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Letter to the Editor

INHIBITORY EFFECT OF EPINEPHRINE AND NOREPINEPHRINE ON CARRAGEENIN-INDUCED PLANTAR EDEMA IN THE RAT

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KEYWORDS: *carrageenin, epinephrine, norepinephrine, edema, inflammation, catecholamine*

INTRODUCTION

In this short paper we report that carrageenin-induced plantar edema in the rat paw is inhibited by epinephrine or norepinephrine. When these catecholamines are administered in combination with carrageenin, they inhibit the inflammatory response in the following three hours, at which point the effects gradually subside.

DISCUSSION

Inflammation is a typical immune mechanism, which is established in the presence of chemical, physical, or biological pathogens with tissue and vascular damage, but also some self-type of immune reactions can cause inflammation and cell death (1). Irritating foreign substances injected into tissue can cause inflammation and edema (2). Carrageenin is a complex molecule extracted from red algae (Chondrus crispus and Gigartina stellata) which contains two polysaccharides (3). When carrageenin is injected into the plantar rat paw, it causes an increase in the levels of fatty acids, including hydroxyeicosatetraenoic acid (5-HETE), which mediates inflammation (4). Tissue edema is a clinical manifestation with abnormal accumulation of lymphatic fluid at the inflamed site, characterized by swelling, reduced function, and often pain (5).

Catecholamines are water-soluble chemical compounds (hormones) released by the adrenal glands under conditions of stress, that circulate in the blood bound to plasma proteins (6). The most well-studied catecholamines are epinephrine (adrenaline) and norepinephrine (noradrenaline) which derive from dopamine, which in turn derives from tyrosine (7) (Fig.1).

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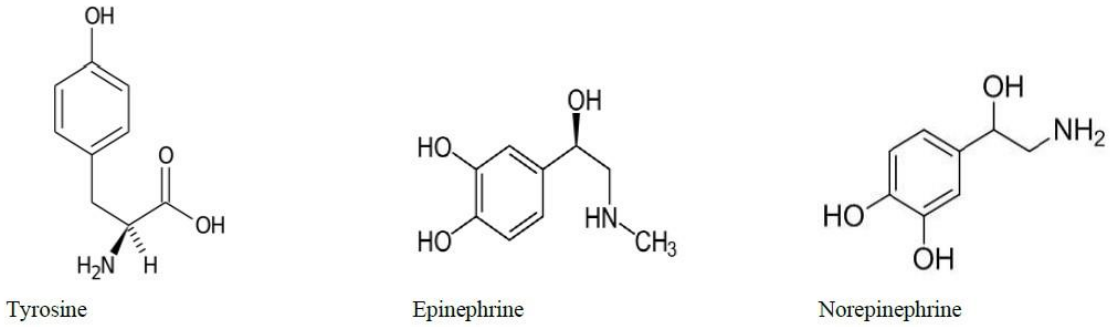

Fig. 1. *The chemical structures of epinephrine (adrenaline), norepinephrine (noradrenaline), and tyrosine.*

Epinephrine and norepinephrine, secreted by the adrenal gland and local sympathetic neurons, regulate the immune system by activating the β2-adrenergic receptor on immune cells, producing cell migration and cytokine secretion (Adrenergic regulation of immune cell function and inflammation (8). The aim of this study is to investigate whether these two catecholamines inhibit carrageenin-induced inflammation.

In this study, carrageenin is injected alone intraplantar and in combination with the epinephrine or norepinephrine in order to inhibit plantar edema (9). Male Wistar rats (100-180 g) were used and anti-edematous activity of epinephrine and norepinephrine by simultaneous injection with carrageenin was investigated and after 3 h the edema was measured. Paw edema was induced by intraplantar injection of 0.1 ml of 1% solution of carrageenin. The volume was measured in a nanogram of the rat paw.

Using this experimental model, we report the inhibitory action of epinephrine and norepinephrine on carrageenin-induced plantar edema in rats. The inhibitory action of these two catecholamines is short-lived, starting immediately after injection and reaching the plateau at 3 h after treatment, an effect that gradually decreased. The efficacy of epinephrine and norepinephrine is high except for dopamine, for which higher doses are needed (data not shown). The inhibitory effect of norepinephrine was slightly less pronounced than that of epinephrine.

The results obtained demonstrate that carrageenin causes a strong inflammatory effect (Table I) correlating to the amount of edema on the sole of the rat's foot (10). When epinephrine and norepinephrine are administered with carrageenin, the inflammatory effect is strongly inhibited. This inhibition appeared only for a short period of time (3 h) and then gradually decreased over time.

Table I. *In this table we show that carrageenin causes severe edema in the plantar of the rat paw and when it is given in combination with epinephrine or norepinephrine, strong inhibition is obtained for 3 hours. The experiment was performed three times in triplicate.*

Treatment	ng/paw	nmol/paw
Carrageenin 1mg (0.1 ml of 1% solution)	450 (+/- 90)	2.35
Carrageenin 1mg (saline solution)	430 (+/- 70)	2.01
Carrageenin + Epinephrine 10^{-7}M	44 (+/- 12)	0.13
Carrageenin + Norepinephrine 10^{-7}M	75 (+/- 27)	0.44

Epinephrine and norepinephrine are endogenous anti-inflammatory catecholamines which are released after an inflammatory process as a defensive response of the body to try to restore the physiological tissue state (11).

