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SEROTONERGIC MECHANISMS IN PSORIASIS

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ABSTRACT

Psoriasis is a chronic inflammatory disease with a prevalence in Sweden of 2-3%. Stress worsens psoriasis. Serotonin, a monoamine and a neurotransmitter, is a well-documented signal substance in situations like stress and in regulation of inflammatory processes. Serotonin produces its effects via about 21 receptors (R), of which the most characterized are 5-HT1AR and 5-HT2AR. The amplitude and duration of activation of the serotonergic system is determined by the serotonin transporter protein (SERT).

The aim of the thesis is to elucidate the serotonergic mechanisms involved in psoriasis.

In the first study there was a decreased expression of 5-HT1AR and an increased 5-HT2AR expression in the involved skin as compared to normal skin. This differential expression concord with their antagonistic effects, 5-HT1AR inhibiting inflammation and 5-HT2AR promoting inflammation. These two receptors could be potential targets for anti-inflammatory therapies in psoriasis. The expression of 5-HT3R was absent in involved skin and evident in the basal epidermis of the non-involved skin.

In Study II the expression of SERT was increased in involved skin as compared to non-involved and normal skin and there was co-localization with caspase-3, a key regulator of apoptosis. This indicates that SERT might play a role in regulating apoptosis in inflammatory cells in psoriasis. SERT might thus constitute a valuable therapeutic target.

In Study III an increased SERT expression in inflammatory cells in the epidermis was positively correlated to psoriasis severity and to chronic stress. This implicates that SERT expression could be of importance for psoriasis severity and chronic stress.

In the last study, a broad population-based cohort study, SSRI use among patients with plaque psoriasis was associated with a decreased need for systemic psoriasis treatment. SSRI may have a protective effect in psoriasis.

In conclusion, a role for the serotonergic system was implicated for the chronic inflammation in psoriasis.

LIST OF PUBLICATIONS

I. Nordlind K, **Thorslund K**, Lonne-Rahm S, Mohabbati S, Berki T, Morales M, Azmitia EC. Expression of serotonergic receptors in psoriatic skin. *Arch Dermatol Res* 2006;298: 99-106.

II. **Thorslund K**, El-Nour H, Nordlind K. The serotonin transporter protein is expressed in psoriasis, where it may play a role in regulating apoptosis. *Arch Dermatol Res* 2009;301:449-457.

III. **Thorslund K**, Amatya B, Eriksson Dufva A, Nordlind K. Expression of the serotonin transporter protein is correlated with the severity of psoriasis. *In manuscript.*

IV. **Thorslund K**, Svensson T, Nordlind K, Ekbom A, Fored CM. Serotonin reuptake inhibitors may have a protective effect in psoriasis: population based cohort study. *Manuscript submitted.*

CONTENTS

LIST OF ABBREVIATIONS

5-HIAA	5-hydroxyindole acetic acid
5-HT	5-hydroxytryptamine; serotonin
5-HT1AR	5-hydroxytryptamine 1A receptor
5-HT2AR	5-hydroxytryptamine 2A receptor
5-HT3R	5-hydroxytryptamine 3 receptor
ACTH	Adrenocorticotrophic hormone
ATC	Anatomical Therapeutic Chemical
BDI	Beck's Depression Inventory
BDNF	Brain derived neurotrophic factor
CGRP	Calcitonin gene-related peptide
CI	Confidence interval
CNS	Central nervous system
CRH	Corticotrophin releasing hormone
DDD	Defined Daily Dose
DOI	2,5-dimethoxy-4-iodoamphetamine
FITC	Fluorescein isothiocyanate
GABA	Gamma-amino butyric acid
HPA-axis	Hypothalamic pituitary adrenal axis
ICD	International Classification of Disease
IFNα	Interferon alpha
IFNγ	Interferon gamma
LAMP	Lysosome-associated membrane glycoprotein
MADRS	Montgomery Åsberg Depression Rating Scale
MAO	Monoaminoxidase
NFκβ	Nucleus factor kappa beta
NGF	Nerve growth factor
NK1R	Neurokinin 1 receptor
NPR	National Patient Register
OR	Odds ratio
PASI	Psoriasis Area and Severity Index
PBS	Phosphate-buffered saline
PDR	Prescribed Drug Register
PIN	Personal identification number
SERT	Serotonin transporter protein
SP	Substance P
SSRI	Serotonin reuptake inhibitors
TNFα	Tumor necrosis factor alpha
TPH	Tryptophan hydroxylase

1 INTRODUCTION

1.1 PSORIASIS

1.1.1 Epidemiology

Psoriasis is a common chronic inflammatory disease, affecting around 2% of the population in Sweden.[1-3] Psoriasis is equally common in males and females[4] and the disease can appear at any time of life[5] with a mean age of onset estimated at 33 years, with 75% of cases occurring before 46 years of age.[6]

1.1.2 Clinical features

The most common type of the disease is psoriasis vulgaris, accounting for 90% of all cases, in which papulosquamous plaques are well delineated from surrounding normal skin. The plaques are red or salmon-pink in color, covered by white or silvery scales, and may be thick, thin, large or small. They are most active at the edge: rapidly progressing lesions may be annular, with normal skin in the centre. Plaques are usually distributed symmetrically, and occur most commonly on the extensor aspects of elbows and knees, scalp, lumbosacral region and umbilicus. Active inflammatory psoriasis is characterized by the Koebner phenomenon, in which new lesions develop at sites of trauma or pressure.[7]

Other forms of psoriasis, accounting for 10% of all cases, includes guttate psoriasis, generalized pustular psoriasis (von Zumbusch psoriasis), palmoplantar pustulosis and erythrodermic psoriasis. Guttate psoriasis, an acute form, in which small papules erupt on the trunk, is affecting mostly children and adolescents, being self-limiting and resolving within 3–4 months of onset. Generalized pustular psoriasis (von Zumbusch psoriasis) is another acute form in which small, monomorphic sterile pustules develop in painful, inflamed skin. Palmoplantar pustulosis, consisting of yellow-brown, sterile pustules on palms and soles, predominantly affects women (9:1 ratio) and is still regarded a variant of psoriasis in the textbooks. However, different demographics and genetics imply that the condition may therefore be a comorbidity rather than a variant of psoriasis. Erythrodermic psoriasis is a life-threatening condition, where the whole body surface is affected by psoriasis, which can lead to hypothermia, hypoalbuminaemia, and high output cardiac failure.[5]

1.1.3 Pathogenesis

The scales of psoriasis vulgaris are a result of a hyperproliferative epidermis with premature maturation of keratinocytes and incomplete cornification with retention of nuclei in the stratum corneum (parakeratosis). The epidermis is thickened (acanthosis) with elongated rete ridges as a result of an increased mitotic rate of the basal keratinocytes as compared to normal skin. The inflammatory infiltrate mainly consists of dendritic cells, macrophages and T cells in the dermis and neutrophils, with some T cells in the epidermis. The redness of the lesions is due to increased numbers of

tortuous capillaries that reach the skin surface through a markedly thinned epithelium.[5,7,8]

1.2 NEUROIMMUNE INTERACTION – PSYCHONEUROIMMUNOLOGY

The skin constitutes one of the major targets for the immune system. There is a complex bidirectional brain-immune network, providing a biological basis for the ancient anecdotal notion that the mind can play a role in health and disease by its ability to influence relevant biological processes.[9] This brain-immune network, the so-called 'brain-skin-axis' can be activated by stress among other stimuli.

