified to accommodate adenovirus-mediated transfer	To assess the role of *Akt* signaling in VP (Isabelle *et al.*, 2002)
Study of synovial infusion of Bradykinin, prostaglandin E1 and histamine on synovial VP	Mongrel dogs	Exudation of a radiolabelled dextran marker from the circulation into the synovial cavity	The effects of infusion of prostaglandin E1 combined with either bradykinin or histamine were greater than mere summation (Grennan Mitchell, 1977)
Study of quantitation of hemorrhage, leukocyte infiltration, Cytokines, and VP	Albino rabbits	Inflammation is induced in the retina by injection of 300 U of rh IL-1b into the vitreous of the eye directly over the optic nerve head	Local administration of TGFβ serves to suppress IL-1-induced hemorrhage & leukocyte infiltration into retina while having no effect on IL-1-induced vascular permeability, & that TGFβ treatment alone also serves to increase VP. (59)
Vasoactive mediators such as histamine, serotonin, bradykinin, arachidonic acid metabolites and PAF to VP changes	Sprague-Dawley rats	EBD extravasation in trachea, thymus, seminal vesicle and stomach, rat model of acute endotoxemia.	The LPS-induced increase in vascular permeability is mediated by secondary vasoactive mediators among which PAF plays a pivotal role, although their relative contribution may vary from tissue to tissue (60).
Study of increased VP and oedema induced by endothelins by intrathecal route.	Wistar rats	Determination of VP by Evans blue dye method	To compare i.t. & i.v. effects of endothelin-1 & endothelin-3 on increased VP in several peripheral organs & on pulmonary oedema in the conscious rat. To ascertain the participation of intraspinal endothelin ET_A receptors in the pulmonary hypermeability & oedema induced by endothelin-1. To examine contribution of Symp NS & other endogenous putative mediators in this central action of endothelin-1 on the lung (Philippe and Rejean, 1998).
Study of role of bradykinin and PAF.	Kininogen-defiecient and Brown-Norway rats	Endotoxin induced acute VP in Kininogen- defiecient and Brown-Norway rats	The study assessed that; the bradykinin is one of the major mediators in the endotoxin induced acute VP increase in rat skin in addition to PAF (Akinori *et al.*, 1995).
Study of effect of melatonin on the inflammatory increase in VP	Wistar rats	VP stimulated by a nonspecific pro-inflammatory agent (carrageenan), by drugs that disrupt endothelial cells junction (histamine, serotonin & bradykinin) or drugs that promote neutrophil recruitment (LT B_4 & fMLP) in rat dorsal skin	The study suggests that VP reduction induced by local melatonin injection is mediated by a reduction of endothelial cells ability to interact with neutrophils (Celina *et al.*, 2006).

*5-HT, 5-Hydroxy tryptamine; VP, Vascular permeability; LPS, lipopolysaccharide; PAF, platelet activating factor; TGF β , transforming growth factor β; IL, Interlukin; i.t., intrathecal; i.v., intravenous; Symp.NS, sympathetic nervous system; fMLP, N-formyl-methionyl-leucyl-phenylalanine; LT B_4, leukotriene B_4.

CONCLUSION

In experiments studying of inflammation, various rodent animal models are used for the screening of anti-inflammatory drugs. In studies of paw edema, ear edema, skin reaction, the cotton pellet implant inflammation model, rat air-pouch inflammation model Freund's complete adjunct (FCA), and rat pleurisy model are usually used for measuring vascular permeability as an index of inflammatory reaction. Vascular permeability by any measure is dramatically increased in acute and chronic inflammation, cancer, wound healing and other pathological conditions. This review discussed the role of vascular permeability in physiological and pathological conditions along with the pharmacological approach for the assessment of vascular permeability in various disorders using EBD.

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