In this short article, the powerful inflammatory effect of carrageenin is confirmed, and we demonstrate that when this proinflammatory compound is administered in combination with epinephrine or norepinephrine, inflammation is inhibited in the first phase of induction, an effect that diminishes over time until it disappears (12). In addition, in our experiments, epinephrine and norepinephrine were increased in the urine of the rats treated with carrageenin induced edema, an effect that may be species specific and thus not detectable in humans (13). It has been reported that in humans, inflammatory stimuli such as ultraviolet radiation causes a decrease in epinephrine or norepinephrine (Nicotinic acetylcholine receptors in glucose homeostasis: the acute hyperglycemic and chronic insulin-sensitive effects of nicotine suggest dual opposing roles of the receptors in male mice (14,15).

CONCLUSIONS

In conclusion, epinephrine or norepinephrine can inhibit the edematous inflammatory response of carrageenin in the first 3 hours after the combined treatment, followed by a gradual decrease in inhibition until it disappears (16).

Conflict of interest

The author declares that they have no conflict of interest.

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HAEMORRHAGE AND STROKE: IMMUNITY AND INFLAMMATION

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ABSTRACT

In ischemic stroke, microglia and the blood-brain barrier (BBB) are activated. This phenomenon leads to the activation of immune cells such as monocytes/macrophages, lymphocytes, mast cells (MCs), and neutrophils. The recruitment of these cells to the site of tissue damage leads to the release of pro-inflammatory cytokines, while in normal tissue, these cells are beneficial for the brain's physiological activity. The BBB protects brain tissue and can be disrupted after stroke, an effect mediated by pro-inflammatory cytokines. However, the relationship between the BBB, microglia and pro-inflammatory compounds in hemorrhagic stroke still needs to be determined.

KEYWORDS: *haemorrhage, stroke, immunity, inflammation, cytokine, microglia, BBB*

INTRODUCTION

Ischemic stroke is characterized by decreased blood flow in the brain tissue and the activation of microglia, which are macrophage-like immune cells resident in the central nervous system (CNS) (1). Microglia are brain cells adjacent to the microvasculature in the basal ganglia, substantia nigra, and hippocampus. These cells, like activated peripheral blood monocytes, produce inflammatory cytokines and chemokines but can also release activated mast cells (MCs), producing proteases and reactive oxygen species (ROS) (2). The release of these pro-inflammatory compounds in stroke recruits immune cells to the site and exacerbates brain damage, resulting in neuronal destruction (3). In addition, the recruitment of immune cells from the bloodstream into the damaged CNS leads to the upregulation of adhesion molecules and cytokine/chemokine receptors with traumatic brain injury (4).

Conversely, the arrival of immune cells occludes vessels after ischemia, causing neuroprotection (5). These cellular dynamics are still unclear and can be the subject of future studies. Ischemic brain tissue produces damage-associated molecular patterns (DAMPs) with immune and inflammatory responses contributing to stroke (6).

DISCUSSION

Immune cells, including T cells, macrophages, microglia, MCs, and neutrophils, are present after stroke and cause damage by worsening ischemic brain damage (7). Cytokines such as IL-1 and TNF are upregulated in stroke and participate in brain inflammation. The secretion of pro-inflammatory cytokines, including IL-1, IL-6, and tumor necrosis factor (TNF) produced by macrophages and microglia, causes brain inflammation (8).

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Microglia, which mediate their effect through the CD36 receptor and other molecules, also produce high levels of ROS, which together with pro-inflammatory cytokines, characterize ischemic stroke. These reactions mediate the acute phase of stroke (9). However, it is not yet clear whether microglia influence neurogenesis (10). Non-activated microglia protect the CNS, but on the contrary, after activation, they polarize in M1 cells through TLR4, interferon gamma (IFN-γ) or granulocyte-monocyte-colony stimulating factor (GM-CSF) receptors, with generation of pro-inflammatory cytokines and arachidonic acid products (11) (Fig.1).

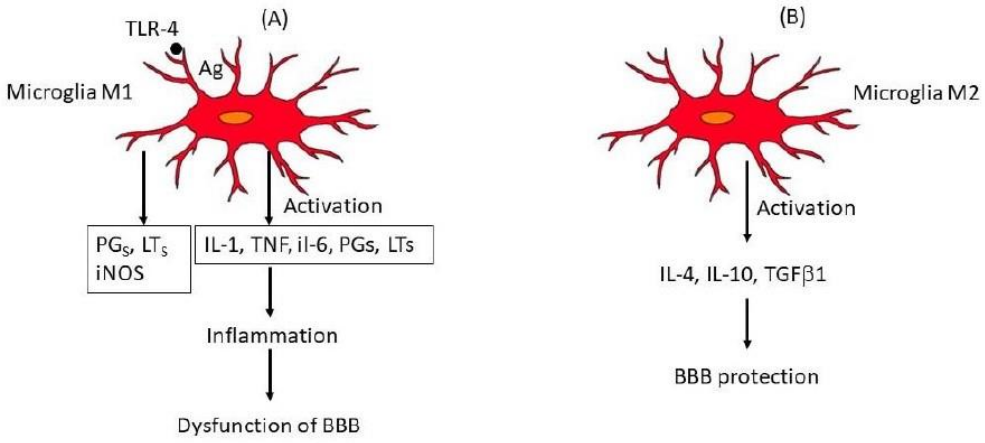

Fig. 1. *In panel A, we show that the antigen binds TLR4 in Microglia M1 which activates the generation of prostaglandins (PGs) leukotrienes (LTs), and Inducible Nitric Oxide Synthase (iNOS) causing inflammation and dysfunction in the Blood-Brain Barrier (BBB). In panel B, the activation of microglia (M2) leads to secretion of anti-inflammatory cytokines which protect the BBB. PGs: Prostaglandins; LTs: Leukotrienes; TLR: Toll-like receptor.*

These effects cause a breakdown of the BBB with brain damage. The M2 microglia cells, producing anti-inflammatory cytokines such as IL-10, IL-4, TGFβ1 and probably IL-37 and IL-38, tend to dump inflammation with the benefit of the brain system (12) (Table I). The disruption of the BBB can lead to the activation of microglia which are physiologically responsible for the protection and repair of the CNS. The production of IL-10 by the M2 microglia phenotype allows the recruitment of Treg cells, with increased expression of CD86 and MHC-II, counteracting inflammation (13).