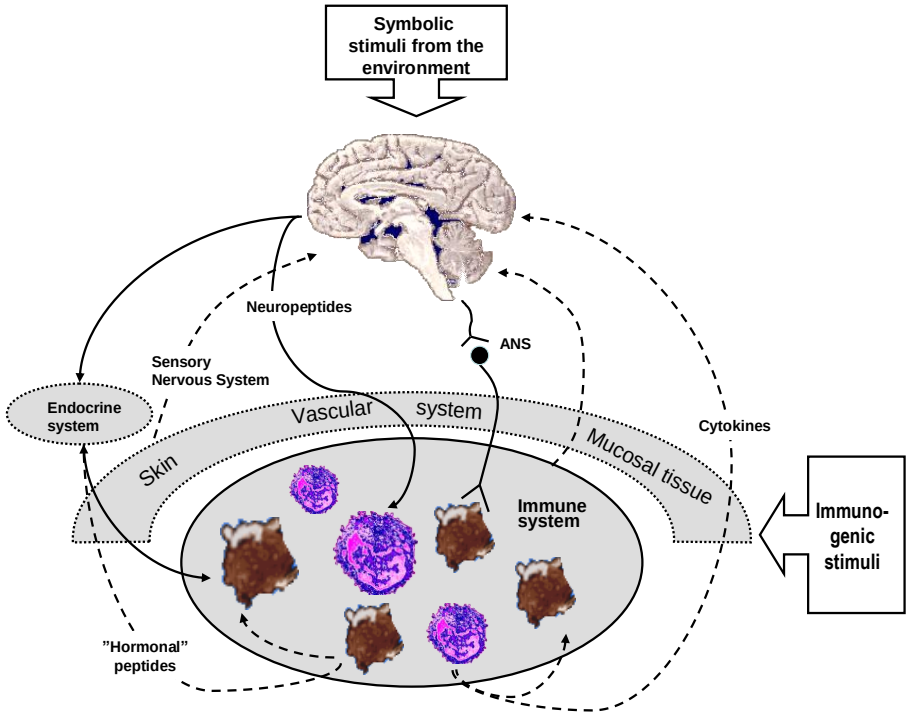

Figure 1. *The bi-directional brain-immune network – 'The brain-skin-axis'.*[9]

Stress can activate three independent pathways that are linked with the immune system. The first pathway is the hypothalamic pituitary adrenal axis (HPA-axis), where a stressful stimulus releases corticotrophin-releasing hormone (CRH) from the hypothalamus. CRH then increases the production of adrenocorticotrophic hormone (ACTH) from the pituitary. ACTH is in turn released into the circulation and transported to the adrenal gland where it stimulates the release of glucocorticoids and the stress hormone cortisol from the adrenal cortex. Cortisol then acts as a negative feedback loop inhibiting the release of CRH from the hypothalamus.[10] In chronic inflammation this negative feedback loop can be disturbed due to low levels of cortisol in serum, leading to a continuous activation of the HPA-axis.

The second pathway is the activation of sensory nerves in the brain and skin resulting in release of neuroendocrine and neural mediators such as CRH, neurotrophins and α-melanocyte stimulating hormone, and different neuropeptides active in the skin – substance P (SP) and calcitonin gene-related peptide (CGRP).

The third pathway is the activation of the autonomic nervous system (ANS).

1.3 NEUROIMMUNE INTERACTION IN PSORIASIS

There is an association between the nervous system and psoriasis. In 1971 Dewing[11] accidently sectioned a spinal nerve and the psoriasis in the corresponding nerve area declined. In retrospective studies there was a correlation between stress experience or stressful life events and the onset of guttate psoriasis or the severity of symptoms in psoriasis vulgaris. Some studies even demonstrated that stress-experience associates with stress axis modulation in psoriasis patients.[12-14] In prospective studies chronic stress worsens psoriasis.[15,16] Exactly which mediators are involved in the crosstalk between the psoriatic skin and the central nervous system (CNS) are not known. There are several neurotransmitters that can be involved.

Nerve growth factor (NGF), a neurotrophic factor, which is expressed both in the nervous system and in the peripheral organs, influences an inflammatory reaction by regulating neuropeptides, angiogenesis, cell trafficking molecules and T cell activation. NGF augments tissue innervation and plays a critical role in regulating certain neuropeptides such as SP and CGRP. Keratinocytes in involved and non-involved psoriatic tissue express high levels of NGF compared to controls, and there is a marked up-regulation of nerve growth factor-receptor (NGFR) in the terminal cutaneous nerves of psoriatic lesions.[17] NGF is also a mediator of stress responses.[18,19]

In the involved skin of psoriasis patients there are more nerve fibers containing the sensory neuropeptide SP. These nerve fibers also maintain increasing numbers of contacts to Neurokinin 1 receptors (NK1R)-positive mast cells, a target for SP.[20,21] Release of SP, which then activates mast cells, could lead to an increased TNFα release.[22,23] TNFα is an important mediator maintaining chronic inflammation and a target for the biologics used in the treatment of psoriasis. It seems that acute stress suppresses TNFα due to the secretion of catecholamines and glucocorticoids while chronic stress increases TNFα, possibly due to the secretion of neuropeptides.

One important mediator of stress-induced responses is the neurotransmitter serotonin.

1.4 SEROTONIN

Serotonin (5-hydroxytryptamine; 5-HT), a monoamine, is one of the oldest signalling substances to appear during evolution. It was first discovered by Rapport *et al.* in 1948 as a potent vasotonic factor and was therefore given the name "tonin".[24,25] Serotonin has an important role in normal physiology, including appetite, thermoregulation, sleep and pain perception.[26] Moreover, stress and stress-related hormones increase serotonin synthesis.[27,28] At the cellular level serotonin has effects on proliferation, migration and apoptosis.[29] Like other neurotransmitters such as acetylcholine, glutamate and gamma-amino butyric acid (GABA), serotonin acts via two categories of receptors: ionotropic and metabotropic, where the ionotropic receptors have a low affinity for their neurotransmitter ligand but a rapid activation constant, and the metabotropic receptors (G-protein activated) exhibit both a high affinity for their neurotransmitter and a slow activation constant.[26]

1.4.1 Serotonin synthesis

Serotonin is synthesized via enzymatic cleavage from its precursor tryptophan by a rate limiting enzyme, tryptophan hydroxylase (TPH).[30] Hydroxytryptophan is then further decarboxylated to serotonin by L-aromatic amino-acid decarboxylase with the cofactor pyridoxal phosphate.

Tryptophan is an essential amino acid in humans obtained from dietary sources. Tryptophan is very effective in capturing light, and it is formed in the reactive core of chlorophyll in plants.[31] Serotonin is involved in root growth and helps out as a potent antioxidant in cell metabolism and leaf motility in plants. In humans TPH is found in brainstem neurons, pineal glands, lungs, gut and also skin, where it has been localised to blood vessels, mast cells, melanocytes, keratinocytes and fibroblasts.[32]

1.4.2 Serotonin degradation

Degradation of serotonin mainly occurs through oxidative deamination in the liver and in lung endothelial cells, where serotonin is stored in vesicles until being released and catalysed by monoaminoxidase (MAO) which is expressed in mammalian skin.[33] MAO deaminates serotonin to 5-hydroxyindole acetaldehyde, which is followed by its transformation to 5-hydroxyindole acetic acid (5-HIAA). 5-HIAA is excreted in the urine and serves as an indicator of serotonin transmission in the body.

Stress induces activation of the HPA-axis resulting in increased levels of glucocorticoids, which stimulate serotonin turnover and synthesis. It has been shown that treatment with dexamethasone, a synthetic adrenal steroid, increases TPH protein concentration in brainstem neurons.[27] In the periphery, 5-HIAA and serotonin turnover is increased in patients showing an elevated cortisol level.[34]

1.4.3 Localisation

Serotonin occurs at the highest concentrations in three locations in the body:

1. In the wall of the intestine. About 90% of the total amount of serotonin in the body is present in enterochromaffin cells, which are mainly located in the stomach and small intestine.
2. In blood. Serotonin is present in high concentration in platelets, which accumulate it from the plasma by an active transport system and release it when they aggregate at sites of tissue damage.
3. In the CNS, acting as a neurotransmitter.[35]

1.4.4 Role of serotonin in skin inflammation

In the inflammatory skin serotonin expression is limited to platelets.[36,StudyII] In chronic eczema and psoriasis serotonin expression has been evident in the cytoplasm of prickle cells, sweat gland cells, sebaceous gland cells, and hair roots, while no expression was evident in normal skin.[37,38] A recent report determined that there was an expression of serotonin in mast cells in urticaria pigmentosa.[39] In earlier studies serotonin was also expressed in melanocytes.[40,41]

1.5 SEROTONIN RECEPTORS

The actions of serotonin are mediated through interaction with membrane-bound receptors which are categorized into 7 families (5-HT1 – 5-HT7) with at least 21 subtypes.[26] Most of these are, as described above, metabotropic receptors coupled to G proteins, except the 5-HT3 receptor family which are ionotropic receptors.