Table I. *Activation of microglia in response to pathological stressor.*

Pro-inflammatory receptors:	TLR4, IFN-γR, GM-CSFR
Compounds:	TNF, IL-1, IL-6, CCL2, XCXL10, Inos
Anti-inflammatory receptors:	IL-4R, IL-10R, VEGFR2,
Compounds:	IL-10, TGF-β1, IL-37, IL-38

We know that immunological processes contribute to hypertensive phenomena. In particular, activated T cells and macrophages arrive in abundance in the perivascular regions of the arteries and release cytokines such as IL-1, TNF, IL-6, IL-17, and IFN-γ, causing vascular resistance and renal fibrosis (14). By causing inflammation, both the innate and adaptive immune systems can provoke damage and malfunction of the organs with the possible consequence of cerebral stroke (15). Microglia, astrocytes, and oligodendrocytes are part of the brain system and play an important role in the formation and healing of ischemic stroke. Thus, they can mediate both harmful and healing effects (16). Oligodendrocyte antigens implicated in neuronal inflammation activate T lymphocytes, involved in the tissue repair process. By producing inflammatory cytokines, active microglia damage the CNS, but in post-ischemic stroke they can generate anti-inflammatory cytokines such as IL-10, IL-37, and IL-38, which regulate the immune response with benefits and recovery of the tissue pathological state (17).

Microglial cells crosstalk with endothelial cells (ECs) contributing to the formation and functioning of the BBB. The CNS is separated from the peripheral circulatory system through the BBB which is composed of cells and biological reactions (18). The BBB, where ECs play a crucial role, protects brain tissue from external insults and keeps the neuronal

system safe. Therefore, the BBB is important for the protection of the CNS and the proper functioning of neurons (19). The contact of the ECs of the CNS with other cells such as pericytes and astrocytes, constitutes the BBB and is critical for its efficient functioning. The BBB, with its vasculature, restricts the trafficking of immune cells in the brain, an effect that is impaired when the BBB is disrupted (20). ECs, together with neurons and microglia, form part of the BBB which, when disrupted or dysregulated, leads to the generation of neurological diseases (21). Activation of ECs in brain tissue induces expression of adhesion molecules ICAM-1 and VCAM-1. ICAM-1 and VCAM-1, and other adhesion molecules such as PECAM-1, participate in the migration of immune cells and, particularly, of CD4+ T cells (22).

Through the interaction with an integrin, the activated CD4+ T cells contribute to the inflammation by crossing the basement membrane of the ECs and in doing so, they can reach the brain tissue (23). The increase in expression of metalloproteins in the inflammatory process contributes to the migration of CD4+ T lymphocytes and neutrophils into the CNS with the secretion of cytokines and chemokines and damage to the glial system. In these biological dynamics, blood vessels play a crucial role in initiating CNS pathologies, including the inflammation that often accompanies these neuropathies such as ischemic stroke (24).

CONCLUSIONS

Therefore, resting microglia cells help to protect the brain tissue, however when they are activated, they can be detrimental to the CNS and may disrupt the BBB, mediating ischemic stroke and hemorrhage (25).

The study of cellular functioning in the BBB allows for better identification of new diagnostic and therapeutic elements which are very important in this field of medicine, where many points are still waiting to be clarified. After a stroke, there can be a loss of BBB that is measurable by various methods such as magnetic resonance imaging, computed tomography, etc. (26). Accurate and early examination with these methodologies can prevent intracerebral hemorrhage in individuals presenting with an acute ischemic stroke. Failure of the BBB leads to an accumulation of immune cells in the brain with the secretion of numerous cytokines and growth factors such as VEGF, which are instrumental in causing vascular permeability and neuroinflammation (27).

Thus, after a stroke, the BBB breaks down and fails to function, resulting in the involvement of immune cells that contribute to inflammation and vascular damage. However, more studies are needed to clarify the exact role of pro and anti-inflammatory cytokines in stroke and haemorrhage (28).

Conflict of interest

The author declares that they have no conflict of interest.

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EXPERIMENTAL STUDY ON VASOCONSTRICTION AND INFLAMMATION: ROLE OF LIPOXYGENASE PRODUCTS

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ABSTRACT

A sufficient blood supply is vital for the physiological functioning of the brain, and a lack of cerebral blood flow leads to neurodegenerative diseases. Immune cells defend the brain system, but they can be protagonists of inflammatory processes in pathological cases. Vasoconstriction and inflammation are pathological elements of the brain. Here, we study the pathogenetic mechanisms of vasoconstriction and inflammation in a rodent model in relation to arachidonic acid compounds. Experimentally induced inflammation was treated with various lipoxygenase and cyclooxygenase inhibitors. Lipoxygenase was inhibited by specific compounds but not by cyclooxygenase inhibitors, although both were anti-inflammatory.

KEYWORDS: *lipoxygenase, arachidonic acid, leukotriene, hydroxyeicosatetraenoic acid, leukocyte, SRS-A*

INTRODUCTION

The brain requires a sufficient blood supply for physiological functioning. A deficiency of cerebral blood flow leads to neurodegenerative diseases (1). In recent years, the importance of lipoxygenase as a key enzyme in inflammatory reactions and allergic processes has been increasingly recognized. The metabolites of arachidonic acid generated after stimulation of the lipoxygenase pathway (2), e.g., the hydroxy fatty acids and the leukotrienes, can regulate both cellular and humoral components of inflammatory and allergic reactions (6). In the first figure, some activities of lipoxygenase products are shown (Fig.1).

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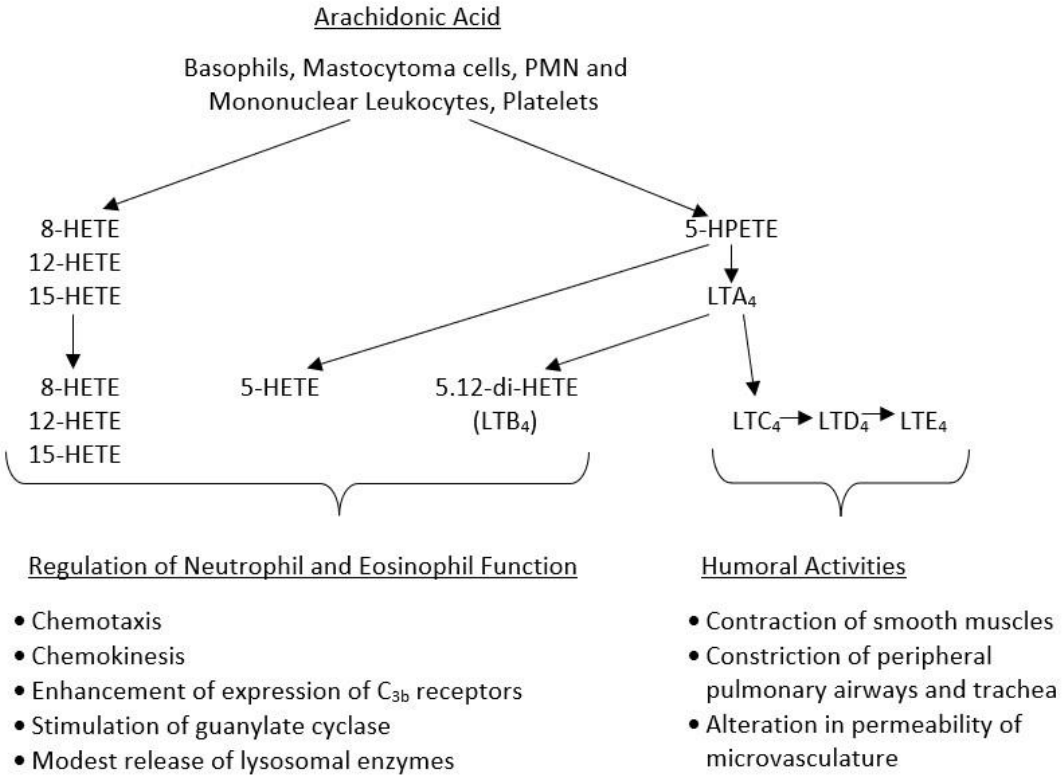

Fig. 1. *Generation and biological properties of products of arachidonic acid. HPETE = hydroperoxyeicosatetraenoic acid, LT = leukotriene.*

The hydroxyeicosatetraenoic acids (HETEs) possess leukotactic and leukinetic activities in which the potencies differ from the kind of metabolite (3). Leukotriene B4 is the most potent chemotactic factor for polymorphonuclear leukocytes. Vascular effects of this metabolite have also been observed due to increased permeability (4).

The stimulation of the leukocyte migration and the vascular effects are reactions which are important for the responses during inflammatory processes (5). Remarkably, some lipoxygenase products have modulating influences on the immune system (6). Thus, the expression of C3b receptors is enhanced, and various lipoxygenase products induce T-suppressor cells. Another important action for inflammatory and allergic reactions is the release of lysosomal enzymes (7).

The mechanism of action of the lipoxygenase products is possibly mediated via stimulating the guanylate cyclase and increasing the cGMP level (8). The leukotrienes C4, D4, and E4, components of SRS-A, are mediators released mainly during allergic reactions while also having been reported to play a role in inflammation (9). They have humoral activities and contract specific smooth muscles, e.g., ileum, peripheral pulmonary airways, and trachea. In addition, these compounds increase vascular permeability, leading to plasma exudation (10,11).

Due to the multiple actions of the lipoxygenase products, potent lipoxygenase inhibitors with favorable pharmacokinetics should, therefore, influence inflammatory and allergic reactions. Combining such agents with antagonists of other mediators of inflammation and allergic reactions could lead to more successful treatment (12).

The study of the pharmacological properties of drugs influencing inflammation and allergy is complicated by several different mediators that can be released during these reactions (13). It is further complicated by the fact that some of these mediators can have opposite effects and by the variety of animal models employed, some of which may not simulate the pathological situation in man (14).

An optimal test hierarchy should include investigating drugs' influence on the isolated enzyme, and such models in which mediators synthesized by the lipoxygenase are important for the pathological process (15).

We tested some compounds (Table I) to inhibit the reticulocyte lipoxygenase from rabbit and soybean lipoxygenase 1.

Table I. *Investigated compounds.*

BW 755 C	Indomethacin
Nordihydroguaiaretic acid (NDGA)	Acetylsalicylic acid (ASA)
Salicylhydroxamic acid (SHAM)	Phenylbutazone
FPL 55712	Noradrenaline
Propyl gallate	Adrenaline
Benoxaprofen	Isoprenaline
Diclofenac -Na	H 252
M 516	

The reticulocyte lipoxygenase converts arachidonic acid into the major product 15-HETE, and 12-HETE as the minor component (16), and the soybean lipoxygenase-1 forms 15-HPETE from arachidonic acid (17).