The human skin expresses these membrane-bound receptors. Detailed molecular analyses have identified mRNA encoding for 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, 5-HT2C and 5-HT7 receptors.[42] *In situ* studies have also demonstrated expression of the 5-HT3R in the basal epidermal layer in the skin,[43,StudyI] while pharmacological studies indicate that 5-HT3R are expressed on sensory nerve endings.[44]

The best-characterized receptor is 5-HT1AR, which is a high-affinity receptor. It is stimulated by normal circulating levels of day-time serotonin and mediates promotion of mitogen-activated T and B cell survival, associated with increased translocation of the transcription factor Nucleus factor kappa beta (NFκβ) to the nucleus.[45] Activation of 5-HT1AR can also induce the release of S100beta from astrocytes.[46] It has been reported that 5-HT1AR is important in preventing apoptosis in neuronal cells and hippocampal HN2-5 cells.[47] Conversely it may be pro-apoptopic through c-Jun N-terminal kinase in Chinese hamster ovary fibroblasts.[48] Hence activation of the 5-HT1AR leads to neurite outgrowth[49] and maybe neuronal survival as well.[50]

The 5-HT2AR is a low-affinity receptor and only activated when serotonin levels are high. Activation of 5-HT2AR is associated with an increase in kinase activity and with cell division and apoptosis through increasing phosphatidylinositol-hydrolysis, which leads to increased intracellular Ca^{2+} levels. Thus 5-HT2AR increases cell proliferation.[29] In rats, cutaneous expression of 5-HT2AR was detected in unmyelinated axons at the dermal – epidermal junctions and in the nerve endings of Pacinian corpuscles.[51] In eczematous skin of mice there was 5-HT2AR immunoreactivity in the epidermis and on nerve endings.[52]

The 5-HT7R has been suggested to have a role in pain and migraine, via actions on blood vessels, but also on neurogenic inflammation.[26]

1.5.1 Role of serotonin receptors in skin inflammation

1.5.1.1 5-HT1A receptor

Serotonin promotes inflammation by increasing the number of mast cells at the site of tissue injury, via the 5-HT1AR.[53] Expression of 5-HT1AR in inflamed skin is associated with melanocytes, keratinocytes in the upper part of the epidermis, mast cells and in vessel walls. In the same study of human contact allergy skin there was a decrease in the number of 5-HT1AR positive cells in the eczematous skin compared to normal skin.[36]

Buspirone, a non-selective 5-HT1AR agonist, applied topically or orally, was able to diminish contact allergy in mice.[54] Tandosporine, another agonist, has been reported to decrease stress and to attenuate itching in patients with atopic dermatitis.[55,56]

1.5.1.2 5-HT2A receptor

In humans Study I revealed expression of 5-HT2AR in mononuclear cells in the papillary dermis of psoriatic skin. These were CD3 positive, indicating these cells to be lymphocytes. These cells are increased in involved compared to non-involved psoriatic skin and normal healthy skin, as well as increased in allergic contact dermatitis skin compared to control skin.[36]

Different 5-HT2AR antagonists have been reported to be able to decrease contact allergic reactions in mice.[57,58] Of such antagonists, ketanserin inhibits the established but not challenge-induced phase of allergic contact dermatitis to nickel in humans.[59,60]

In aortic smooth muscle cells activation of 5-HT2AR, with the agonist - 2,5-dimethoxy-4-iodoamphetamine (DOI) provides an inhibition of TNFα-mediated inflammation.[61]

1.5.1.3 5-HT3 receptor

When injected intradermally serotonin gives rise to pruritus in humans.[44] This may be mediated through different receptors such as the 5-HT3 receptor. Thus ondansetron, a 5-HT3 antagonist, is able to decrease pruritus. In addition, pruritus in malignant disease has been treated with paroxetine, a selective SSRI, and the antipruritic action was connected with a down-regulation of 5-HT3 receptors.[62]

1.6 SEROTONIN TRANSPORTER PROTEIN

Serotonin in the plasma can be transferred to platelets by the serotonin transporter protein (SERT), which is a 12-membrane spanning molecule that regulates both the release and uptake of serotonin. The release of serotonin from platelets is mediated by SERT. SERT moves serotonin into the cells in most situations and there are a large number of pharmaceutical drugs that can inhibit this reuptake process, namely serotonin reuptake inhibitors (SSRI) that are used in the treatment of depression.

Some individuals are prone to be more sensitive than others to stress and depression due to polymorphisms in the promoter or intron region of the SERT gene.[63,64] In psoriasis no significant differences in genotype distribution and allele frequencies were apparent in a German population.[65]

1.6.1 Role of serotonin transporter protein in inflammation

No earlier studies have focused on SERT expression in the skin. Platelet SERT appears to be identical to that carried by neurons and stored serotonin can be rapidly released in response to a variety of inflammatory signals.[66] Lymphocytes also carry SERT. The high affinity uptake of serotonin by peripheral blood lymphocytes has been described with corresponding inhibition by SSRI.[67]

The results of studies of intestinal inflammation in experimental animals and humans regarding mucosal expression of SERT have been inconsistent. For instance, the levels of SERT mRNA have been reported to be elevated in the small intestines of patients with irritable bowel syndrome (IBS),[68] reduced in rectal biopsies of patients with

ulcerative colitis and IBS,[69] and in guinea pig colitis[70] and normal in rectal and sigmoidal mucosal biopsies of patients with IBS.[71]

Other important cytokines in chronic skin inflammation might be affected by SERT. In cultured colonic epithelial cells SERT function and expression decreases IFNγ and TNFα.[72] It has been reported that SERT synthesis may be stimulated by another cytokine important for psoriasis pathogenesis, IFNα.[73]

1.7 SEROTONIN REUPTAKE INHIBITORS

Serotonin reuptake inhibitors (SSRI) are a family of antidepressants with more-or-less the same mode of action. The most common SSRI in Sweden are: citalopram, escitalopram, paroxetine, fluoxetine, fluvoxamine and sertraline.

The mechanism of action of SSRI is simply explained by selective inhibition of SERT. SSRI immediately blocks the serotonin reuptake pump, and this action causes a sudden increase in serotonin, predominantly in the somatodendritic area as compared to the axon terminals where serotonin is presumably needed in order to exert its therapeutic actions. This might explain why SSRI do not have a rapid onset of therapeutic effect. In the longer term, this sustained increase of serotonin causes the somatodendritic 5-HT1A autoreceptors to desensitize and the neuronal impulse flow is turned on, which means that more serotonin releases from the axon terminal. The final step of action is the desensitization of post-synaptic serotonin receptors, leading to serotonin release in the axon terminal.[74]

There might be other pathways of action for SSRI in the treatment of depression such as neuroplasticity. Treatment with escitalopram in the rat frontal cortex activated intracellular pathways linked to brain-derived neurotrophic factor (BDNF) and increased the levels of Pro-brain-derived neurotrophic factor, hence contributing to synaptic plasticity.[75]

1.7.1 Role of serotonin reuptake inhibitors in skin inflammation

Treatment with SSRI can lead to cutaneous side-effects such as spontaneous bruising, pruritus, urticaria, angioedema, erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, erythema nodosum, alopecia, hypertrichosis and leukocytoclastic vasculitis.[76] Interestingly, case reports on the effect of SSRI in psoriasis have yielded conflicting results; one study indicated improvement of psoriasis after use of paroxetine[77] and three studies indicated a flare-up or worsening of the psoriasis following fluoxetine and paroxetine treatments.[78-80]

Patients with depression have elevated blood levels of cytokines as compared to healthy controls, and these levels are reduced upon treatment with SSRI.[81,82] In patients who failed to respond to SSRIs, no reduction in cytokine levels was observed,[83] suggesting a connection between SSRI, depression and the immune system.

In humans and in an animal arthritis model there was a suppressed inflammatory cytokine production through treatment of fluoxetine and citalopram, i.e. anti-inflammatory effects.[84] In rats fluoxetine has also suppressed inflammatory responses in lung tissue and inhibited the expression of inflammatory cytokines, interleukin-1β, TNFα, monocyte chemotactic protein and intercellular adhesion molecule-1.[85] There is

further evidence for an anti-inflammatory effect of SSRI. In a rat model of periodontitis fluoxetine suppressed the inflammatory response and protected against periodontal bone resorption and destruction of collagen fibers.[86] In a murine colitis model fluoxetine inhibits NFκB activation in intestinal epithelial cells and hence ameliorates acute murine colitis.[87] In animal models of septic shock and allergic asthma, fluoxetine reduced the inflammatory reaction.[88]

There are no earlier studies of SSRI and skin inflammation.

1.8 SWEDISH HEALTHCARE REGISTERS

Sweden has a long tradition of healthcare registers. Already in 1749 there was a nationwide cause-of-death register introduced.[89] The structure of the Swedish healthcare system, reliable healthcare registers and the unique personal identification number (PIN)[90] offers exceptional possibilities for performing register-based epidemiological studies in Sweden.

1.8.1 National Patient Register

In 1964 the National Board of Health and Welfare launched the National Patient Register (NPR). Since 1987 the register has a nearly complete coverage of all inpatient care (both public and private). From 2001 data from specialized out-patient care is included (both public and private) with primary care being excluded.[91] It is estimated that the outpatient register has 80% coverage[92] due to lower reporting from private caregivers and low reporting data in psychiatric care. Private care is estimated to sustain less than 10% of all healthcare in Sweden. Regarding accuracy, a validation from the Swedish National Board of Health and Welfare in 2007 reported that more than 98% of all diagnoses had been technically correctly coded with only 1-2% dropouts and 1% missing data. [91] Other studies with validations of medical records of the inpatient care registered in NPR have shown varying positive predictive values between 85-94%.[92,93] No validation of the specific diagnosis of psoriasis vulgaris has been performed.

Since 1997 diagnoses in the register are coded according to the International Classification of Diseases, 10th revision (ICD-10). Register information includes patient characteristics (PIN, sex, age, and county of domicile), administrative data, hospital identification and medical data (major interventions and discharge diagnoses, with one main diagnosis and up to seven secondary discharge diagnoses and dates of diagnosis).

1.8.2 Prescribed Drug Register

In 1999 the Prescribed Drug Register (PDR) was started and since 1st July 2005 the PIN was included. The register offers complete coverage of all dispensed drugs in Sweden, where each dispensation is connected to an individual. It is updated every month and contains approximately 90 million prescriptions annually. Only dispensed drugs are captured since the register is pharmacy-based.[94] All pharmacies in Sweden report to the register. Pharmacy dispensing is considered as the golden standard in drug exposure information.[95]

The register measurements units are PIN, type of drug described, date of prescription and dispensing and Defined Daily Doses (DDDs). All drugs are classified according to the Anatomical Therapeutic Chemical (ATC) classification system. The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults.[96] The database is complete for the entire population in the country (patient identity data are missing for <0.3% of all items).[94]

2 AIMS OF THE STUDIES

2.1 OVERALL AIM

To study the serotonergic mechanisms in psoriasis and its impact on disease.

2.2 SPECIFIC AIMS

- Study the expression of serotonergic receptors (5-HT1AR, 5-HT2AR, and 5-HT3R) in involved and non-involved psoriatic skin as compared to normal skin and characterization of cells and structures expressing these receptors (Study I).

- Study the expression of SERT and serotonin in involved and non-involved psoriatic skin as compared to normal skin, characterization of cells and structures expressing SERT and serotonin and their relation to apoptosis (Study II).

- Study if the altered SERT expression determined in Study II correlates to psoriasis severity, depression, and chronic stress (Study III).

- Study if modulation by the serotonergic system can improve or worsen psoriasis by studying SSRI and its effect in psoriasis (Study IV).

3 PATIENTS AND METHODS

STUDIES I – III

3.1 SUBJECTS

Patients were recruited from the Department of Dermatology, Karolinska University Hospital with stable plaque psoriasis, without ongoing systemic treatment who had not received topical treatment (except emollients) for at least two weeks. Control patients were recruited from the same area and were healthy regarding inflammatory dermatoses (Studies I-II).

In Study III patients were recruited from the Department of Dermatology, Karolinska University Hospital and from treatment units at the Psoriasis Association in Stockholm with the same criteria as described above.

3.2 CLINICAL MEASUREMENTS (STUDY III)

3.2.1 Psoriasis Area and Severity Index

The clinical severity of psoriasis in each patient was assessed using the Psoriasis Area and Severity Index (PASI).[97]

3.2.2 Salivary cortisol test

Nineteen of the 20 patients with psoriasis took part in the salivary cortisol test. Salivary cortisol samples were collected in plastic vials at 08.00h for four consecutive days. At 22.00h on the third day each patient was asked to take 0.25 mg dexamethasone orally, which was followed by a cortisol test the following morning. These samples were stored at -20°C until analysed. The cortisol concentration was determined using a radioimmunoassay kit (Spectria Cortisol; Orion Diagnostica, Espoo, Finland). A salivary cortisol quotient was determined by dividing the mean of the three initial cortisol values by the last cortisol value following the dexamethasone challenge. A low ratio is an indicator of chronic stress.[98,99]

3.2.3 Beck's Depression Inventory

Symptoms of depression of each patient with psoriasis were assessed using Beck's Depression Inventory (BDI), which consists of 21 questions. A score of 21 or more represents depression.[100]

3.3 IMMUNOHISTOCHEMISTRY

Punch biopsies of 3 mm diameter were obtained from the edge of the involved and non-involved skin (at least 5 cm away from the plaque) on the lower back of patients. Biopsies were taken from the same site from the healthy controls.

The skin specimens were first immersed in phosphate-buffered 4% formaldehyde containing 0.17% picric acid for 2 h and thereafter rinsed in 0.1 M Sörensen's phosphate-buffered saline (PBS) supplemented with 10% sucrose for at least 24 h. The samples were then frozen rapidly on dry ice and stored at -70^{o} C until further processing. While still frozen the biopsy material was embedded in Tissue Tek OCT Compound (Sakuro Finetek, Zoeterwoude, Netherlands), cut to 14-µm thick sections with a Microm HM500 cryostat (Heidelberg, Germany), and mounted on SuperFrost/Plus glass slides (six sections on each slide) for immunohistochemical staining.

3.3.1 Biotinylated streptavidine method

After blocking of non-specific binding sites with normal serum, sections were incubated with the primary antibodies overnight at 4°C. The following day sections were incubated with a biotinylated secondary antibody (Vector Laboratories, Burlingame, CA, USA) for 40 min at room temperature. Finally, a fluorochrome was added to visualize the primary antibody. Fluorochromes used were a Cy2-labelled streptavidine (Amersham Pharmacia Biotech, Uppsala, Sweden) with a green fluorescence (Study III) and streptavidine-conjugated Texas Red (Vector Laboratories) with a red fluorescence (Studies I-III).

Table 1. *Primary antibodies for immunohistochemical staining*

Antibody	Type	Company	Dilution	Used in study	Fluorochrome
5-HT	Poly (rabbit)	Diasorin, Millwater, USA	1:10000	II	Cy2
SERT	Mono (mouse)	MabTechnologies, Stone Mountain, USA	1:2500 – 1:10000	II, III	Cy 2, Texas Red
5-HT1AR	Poly (rabbit)	Azmitia et al.[101]	1:1000	I	Texas Red
5-HT2AR	Mono (mouse)	Pharmingen, San Diego, USA	1:150	I	Texas Red
5-HT3R	Poly (rabbit)	Morales et al.[102]	1:4000	I	Texas Red

For 5-HT and 5-HT1AR antibodies preadsorption with each antigen eliminated the immunostaining. For the 5-HT3R antibody a preimmune serum was used as control. For the monoclonal antibodies (SERT and 5-HT2AR) control staining with non-specific mouse IgG of the same isotype (Dako, Glostrup, Denmark) and in the same dilution as the primary antibodies were negative. Additionally, for each observation and every patient, the primary antibody was omitted in two sections and no staining was observed.

3.3.1.1 Double staining

The sections were treated as described above and then subjected to a second blocking of non-specific binding sites. Next, another primary antibody was added and the sections were incubated overnight at 4°C. The following day sections were incubated with secondary antibodies that were associated with a fluorochrome, either FITC-conjugated (green label) or Alexa Fluor-conjugated (green label). As a control the primary antibody was omitted in two sections and no staining was recorded. In two sections the second primary antibody was being stained in the absence of the first primary antibody.

Table 2. *Antibodies for double staining procedures.*

Antibody	Type	Company	Dilution	Stained With	Used in study	Fluorochrome
Caspase-3	Poly (rabbit)	R&D Systems, Minneapolis, USA	1:100	SERT	II	FITC-conjugated swine-antirabbit
CD1a	Mono (mouse)	Becton-Dickinson, Franklin Lakes, USA	1:100(I) ,1:320 (II)	5-HT1A (I), SERT (II)	I,II	Texas Red (I), FITC-conjugated donkey anti-mouse (II)
CD1a	Mono (mouse)	BD Pharmingen, Franklin Lakes, USA	1:20 (I), 1:25 (III)	5-HT2A (I), SERT (III)	III	FITC-conjugated donkey anti-mouse(I) Alexa Fluor-conjugated donkey anti-mouse (III)
CD3	Mono (mouse)	Becton-Dickinson	1:100	5-HT1A	I	Texas Red
CD3	Poly (rabbit)	Novocastra Lab, Newcastle, UK	1:50	SERT	II	FITC-conjugated swine-antirabbit
CD4	Mono (mouse)	Becton-Dickinson	1:40	5-HT1A	I	Texas Red
CD8	Mono (mouse)	Becton-Dickinson	1:40	5-HT1A	I	Texas Red
LAMP	Mono (mouse)	Immunotech SAS, Marseille, France	1:25	SERT	III	Alexa Fluor-conjugated donkey anti-mouse
NK1-beteb	Mono (mouse)	Sanbio BY, Am Uden, Netherlands	1:80	5-HT1A	I	Texas Red
Tryptase	Mono (mouse)	Chemicon, Temecula, USA	1:5000	5-HT1A	I	Texas Red
Tryptase	Poly (rabbit)	Gift from Harvima[103]	1:1000	SERT	II	FITC-conjugated swine-antirabbit

3.3.2 Microscopy

Sections were mounted with glycerol/gelatin (Merck, Darmstadt, Germany), covered with glass coverslips and then examined using a fluorescence Zeiss Axioskop 2 MOT microscope (Carl Zeiss, Stockholm, Sweden) in Studies II and III.