The anti-inflammatory and antiallergic activities of the compounds have been investigated by using the carrageenin oedema of rat paw as a model of acute inflammation, the adjuvant arthritis of the rat as a model of chronic inflammation, and the active anaphylactic oedema of rat paw as well as the active cutaneous anaphylaxis of the rat, as allergic models (18).

MATERIALS AND METHODS

Reticulocyte lipoxygenase was isolated from the defibrinated blood of rabbits and made anaemic by repeated bleeding or treatment with phenylhydrazine. The purification procedure involved ammonium sulfate precipitation, anion exchange chromatography on DEAE-Sephadex A-50, and isoelectric focusing in ampholine, pH 5 to 7.

Soybean lipoxygenase was obtained from Boehringer Mannheim GmbH (FRG). The activity of the lipoxygenase was measured polarographically through an oxygen electrode. The reaction mixture contained 0.53 mM linoleic acid, 0.2% sodium cholate, and 5% ethanol in 0.1 M potassium phosphate buffer, pH 7.4 and 9.0, respectively, and the final volume was 2.0 ml. Inhibitors were preincubated with the enzyme for 10 min. The assays were performed at 20° C.

Carrageenin oedema of the rat paw was induced in female Wistar rats by subplantar injection of 0.1 ml of a 1% carrageenin solution. The drugs were given orally and simultaneously with the carrageenin injection. Adjuvant arthritis was induced in female Wistar rats by subplantar injection of 0.1 ml Freund's complete adjuvant (0.5% suspension of heat-killed mycobacterium. in paraffinic perliquidum). The drugs were given orally once a day.

For producing the anaphylactic reactions, female Wistar rats were sensitized by i.m. injecting 0.2 ml of a 0.5% solution of bovine serum albumin in Bordetella pertussis vaccine containing $2x10^{10}$ bacteria. Two weeks later, the animals were challenged by subplantar injection of 500 μg bovine serum albumin in 0.1 ml 0.9% NaCl solution for producing the active anaphylactic oedema and by intracutaneous injection of 0.5 μg bovine serum albumin in 0.05 ml 0.9% NaCl solution for performing active cutaneous anaphylaxis, respectively. The drugs were administered orally 1 hour before the provocation of the edema and intraperitoneally 20 minutes before the cutaneous anaphylaxis, respectively.

RESULTS AND DISCUSSION

Generally, soybean lipoxygenase seems more insensitive to the inhibitors than reticulocyte lipoxygenase (19) (Table II). Therefore, the most potent inhibitors are BW 755 C, inhibiting cyclooxygenase (15), NDGA, SHAM, and propyl gallate.

Table II. *Inhibition of the activity of the reticulocyte lipoxygenase and soybean lipoxygenase-1.*

Compound	Inhibitory potency Reticulocyte lipoxygenase	Soybean lipoxygenase-1
BW 755 C	$IC_{50}= 3x10^{-6}$ M/l	$IC_{50}= 1.7x10^{-4}$ M/l
NDGA	$IC_{50}= 4x10^{-6}$ M/l	$IC_{50}= 2x10^{-5}$ M/l
SHAM	$IC_{50}= 5.6x10^{-5}$ M/l	$IC_{50}= 4.5x10^{-4}$ M/l
FPL 55712	$IC_{50}= 4x10^{-4}$ M/l	10^{-3}M/l 39% inhib.
Propyl gallate	$IC_{50}= 4x10^{-5}$ M/l	$IC_{50}= 9.4x10^{-5}$ M/l
Benoxaprofen	10^{-3}M/l 50% inhib.	10^{-3}M/l no inhib.
Diclofenac	10^{-3}M/l 53% inhib.	10^{-3}M/l no inhib.
Indomethacin	10^{-3}M/l 51% inhib.	10^{-3}M/l no inhib.
ASA	10^{-3}M/l no inhib.	10^{-3}M/l no inhib.
Phenylbutazone	10^{-3}M/l no inhib.	10^{-3}M/l no inhib.
Adrenaline	10^{-3}M/l no inhib.	10^{-3}M/l no inhib.
Noradrenaline	10^{-3}M/l 27% inhib.	10^{-3}M/l no inhib.
Isoprenaline	10^{-3}M/l no inhib.	10^{-3}M/l no inhib.
H 252	10^{-3}M/l no inhib.	10^{-3}M/l no inhib.
M 516	$IC_{50}= 6x10^{-5}$ M/l	$IC_{50}= 5.4x10^{-4}$ M/l

Remarkably, the SRS-A antagonist FPL 55712 inhibits the lipoxygenase activity, too and does not only display its antiallergic activity by antagonising the actions of the leukotrienes at the receptor site. The antiallergic and anti-inflammatory agent benoxaprofen blocks the lipoxygenase activity very weakly in our system (20). This drug selectively inhibits the 5-lipoxygenase, which is responsible for the biosynthesis of the precursors of LTC_4, D_4, and E_4 (21).

Of the anti-inflammatory drugs, only diclofenac and indomethacin exert weak inhibitory activities against the reticulocyte lipoxygenase. Catecholamines inhibit inflammatory responses (22) but do not affect lipoxygenase activity. The compound M 516 is shown to be a strong, selective inhibitor of lipoxygenase because it does not inhibit cyclooxygenase (23).

In investigating the compounds in the *in vivo* models, BW 755 C inhibits the carrageenin oedema after systemic and local administration (Tables III, IV).