In Study I the sections were mounted with glycerol/PBS and then covered with glass coverslips. For microscopic evaluation a Nikon Microphot-FXA microscope was used (Nikon Corporation, Yokohama, Japan).

The numbers of labelled cells in involved, non-involved and normal skin respectively, were counted by the same observer. For each patient the numbers of cells were counted in a blinded way in four different sections. The average values were used to calculate the mean ± SD value for all of the specimens. In Study I the 5-HT1A and 5-HT2A cells were counted in the papillary dermis. In Studies II and III the SERT-positive cells were counted separately for the epidermis and papillary dermis.

3.3.2.1 Double staining

In Study I the 5-HT1A and 5-HT2A positive cells in the papillary dermis were counted in two sections per sample and then the double stained cells with each antibody (tryptase, CD1a, CD3, CD4, CD8, and NK1-beteb) were counted and expressed as "majority of 5-HT1A cells were positive for…".

In Study II 7/10 biopsies were double stained and two sections per patient were counted and expressed as mean percentages. In this case each positive, double-stained cell with antibody (CD1a, CD3 and tryptase) was counted and then the expression of SERT was counted and expressed as a percentage of CD1a-, CD3-, and tryptase positivity, respectively. For caspase-3 the SERT-positive cells were counted and then the caspase-3-positive cells were counted and expressed as a percentage of SERT-positive cells.

In the case of double staining in Study III 7/20 subjects were examined and the numbers of epidermal SERT-positive cells were counted in two sections per sample and then the epidermal cells double stained for the other primary antibodies (CD1a and LAMP) were counted and expressed as a percentage of SERT-positive cells.

3.4 STATISTICAL ANALYSIS

To compare the total numbers of labelled cells in involved and non-involved skin compared to normal skin the Mann-Whitney U-test was used (Studies I, II) For within-group analysis to compare between involved and non-involved skin the Wilcoxon signed rank test was used (Studies I,II,III).

In Study III the Pearson correlation coefficient was used to measure the strength of a linear correlation between number of cells expressing SERT in the epidermis and papillary dermis, and in PASI and BDI, respectively. The Spearman correlation coefficient was used to measure the strength of a linear correlation between number of cells expressing SERT in the epidermis and papillary dermis and salivary cortisol ratio levels.

A p-value of <0.05 (two-tailed) was considered to be statistically significant. All the analyses were carried out using SAS software (SAS Institute, Cary, NC, USA).

In Study III missing data was handled by exclusion from correlation for one patient regarding salivary cortisol level and for one patient regarding numbers of SERT-positive cells in the papillary dermis and epidermis.

STUDY IV

3.5 SUBJECTS

The source population was all Swedish residents ever diagnosed with plaque psoriasis by dermatologists (ICD; L40.0, L40.5, L40.8-9) and registered in the National Patient Register (in-hospital care 1997-2006 and outpatient care 2001-2006). In total, 69830 patients were identified.

3.5.1 Exposed cohort

The cohort of SSRI-exposed subjects (n=1282) had a prescription dispensed of fluoxetine, citalopram, paroxetine, sertraline, fluvoxamine, or escitalopram twice during a period of six months at a Swedish pharmacy between 1st July 2006 and 1st April 2008. To assure incident SSRI treatment we conformed to a wash-out period of at least one year without any dispensed SSRI drugs. Cohort entry was defined as date for dispensed SSRI prescription. Psoriasis disease severity was classified according to the last psoriasis treatment dispensed (systemic or non-systemic) up to six months prior to cohort entry. Severe psoriasis was defined as use of systemic treatment (methotrexate, cyclosporine, acitretin, and biologics (etanercept, infliximab, efalizumab and infliximab)), while patients without systemic treatment were classified as having mild psoriasis. This method for psoriasis classification has previously been used and validated in several publications.[104-107]

3.5.2 Reference cohort

The reference cohort included patients from the source population with psoriasis but unexposed to SSRI (n=1282). Reference patients were matched for age, sex, county of domicile, psoriasis severity (systemic or non-systemic psoriasis treatment), and for seasonal variation. Seasonal variation was matched between the two cohorts as same date of psoriasis drug disposure ± 2 months.

3.5.3 Main outcome measure

The main outcome measure was change in psoriasis severity by means of switching psoriasis treatments from systemic to non-systemic or *vice versa* six months after exposure to SSRI. Outcome was measured between six-to-twelve months after cohort

entry, defined as first psoriasis drug dispensation or absence of psoriasis drug dispensation.

3.6 STATISTICAL ANALYSIS

To measure change in psoriasis severity we studied switching or not switching in psoriasis treatments, from systemic to non-systemic treatment and *vice versa* in our two cohorts – exposed and unexposed to SSRI (binary outcome and binary predictor). Conditional logistic regression was used to determine the odds ratio (OR) and 95% confidence interval (CI) of switching psoriasis treatments after exposure to SSRI. The OR was adjusted for age, sex, and county. Age was divided into 10-year strata and counties in Sweden divided into three regions, one rural and two urban. We also performed a sensitive analysis for DDD of SSRI at three levels: low, medium, and high. SAS 9.2 was used for statistical analyses.

STUDIES I-IV

3.7 ETHICS APPROVAL

All studies in this thesis were approved by a Regional Ethical Review Board. For Studies I-III informed consent was obtained from the patients prior to enrolment in the studies. For Study IV the Regional Ethical Review Board in Stockholm did not require informed consent from the patients.

4 RESULTS

4.1 STUDY I

4.1.1 5-HT1A receptor

There was a decreased expression of 5-HT1AR positive cells in the papillary dermis in involved ($p<0.001$) and non-involved skin ($p<0.001$) compared to normal skin. No difference was recorded between the involved and non-involved skin. The majority of 5-HT1AR-positive cells were tryptase positive, with a few CD4 and CD8 positive cells. There was also 5-HT1AR immunoreactivity in the vessel walls.

In the normal and non-involved skin there were immunoreactive cells in the basal epidermis. Double staining of these cells with NK1-beteb indicated that they were melanocytes. There was also 5-HT1AR immunoreactivity of a reticular pattern in the upper part of the epidermis, staining 25% of the keratinocytes in the normal and non-involved skin and up to 40% in the involved skin.

4.1.2 5-HT2A receptor

There was an increased expression of 5-HT2AR-positive cells in papillary dermis in involved skin as compared to both normal ($p<0.001$) and non-involved skin ($p<0.01$). Nearly all 5-HT2AR positive cells were CD3 positive.

4.1.3 5-HT3 receptor

There were 5-HT3R positive cells in the basal part of the epidermis in non-involved skin, which were absent in the involved skin. The acrosyringium of involved skin exhibited immunoreactivity.

4.2 STUDY II

4.2.1 Serotonin

Serotonin was present in the platelets of the involved skin. There was also a focal expression in keratinocytes in 2/10 biopsies of the involved skin. These two biopsies had a high grade of inflammation and acanthosis. In the non-involved skin 1/10 biopsies exhibited diffuse keratinocyte expression. Normal skin had expression of serotonin in 1/10 biopsies with a weak staining in the apical part of epidermis.

4.2.2 SERT

There was an increased expression of SERT-positive inflammatory cells in the epidermis in the involved skin as compared to normal skin ($p<0.05$). There was also an

increased expression of SERT-positive mononuclear cells in the papillary dermis in the involved skin as compared to both non-involved ($p<0.01$) and normal skin ($p<0.01$).

In the involved skin 7/10 biopsies had focal or basal epidermal immunoreactivity as compared to apical epidermal immunoreactivity in 5/10 biopsies of the non-involved skin. In normal skin weaker apical epidermal immunoreactivity was evident in 8/10 biopsies. Intense SERT immunoreactivity was evident in adnexal structures, irrespective of skin inflammation.

Double staining revealed that the SERT-positive cells could express CD1a, CD3, and tryptase. A majority of the SERT-positive cells in the papillary dermis expressed caspase-3. Among the SERT-positive dendritic epidermal cells, only a few stained for caspase-3.

4.3 STUDY III

PASI values of the patients varied from 4-45 with a mean value ± standard deviation of 17±10. The mean of the cortisol ratio levels was 2.9±2.7 and the BDI score was 10.0±9.4.