Table III. *Inhibition of the carrageenin edema of rat paw ($^{++}$);($^{+}$) significantly different from control group ($p<0.01$; $p<0.05$) according to Student's t-test.*

Compound Dose/Administr.	% Inhibition 0.5 h	1 h	2 h	3 h	4 h	5 h
BW 755 C 50 mg/kg p.o.		35^{++}	37^{++}	62^{++}	62^{++}	60^{++}
BW 755 C 1 mg local.		29^{+}	33^{+}	21^{+}	-20	-10
NDGA 200 mg/kg p.o.		0	0	0	0	0
SHAM 200 mg/kg p.o.		9	0	0	0	7
FPL 55712 0.2 mg local.	45^{++}	25	-37	-4	-7	5
Propyl gallate 250 mg/kg p.o.				39^{++}		19
Propyl gallate 1 mg local.			0	20		9
Benoxaprofen 20 mg/kg p.o.		41^{+}	35^{+}	23	16	19
Diclofenac 2.5 mg/kg p.o.				49^{++}		22
Indomethacin 0.1 mg/kg p.o.			35^{++}	41^{++}		34^{++}
Indomethacin 0.1 mg local.			38^{++}	49^{++}		45^{++}
ASA 250 mg/kg p.o.				53^{++}	57^{++}	53^{++}
Phenylbutazone 100 mg/kg p.o.				57^{++}	62^{++}	54^{++}
Phenylbutazone 0.5 mg local.		38^{+}	57^{+}	45^{+}	38^{+}	22^{+}
Adrenaline 0.01 ug local.	49^{++}	54^{++}	7	9		17
Noradrenaline 0.01 ug local.	27^{+}	18	13	4		6
Isoprenaline 0.01 ug local.	29^{+}	36^{+}	10	7		7
H 252 65 mg/kg p.o.			58^{++}	50^{++}	26	7
M 516 68 mg/kg p.o.			41^{+}	45^{+}	45^{+}	34
M 516 1 mg local.		37^{+}	49^{+}	0	-18	2

Table IV. *Inhibition of the adjuvant arthritis of rat ($^{++}$);($^{+}$) significantly different from the control group ($p<0.01$; $p<0.05$) according to Student's t-test.*

Compound Dose/Administr.	% Inhibition 2 d	4 d	16 d
BW 755 C 50 mg/kg p.o.	23^{+}	20^{+}	
Propyl gallate 250 mg/kg p.o.	2	6	0
Benoxaprofen 20 mg/kg p.o.	33^{+}	35^{+}	
Diclofenac 2.5 mg/kg p.o.	41^{++}	51^{++}	52^{++}
Indomethacin 1 mg/kg p.o.	25^{+}	42^{++}	14^{+}
ASA 250 mg/kg p.o.		44^{++}	49^{++}
Phenylbutazone 100 mg/kg p.o.		52^{++}	59^{++}
Adrenaline 0.25 mg/kg s.c.		9	0
M 516 68 mg/kg p.o.	0	0	

Strong inhibitory properties on the anaphylactic models are also evident, as shown in Tables V and VI (Tables V, VI). These results conclude that there is the best correlation between *in vitro* and *in vivo* activities. However, the *in vivo* effects can also be caused by inhibiting cyclooxygenase.

Table V. *Inhibition of the active anaphylactic oedema of rat paw ($^{+}$); significantly different from the control group ($p<0.05$) according to Student's t-test.*

Compound Dose/Administr.	% Inhibition 0.5 h	1 h	2 h	3 h	4 h	5 h
BW 755 C 50 mg/kg p.o.	27	29	40^{+}	38^{+}	34^{+}	41^{+}
Benoxaprofen 50 mg/kg p.o.	42^{+}	47^{+}	51^{+}	38^{+}	54^{+}	52^{+}
Diclofenac 3 mg/kg p.o.	-7	0	15	20	14	23
Indomethacin 5 mg/kg p.o.	14	16	25	20	25	28
ASA 200 mg/kg p.o.	12	32	35^{+}	27	52^{+}	31
Phenylbutazone 30 mg/kg p.o.	18	7	2	18	5	27
H 252 100 mg/kg p.o.	14	27	13	4	6	8
M 516 100 mg/kg p.o.	6	3	24	2	-3	1

Table VI. *Inhibition of the active cutaneous anaphylaxis of the rat (+); significantly different from the control group (p<0.05) according to Student's t-test.*

Compound Dose/Administr.	% Inhibition
BW 755 C 50 mg/kg i.p.	43+
Benoxaprofen 50 mg/kg i.p.	48+
Diclofenac 5 mg/kg i.p.	6
Indomethacin 5 mg/kg i.p.	22
Phenylbutazone 30 mg/kg i.p.	12
ASA 200 mg/kg i.p.	27
H 252 100 mg/kg i.p.	22
M 516 100 mg/kg i.p.	18

The antioxidant propyl gallate inhibits carrageenin oedema only after oral administration at relatively high doses. Therefore, it does not affect adjuvant arthritis (24).

SHAM and NDGA show no influence on carrageenin oedema. Despite the suitable inhibitory activities on the lipoxygenase, there is no correlation with the *in vivo* properties of the propyl gallate, SHAM, and NDGA compounds. The cause of this infectivity could be insufficient absorption with ineffective serum and tissue concentration. The SRS-A antagonist FPL 55712 decreases the swelling of the carrageenin oedema in the first hour, suggesting that the C_4, D_4, and E_4 leukotrienes or other lipoxygenase products are released (25).

As an inhibitor of the 5-lipoxygenase, benoxaprofen acts dose-dependently on carrageenin oedema and adjuvant arthritis; these results are also obtained in the anaphylactic models. Acid nonsteroidal anti-inflammatory agents have the strongest inhibitory activities on carrageenin oedema and adjuvant arthritis and are cyclooxygenase inhibitors (26), while acetylsalicylic acid also significantly inhibits the anaphylactic paw oedema but not cutaneous anaphylaxis. Carrageenin oedema is inhibited by the catecholamines after local administration; however, inhibition of adjuvant arthritis could not be found (27).

The compounds H 252, which does not inhibit lipoxygenase activity, and M516, which inhibits lipoxygenase, show moderate dose-dependent inhibitory activities in carrageenin oedema. However, both drugs have no statistically significant influence on the anaphylactic models. The compound M 516 is also ineffective in the primary phase of adjuvant arthritis.

It can be stated that there are no satisfactory correlations between the inhibition of the 15- lipoxygenase and *in vivo* activities. The cause could be the subordinate importance of the products of these lipoxygenases in our *in vivo* models and/or insufficient substance concentrations *in vivo*. The best correlation is obtained with BW 755 C, which influences all *in vivo* models but is also a potent cyclooxygenase inhibitor.

CONCLUSIONS

The importance of lipoxygenase in inflammatory and allergic reactions is established. It oxidises multiple unsaturated fatty acids into many biologically active compounds, such as hydroxyeicosatetraenoic acids and leukotrienes. Therefore, the inhibitors of the lipoxygenase may be possible anti-inflammatory and antiallergic drugs (28).

Here, some compounds were tested for their activity against the isolated lipoxygenase from rabbit reticulocytes and soybean and in four *in vivo* models. Again, conventional anti-inflammatory drugs with high potency are tested as a standard.

The most potent lipoxygenase inhibitors were BW 755 C, NDGA, SHAM, and the compound M 516, which showed anti-inflammatory and antiallergic activities in some *in vivo* models (29). On the other hand, the potent cyclooxygenase

inhibitors' anti-inflammatory agents had no inhibitory action on the lipoxygenase, but they strongly blocked the inflammatory reactions *in vivo* (30).

Conflict of interest

The authors declare that they have no conflict of interest.

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Letter to the Editor

SUBSTANCE P OR PGE2 ALONE AND IN COMBINATION INDUCE IL-1 SECRETION AND INFLAMMATION IN THE RAT PAW

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KEYWORDS: *Substance P, PGE2, inflammation, IL-1, mast cell, immunity*

INTRODUCTION

Substance P (SP), discovered by von Euler in 1931, was described as a potent vessel depressor (1). Later, in 1970, Leeman et al. published the purified chemical structure of SP from bovine hypothalamic tissue (2). In this article, the authors identified SP as an undecapeptide present in numerous organs, tissues, and cells. Moreover, they later discovered the neurokinin (NK1), the receptor of SP.

SP stimulates the turnover of cell membrane phospholipids through the activation of calcium receptors. It also stimulates mast cells (MCs), as reported by Chang and Leeman (2), participating in the inflammatory process. It has been reported that mast cell line (LAD2) secretes IL-1β when it is activated with SP for 24 hours (3). When activated by IgE through their FcεRI receptors, MCs immediately release inflammatory mediators (4) (Fig.1).

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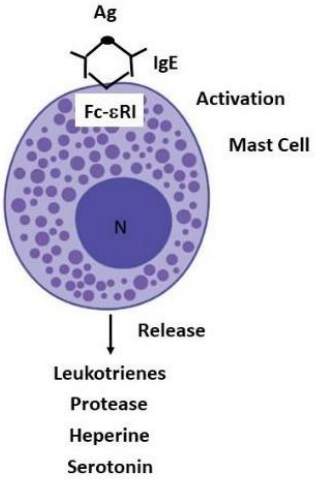

Fig. 1. *This figure depicts the antigen activation mast cell through IgE receptor, releasing leukotrienes, protease, heperine and serotonin.*

In addition, IL-1 stimulation of MCs occurs through the production of caspase-1 and subsequent production of IL-1, which mediates the inflammatory process (5). In fact, in an interesting experiment, it was reported that when SP is simultaneously administered together with IL-33 (SP + IL-33), there is a strong secretion of IL-1β (10 times more than cells treated with IL-1 alone), an effect that further increases inflammation (3).

Moreover, SP administered in combination with IL-33 strongly stimulates the gene expression of tumor necrosis factor (TNF), a potent pro-inflammatory cytokine (6,7). This result demonstrates the cooperation between the SP NK-1 receptor and the IL-33 ST2 receptor in the inflammatory process. SP and IL-33 together markedly enhance TNF synthesis and secretion from human mast cells mediated by the interaction of their receptors (8). SP, binding its receptor NK-1, activates many cells, leading to the release of IL-1β and causing inflammation (Fig.2).

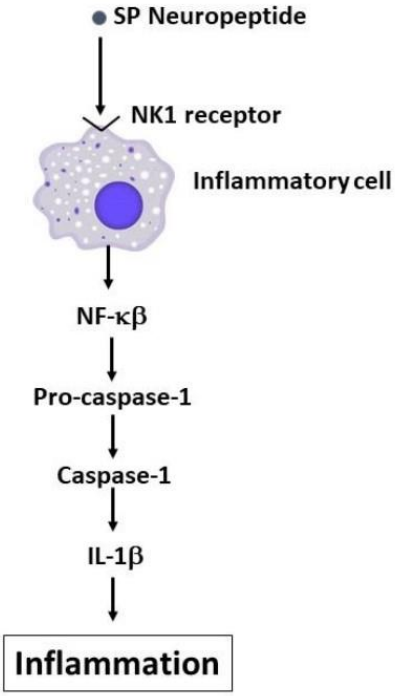

Fig. 2. *Substance P neuropeptide binds its receptor NK1 and activates inflammatory cells to generate IL-1 and therefore, inflammation.*

DISCUSSION

SP mediates the pathogenesis of inflammatory diseases such as psoriasis, where increased levels of this neuropeptide have been found (9). In fact, IL-33 has been reported to enhance the effect of SP inducing vascular endothelial growth factor (VEGF) secreted by MCs, causing increased vascular permeability (10).