As in study II the expression of SERT-positive cells in dermis in the involved psoriatic skin, with a mean ±SD value of 10.5±7.7 was increased as compared to the SERT positive cells in dermis in the non-involved skin, with a mean ±SD value of 0.9±1.8 ($p<0.001$). The expression of SERT-positive cells in epidermis in the involved psoriatic skin, (Fig. 1a) with a mean ±SD value of 5.0±4.4 was increased as compared to the SERT-positive cells in epidermis in the non-involved skin, with a mean ±SD value of 1.9±2.4 ($p< 0.001$).

4.3.1 Correlation data

There was a correlation between PASI and the numbers of SERT-positive inflammatory cells in the epidermis of the involved skin ($r=0.53$; $p<0.05$). There was also a high tendency for a positive correlation between PASI and the numbers of SERT-positive cells in the epidermis of the non-involved psoriatic skin ($r=0.44$; $p=0.06$). Furthermore, a negative correlation between salivary cortisol ratio levels and the numbers of SERT-positive cells in the epidermis of the involved skin ($r=-0.46$, $p<0.05$) was evident. A low level of salivary cortisol ratio indicates chronic stress; our finding therefore indicates that an increased epidermal SERT expression in psoriasis correlates with chronic stress.

No correlation was observed between dermal SERT expression in involved and non-involved skin and PASI or salivary cortisol ratio levels, respectively. No correlation was determined between epidermal and dermal SERT expression in involved and non-involved skin and BDI.

4.3.2 Double staining

Double staining revealed that 73% of the SERT-positive epidermal cells were positive for CD1a and 50% were positive for LAMP. These findings strongly indicate that the SERT-positive epidermal cells are dendritic cells (both immature and mature).

4.4 STUDY IV

Sixty-four percent of the patients were females. The mean age was 55 years among both the exposed and the reference cohort. At cohort entry 89% were classified as having mild psoriasis (Table 3).

Table 3. *Basic characteristics of the two cohorts*

Characteristics	Exposed group n=1282		Reference group n=1282	
Sex				
Female	482	36%	482	36%
Male	800	64%	800	64%
Age				
0-19	28	2%	28	2%
20-39	265	21%	265	21%
40-59	442	34%	442	34%
60-79	421	33%	421	33%
80-	126	10%	126	10%
Median (IQR)	57(41,69)		56(41,68)	
Mean	55.0		54.6	
Psoriasis severity				
Mild (no systemic treatment)	1136	89%	1136	89%
Severe (systemic treatment)	146	11%	146	11%

Among those with mild psoriasis 29 patients switched from non-systemic to systemic treatments after exposure to SSRI as compared to 64 patients in the reference group (OR 0.44 95% CI 0.28-0.68) (Table 4). Among study subjects classified with severe psoriasis, 42 patients switched from systemic to non-systemic treatment after SSRI use as compared to 37 patients in the reference group (OR 1.20 95% CI 0.71-2.02) (Table 4). Both these findings indicate that SSRI may have a protective effect in psoriasis.

To assess whether this effect might be dose-dependent we investigated days covered with DDDs of SSRI and changes in psoriasis severity. Among those with more than 90% of days covered with 1 DDD/day of prescribed SSRI and mild psoriasis, 23 patients switched treatments after SSRI use as compared to 49 patients in the reference group (OR 0.45 95% CI 0.27-0.75). The protective effect of SSRI is also seen among those with 40-90% of days covered with 1 DDD/day of prescribed SSRI, where four patients with mild psoriasis switched treatments after SSRI use as compared to 13 patients in the reference group (OR 0.30 95% CI 0.10-0.93) (see Table 4, manuscript IV).

Table 4. *Number of persons switching and risk of switching psoriasis treatments before vs. after SSRI exposure. The risks are depicted as adjusted odds ratios with 95% confidence intervals adjusted for age, sex, and county.*

Treatment at baseline	Exposed cohort (No=1282)		Reference cohort (No=1282)		Adjusted odds ratio (95%CI)
Non-systemic					
No Switch	1107	97.4%	1072	94.4%	Reference
Switch (NS→S)	29	2.6%	64	5.6%	0.44 (0.28-0.68)
Total	1136		1136		
Systemic					
No switch	104	71.2%	109	74.7%	Reference
Switch (S→NS)	42	28.8%	37	25.3%	1.20 (0.71-2.02)
Total	146		146		

Finally, we also studied whether this protective effect of SSRI was maintained between males and females. When stratified for sex, the odds ratio of switching treatments from non-systemic to systemic treatment after SSRI use was 0.51 for males, (95% CI 0.28-0.96) and 0.37 for females, (95% CI 0.19-0.71) (data not included).

We determined no major difference between the exposed and reference cohort in the use of phototherapy and the geographical distribution of the patients (data not included).

5 DISCUSSION

5.1 SEROTONIN

Serotonin was present in platelets of the involved skin. This is a well-known localization for serotonin. However, we did not detect any serotonin expression in the platelets of non-involved and normal skin. This could most probably be explained by the fact that in a high grade of inflammation, an enhanced vascularization, as in the involved skin will occur compared to in the non-involved and normal skin. There are findings supporting that altered platelet levels of serotonin may be involved in elevated blood pressure levels and cardiovascular disease.[108] Psoriasis is also associated with an increased risk of cardiovascular disease[104,109-111]. It might be that serotonergic pathways can contribute to this comorbidity in psoriasis.

The focal serotonin expression in keratinocytes evident in two patients in the involved skin in Study II is interesting, even though no definitive conclusions can be drawn from such small findings. This is in contrast to earlier studies by Huang *et al.*[38] in which serotonin expression in psoriasis was observed in prickle cells, sweat gland cells, sebaceous gland cells and hair roots with a more prominent staining in 'progressive' psoriasis compared to the 'static' stage of disease. These differences in serotonin expression might be explained by different patient characteristics and different technical procedures. We used immunohistochemistry on biopsies treated with Lanas fix with polyclonal antibody to serotonin, while Huang and colleagues used paraffin-embedded tissue and a "Rabbit anti-serotonin multicolonal antibody".

Whether the focal staining seen in the keratinocytes was cytoplasmatic or membrane attached was not assessable.

5.2 SEROTONIN RECEPTORS

The expression of 5-HT1AR was mainly localized to mast cells and melanocytes. The mast cells produce important inflammatory cytokines and neuropeptides[112] and the number of mast cells are increased in psoriasis.[113] A 5-HT1A-mediated promotion of cell survival and proliferation of mitogen-activated T and B lymphocytes,[45] a 5-HT1AR stimulation that inhibits apoptosis in neuronal cells,[114,115] and a protection of NK cells against oxidation-induced apoptosis[116] may contribute to a prolonged life span of mast cells in psoriasis, leading to prolonged release of proinflammatory cytokines and neuropeptides, thus maintaining chronic inflammation. Moreover, the flare-up of psoriasis during stress conditions might be explained by hormonal effects through 5-HT1AR on mast cells and the subsequent release of inflammatory mediators.

In the brain, the firing rate of serotonergic neurons is inhibited by 5-HT1AR and results in a decreased release of serotonin from these cells. Melanocytes may contain serotonin. [40,41,117] We do not know if a similar mechanism occurs in melanocytes, constituting an autocrine system, where released serotonin would feedback on the cell through the 5-HT1AR.

The increased immunoreactivity in the upper epidermis in involved skin might be explained by the fact that the normal keratinocyte differentiation process is changed

and the presence of 5-HT1AR may suggest an up-regulation of the receptor due to decreased activation.[30] It has been reported that 5-HT1AR is important in preventing apoptosis in neuronal cells and hippocampal HN2-5 cells.[47] A decreased activation of 5-HT1AR may lead to stimulated apoptosis and it could be that the keratinocytes in upper epidermis enter apoptosis, leading to an increased turnover of keratinocytes.

The most important finding is the decreased expression of 5-HT1AR-positive cells in the papillary dermis. These cells were mainly mast cells and some lymphocytes. 5-HT1AR might influence inflammation in psoriasis by mechanisms described above or by another pathway, with altered neuropeptide release supporting TNFα-induced inflammation. Furthermore, a 5-HT1AR agonist (buspirone) could suppress a contact-allergy reaction.[54]

There was an increase of 5-HT2AR-positive cells in papillary dermis and double-staining indicated that they were lymphocytes. DOI, a 5-HT2AR agonist, was able to potently inhibit the effects of TNFα.[61] In another disease with chronic and TNFα-driven inflammation, RA, it was observed that the density of 5-HT2AR was decreased, suggesting a possible role for reduced expression of the 5-HT2AR in the sensitization of cells to TNFα in RA.[118] An up-regulation of 5-HT2AR could reflect a high inflammation or being a stimulator of inflammation. This effect of 5-HT2AR in involved skin was also seen in the delayed type hypersensitive reaction, where a 5-HT2AR antagonist (ketanserin) could suppress a contact-allergy reaction.[57]

The different dynamics of reversed expression in involved skin, with a decrease of 5-HT1AR and increase of 5-HT2AR expression are intriguing since in short 5-HT1AR suppresses inflammation and 5-HT2AR triggers inflammation. These two receptors may work together in an antagonistic way to maintain inflammation in psoriasis.