Prostaglandins (PGs) are eicosanoids, fatty acids with 20 carbon atoms, and are ubiquitous in the human body. PGs are lipids synthesized from arachidonic acid via constitutive cyclooxygenase-1 (COX-1), or COX-2, which is synthesized after trauma or stimuli. The inflammatory stimulus leads to the activation of phospholipase A2 (PLA2) with release of arachidonic acid and the expression of COX2 (11). PGE2 is a pentanoid PG, the most abundant PG in the human body. It is an important mediator of inflammation, and its pharmacological inhibition represents an important therapeutic strategy. In fact, PGE2, as reported by Sir John Vane in 1971, is inhibited by non-steroidal anti-inflammatory drugs through suppression of the cyclooxygenase enzyme (12). PGE2 is a protector of gastric mucosa and is a mediator of pain, inflammation, fever, and platelet aggregation (13). Gastric erosion and ulcers can occur when PGE2 is inhibited by anti-inflammatory drugs. Therefore, PGE2 plays a protective role in the gastrointestinal tract. In contrast, when PGE2 levels are increased in tissue, it is a strong mediator of inflammation, pain, and fever.

It is known that IL-1 and other inflammatory mediators can regulate the proliferation and differentiation of a number of human cells including immune cells. The modulation of inflammatory mediators can be very useful in therapy for immune and inflammatory diseases. IL-1 is produced by activated monocytes and macrophage cells following inflammatory stimulus, while the levels are low or absent in healthy subjects.

In our study, we used Wistar rats that were put to sleep with CO2, and afterwards, PGE2, SP, and the combination of PGE2 plus SP were injected into the footpads of the rats. After 30, 60, 90, and 120 minutes, inflamed tissue and controls (untreated rat paw) were removed. Pieces of inflamed tissue and controls were minced, placed in medium, stirred for 30 min, and the supernatant was collected for IL-1 testing. The supernatant was filtered and the levels of IL-1β were calculated by ELISA method. The ELISA reader was set at 405 nm absorbance, all the samples were read at room temperature, and the standard curve was constructed. All samples were assayed in triplicate. Results are expressed as pg/ml (± SD) (Fig.3).

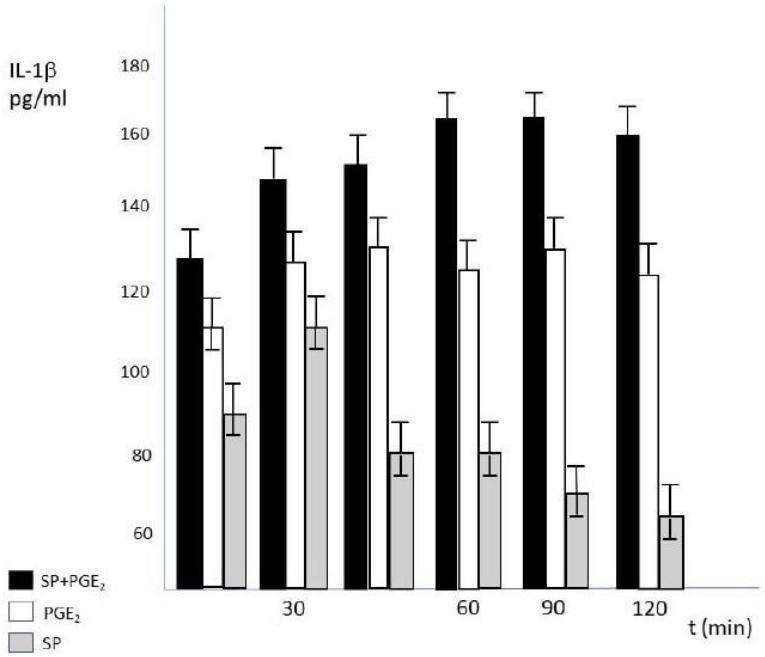

Fig. 3. *In this figure we report the generation of IL-1 after treatment with PGE2, SP, and SP+PGE2 in combination. Here it is shown that the combination of SP+PGE2 generates larger amounts of IL-1 compared to PGE2 and SP alone.*

CONCLUSIONS

In this short article, we report that inflammation occurs when SP and PGE2 are injected alone and in combination into the rat footpad, when compared to the untreated control group. The results have shown that PGE2 is highly inflammatory,

while SP is moderately inflammatory, and when both are combined, levels of IL-1 are higher than PGE2 alone. This effect was shown in all time periods (30 min intervals) of treatment. The inflammatory effect of PGE2 is higher than that of SP because these two compounds act in different ways. The PGE2 plus SP combination is highly inflammatory, higher than PGE2 administered alone, suggesting that SP potentiates the activity of PGE2. In addition, these compounds were found to provoke the generation of IL-1 (Fig.3).

Here, we show that the neurotransmitter SP acts as an inflammatory compound in the first 30 minutes after injection. Afterwards, the inflammatory effect of SP subsequently decreases, while the arachidonic acid product PGE2 remains highly inflammatory. When these two compounds are injected in combination at the same time into the plantar of the rat paw, the inflammatory effect and the level of IL-1 was higher than PGE2 administered alone.

Conflict of interest

The author declares that they have no conflict of interest.

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