The reduced immunoreactivity of 5-HT3R in involved skin in basal epidermis implicates that it may affect keratinocyte proliferation and differentiation. 5-HT3R antagonists inhibit the stimulated release of SP, neurokinin A and CGRP from primary afferents[119] and prevent unmasking of autonomous tachykinin NK2R.[120] Diminished 5-HT3R may therefore affect neuropeptides in psoriasis.

Regarding the role of the serotonergic mechanisms in psoriasis our findings are just hypothesis-generating. To further elucidate the role of the serotonergic receptors in psoriasis it would be interesting to examine their contact with the nerve endings. We know that serotonergic receptors were expressed in immune cells and mast cells in the papillary dermis where they are in close contact with nerve endings. Double staining with a marker for nerve endings could be useful.

Another key issue is the functionality of the receptors. Are we staining functionally active receptors? It would be very useful to perform a ligand-binding study where either skin biopsies or a cell line (for example keratinocytes) would be treated with a specific agonist or antagonist and then the activity of the involved receptors could be measured. So far this technique is not available in skin specimens.

5.3 SERT

The expression of SERT-positive cells in the epidermis and papillary dermis of the involved skin is increased in involved skin as compared to normal skin and the non-involved skin. These findings are in agreement with earlier findings in other inflammatory dermatoses such as atopic eczema[121] and allergic contact eczema.[36] We also determined that increased epidermal SERT expression in inflammatory cells is correlated to psoriasis severity and chronic stress, implicating a role for the serotonergic system in psoriasis.

Double staining revealed that SERT-positive cells in the epidermis were either dendritic cells or lymphocytes and in the papillary dermis lymphocytes or mast cells. One disadvantage with the double staining procedure in Study II was that we did not count the percentage of SERT-positive cells that expressed CD1a, CD3, or tryptase. However, we did that for caspase-3 in Study II and for CD1a and LAMP in Study III. We can conclude that the majority of SERT-positive cells in epidermis are dendritic cells and that the SERT-positive cells in the papillary dermis were mainly mast cells or lymphocytes.

Dendritic cells have a key role in the pathogenesis of psoriasis, bridging the gap between innate and adaptive immunity.[8] Dendritic cells have been reported to express functional SERT at a level that is regulated by their state of maturation. Immature dendritic cells may sequester serotonin released by platelets or mast cells at sites of injury and inflammation and mature dendritic cells may shuttle serotonin between activated and naive T cells, thereby amplifying adaptive immune responses.[122] Serotonin activates different dendritic cell signaling pathways in a maturation-dependent manner[123] and in this respect is able to modulate the release of different chemokines and cytokines in human monocytes.[124] Serotonin could also inhibit apoptosis in human monocytes, allowing these cells to remain in the tissue and to contribute to chronic inflammation.[125] In addition, SSRI suppress the ability of dendritic cells to present bacterial antigens to T cells and could thus down-regulate the resulting T-cell proliferation in a SERT/5-HT-independent manner.[126] Furthermore, migration of dendritic cells may be affected. These modes of actions of SERT might be possible explanations for the positive correlation between psoriasis severity and SERT expression in Study III.

The co-localization of immunostaining of SERT-positive cells in the papillary dermis and caspase-3 might indicate that the immune cells of the skin that enter into apoptosis may express SERT. It has been demonstrated that SERT synthesis may be stimulated by IFNα, an important cytokine for psoriasis pathogenesis.[73] This could lead to a regulatory loop in which chronic inflammation and release of IFNα could stimulate SERT synthesis that would lead to apoptosis of immune cells, thus maintaining chronic inflammation. Modulating the levels of SERT expressed by the immune cells could possibly lead to a change in chronic inflammation. This finding led us to Study IV and to analyse the use of SSRI in psoriasis.

We also determined a modified epithelial SERT expression, with a stronger basal expression in the involved skin as compared to non-involved skin, but also as compared

to a weaker apical staining in normal skin. These findings are just observations in ten patients with no possibility of further statistical analysis. In another disease with chronic inflammation, IBS, there have been numerous reports of modified epithelial SERT expression. Levels of SERT mRNA have so far been reported to be elevated in the small intestines of patients with IBS,[68] reduced in rectal biopsies from patients with ulcerative colitis and IBS,[69] and in guinea pig colitis,[70] and normal in rectal and sigmoidal mucosal biopsies from patients with IBS.[71] This modified epithelial expression indicates that SERT may also have a direct role in epithelial proliferation and differentiation in psoriasis.

As for the serotonin receptors we lack information about the functionality of the SERT expression. At the SERT gene level there were no significant differences in genotype distribution and allele frequencies in a German population.[65] More limitations are the lack of patient characteristics in Studies I and II. We had no information of important potential confounders such as smoking and alcohol. The patients were not receiving systemic or local treatments for the previous two weeks prior to biopsy, but no information regarding possible previous systemic or local psoriasis treatments or other important medications were obtained. In Studies I and II no information about psoriasis severity, chronic stress, gut inflammation or depression was obtained. The present results are limited but they generate further questions and new important theories about the role for the serotonergic system in psoriasis.

5.4 USE OF SSRI IN PSORIASIS

Bearing in mind the possibilities to modify SERT expression our aim was to investigate the role of SSRI in psoriasis. The golden standard for such an investigation would be a randomized clinical trial. With limited resources that was not an option. Instead we conducted a broad population-based cohort study from Swedish healthcare registers with incident SSRI treatment as exposure and change in psoriasis treatments (a surrogate for psoriasis severity) as outcome.

We demonstrated that patients with mild psoriasis have a decreased need for systemic psoriasis treatment after starting SSRI treatment. We also determined that patients with severe psoriasis are more likely to have a decreased need for systemic psoriasis treatment after SSRI use.

One possible explanation would be that treatment of clinical depression will have a direct effect on psoriasis symptoms and thus reduce the need for psoriasis treatment or an indirect effect where improvement in the exposed subjects' mood disorder will increase their ability to comply with non-systemic psoriasis treatments. Another explanation could be a direct biological anti-inflammatory effect of SSRI, either involving serotonin signaling pathways or being independent of the serotonergic system. Animal studies and some human models have indicated that some SSRI may have anti-inflammatory effects.[84,85]

Particular strengths of Study IV include its thorough design with a well-defined study base, which minimizes bias while maximizing generalizability of the results. We

included two strict criteria for exposure to SSRI. First, to assure incident SSRI treatment only patients with a wash-out period of SSRI of one year or longer were included. Second, only patients with at least two dispensed prescriptions of SSRI during six months were included. This reflects a clinically relevant SSRI treatment, since the biological effects of SSRI will take approximately 4-6 weeks to evaluate. Patients who face side-effects after a few weeks were not included in this study, since available register information prevented us from finding out whether these patients stopped treatment because of side-effects or for any other reason. Further strengths are that the diagnosis of plaque psoriasis was made by dermatologists, minimizing misclassification of disease. Both databases used have high internal validity with identity data missing for <0.3% of all items in the Swedish Prescribed Drug Register[94] and 0.5% for the National Patient Register. One limitation is that no validation was conducted regarding the diagnosis of psoriasis vulgaris. However, it is a clinical diagnosis and the ICD-coding system for psoriasis vulgaris is stringent. In RA, another disease with chronic inflammation, one validation study from the NPR reported that the positive predictive value for RA-diagnosis was around 87-94%.[92]

The reference group is strictly matched to allow us to look at the effect of SSRI exposure. However, this does not allow us to draw any other conclusions than those we studied. One important matching factor is the seasonal variation, not only reflecting the clinical impression from Sweden that psoriasis improves during summertime. It is also evident that there is a seasonal variation in the binding potential for the target protein of SSRI, the SERT.[127]

There are some important limitations to consider. By using systemic psoriasis treatment as a construct to measure severe psoriasis it is possible that there is misclassification with regard to psoriasis severity. Patients with severe psoriasis may not receive systemic treatment and may therefore be misclassified into the mild psoriasis cohort. The possibility of misclassification is unlikely to differ between study groups and may be regarded as non-differential. Another limitation is that our study design does not include those who experience side-effects of SSRI and stop the SSRI treatment after one or two months. These individuals could be highly interesting if they develop a flare-up of psoriasis as a side-effect. In that case we would overestimate the protective effect of SSRI in psoriasis. However, this also strengthens our study since we could be certain that the subjects in the two study groups tolerated SSRI and are those who could benefit from the possible treatment effects.

More limitations to consider include the dermatologist's delay in changing psoriasis treatments even though the patient experiences a change in psoriasis severity. Phototherapy is traditionally a fairly common systemic treatment used for psoriasis in Sweden. We did not include this entity as a marker for severe psoriasis since it is not registered in the Prescribed Drug Register. Phototherapy is recorded in the National Patient Register, but there may be variations between counties in the reporting of such outpatient treatment procedures. We therefore investigated use of phototherapy and the geographical distribution of the patients and we determined no major differences between the exposed and the reference groups. We did lack information regarding two important confounders, smoking and alcohol.

In conclusion, our results suggest that SSRI may have a protective effect in psoriasis. To our knowledge this finding has not been previously described.

5.5 METHODOLOGICAL CONSIDERATIONS

Immunohistochemistry is useful in describing expression of receptors and proteins. The method is reproducible and transparent in trained hands. One has to be cautious when interpreting results from the method for several reasons. First of all the sections analyzed are relatively limited parts of a punch biopsy. Furthermore, the representativeness of a punch biopsy may differ depending on which area you biopsy. We therefore tried to be consequent taking the biopsies from the lower back from both involved and non-involved skin and from the reference persons with normal skin. Nevertheless the 'lower back' is a quite broad area and there may be local differences in the skin.

All the staining procedures were conducted consecutively for all the patients, eliminating staining failures. Six sections from one patient were stained on the same glass slide, grouped two-by-two, allowing three possible primary antibodies to be employed in the same staining procedure. We chose to have one group of two sections with the primary antibodies omitted as control, and the other two groups with the primary antibody, a total of four sections per patient. In the case of double staining two sections were controls with primary antibody omitted. Two sections were only stained with the first antibody and the last two sections were double-stained.

Interpreting immunohistochemistry is dependent on the examiner. In all three studies the positive cells were always counted by the same observer blinded to the nature of the specimen (involved skin, non-involved skin, and normal skin), and the patient examined. However, in some cases the acanthosis and broadened rete ridges with thin suprapapillary areas were obvious, indicating involved skin. In two of the studies (II and III), an independent examiner re-counted the cells and no major differences were determined.

Immunohistochemstry only allowed us to describe receptor and protein expression. You cannot say if you have stained an active or inactive receptor or protein and therefore no statements about functionality can be made. It would be important to follow-up the observed findings and try to use other methods for functionality (activation or blocking with receptor agonists or antagonists in a ligand-binding study, either *in vitro* with for example cultured keratinocytes, monocytes or lymphocytes or *in vivo* with skin specimens). We did try to see one form of functionality by correlating the expression of SERT with important patient characteristics.

A particular strength is that we have had an internal control in the case of the non-involved skin. Conversely, psoriasis is a systemic disease and we do not know if there are more important observations or inflammatory processes in the non-involved skin compared to the involved skin. For example, the numbers of mast cells are increased in the non-involved skin in psoriasis,[128] indicating that there is also inflammation in the non-involved skin as well.

As mentioned in the *Discussion* we lack important information about patient characteristics in Studies I and II, which could influence the results.

In Study III we used salivary cortisol ratios as an indicator for chronic stress. The use of salivary cortisol as a measure of stress is debatable.[10] In our study we used both a dexamethasone-suppressed salivary cortisol level and a mean morning cortisol level of three observations. The ratio of the mean of the three values to the last cortisol value was determined, a low ratio being an indicator of chronic stress. This method for chronic stress has previously been used in other studies.[98,121]

In Study III we used the Beck's Depression Inventory for measure of depression. A more commonly used measurement is Montgomery Åsberg Depression Rating Scale (MADRS).

In Study IV we used a population-based cohort study. In a cohort study a number of patients with a specific exposure are followed over time and the occurrence of different outcomes are studied and compared with an unexposed cohort. A cohort study is efficient for unusual exposures but usually not for rare outcomes and gives possibilities to study multiple endpoints. Cumulative risks, incidence rates, relative risks and attributable risks can be calculated. On the negative side is that cohort studies can be costly, time consuming, the validity of the results can be influenced by loss to follow-up, and there is a risk of selection and information bias. [129] This was not the case in our study where register data allowed us complete follow-up and already finished study-time.

6 CONCLUSION

- In psoriatic skin the expression of serotonin is limited to platelets in the involved skin.

- There is a decreased expression of 5-HT1AR in involved skin as compared to normal skin and there is an increased expression of 5-HT2AR in involved skin as compared to non-involved and normal skin. This reversed receptor expression concords with their antagonist function; 5-HT1AR inhibiting inflammation and 5-HT2AR being pro-inflammatory.

- The expression of SERT is increased in involved skin as compared to non-involved and normal skin.

- An increased SERT expression in inflammatory cells in epidermis correlates to psoriasis severity and chronic stress.

- SSRI may have a protective effect in psoriasis, since patients with mild psoriasis have a decreased need for systemic psoriasis treatment and patients with severe psoriasis are more likely to have a decreased need for systemic psoriasis treatment after starting SSRI treatment.

- These findings indicate a role for the serotonergic system in psoriasis both as a neuroimmunological bridge between the psychological symptoms and the disease, as well as an actor in the inflammatory process.

7 QUESTIONS FOR THE FUTURE

- Is the change in serotonin receptors and SERT expression observed by immunohistochemistry related to functional differences?

- Is there a therapeutic potential of selective modulation of 5-HT1A and 5-HT2A receptors for the treatment of psoriasis?

- Are SSRI a possible new and easily accessible treatment option for psoriasis? Future clinical trials would be of interest.

- Are there other treatments strategies to modulate SERT in the skin, such as for example topical treatments, not having the systemic effects of SSRI?

- Could it be that the important serotonergic mechanisms in psoriasis are due to the presence of SERT and serotonin receptors in keratinocytes?

- Is there a correlation between a functional SERT gene polymorphism and the severity of disease?

8 SUMMARY IN SWEDISH

Psoriasis är en kronisk inflammatorisk hudsjukdom, 2-3% av alla svenskar är drabbade. Stress försämrar psoriasis. En viktig signalsubstans vid stressutlöst inflammation är serotonin. Serotonin utöver sina effekter via ett antal olika serotoninreceptorer, varav två stycken är bättre karaktäriserade än andra – serotonin receptor 1A och serotonin receptor 2A. Serotonins inflammatoriska effekt regleras av ett transportörprotein, serotonin transporter protein (SERT). Vid inflammatoriska sjukdomar såsom psoriasis och IBS (Irritable Bowel Syndrome) är nivåerna av detta protein ändrat och det tycks spegla den inflammatoriska aktiviteten.

Vårt mål var att kartlägga vad som driver den inflammatoriska processen vid psoriasis och om det serotonerga systemet kan vara involverat. En ökad förståelse av vad som driver den inflammatoriska processen vid psoriasis kan på sikt leda till nya behandlingsmetoder av sjukdomen.

I de första tre studierna använde vi oss av involverad och icke-involverad hud från psoriasispatienter och från friska individer. Huden fryssnittades och därefter användes en färgningsteknik, där antikroppar som binder till serotonin receptorer eller SERT syns i mikroskopet. På så sätt kan man jämföra förekomsten av dessa receptorer och SERT mellan friska individer och psoriasispatienters involverade och icke-involverade hud.

I den första studien sågs minskad förekomst av serotonin receptor 1A i den involverade huden och en ökad förekomst av serotonin receptor 2A jämfört med den icke-involverade och den friska huden. I den andra studien sågs ökad förekomst av SERT i inflammatoriska celler i den involverade huden jämfört med den icke-involverade och den friska huden. I den tredje studien kunde vi påvisa att ju större förekomst av SERT i överhuden desto större var svårighetsgraden av psoriasis. Dessutom kunde vi påvisa att ju större förekomst av SERT i överhuden desto högre grad av kronisk stress uppvisade patienterna.

Genom att påverka nivåerna av SERT skulle man kanske kunna påverka inflammationsprocessen i psoriasis. Ett sådant preparat är serotoninåterupptagshämmarna (SSRI – ”lyckopiller” vid depression). Detta ledde fram till den fjärde studien, där vi genom de unika svenska hälsoregistren kunde få fram 69835 individer med psoriasis, där 1282 individer hade behandlats med SSRI under åtminstone 6 månader och 1282 individer med samma kön, ålder, boenderegion, svårighetsgrad av psoriasis, och given SSRI behandling under samma tidsperiod på året. När man jämförde dessa två grupper visade det sig att de som fått SSRI i mindre utsträckning behövde ”tyngre” psoriasisbehandling. Således visade studien att det tycks finnas en skyddande effekt av SSRI behandling vid psoriasis.

Med den här avhandlingen konstateras att det serotonerga systemet tycks ha en inverkan på den inflammatoriska processen vid psoriasis.

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