Selective serotonin reuptake inhibitors as a novel class of immunosuppressants

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A B S T R A C T

In the past decades, selective serotonin reuptake inhibitors (SSRIs) have been shown to exert several immunological effects, such as reduced lymphocyte proliferation, alteration of cytokine secretion and induction of apoptosis. Based on these effects, SSRIs were proposed as drugs for the treatment of autoimmune pathologies and graft-versus-host disease. This review summarizes preclinical and clinical evidence supporting a role for SSRIs in autoimmune diseases and graft-versus-host disease, and discusses what is known about the mechanism underlying these effects.

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Contents

1. Introduction ........................................................................................................... 148
2. Effects of SSRIs on immune cell functioning .................................................. 149
   2.1. Proliferation .................................................................................................. 149
   2.2. Cytokine secretion ....................................................................................... 149
   2.3. Apoptosis ..................................................................................................... 149
3. Potential mechanisms of action ......................................................................... 150
   3.1. Involvement of 5HT and its transporter ..................................................... 150
   3.2. Effects on signal transduction pathways .................................................... 151
   3.3. Induction of the apoptotic cascade ............................................................. 151
   3.4. Unexplored mechanisms ............................................................................ 152
4. Animal studies of SSRIs as immunosuppressants ........................................... 152
   4.1. Autoimmune diseases ............................................................................... 152
   4.2. Graft-versus-host disease ......................................................................... 153
5. From bench to bedside ....................................................................................... 153
   5.1. Plasma concentrations .............................................................................. 153
   5.2. Clinical evidence supporting the human use of SSRIs as immunosuppressants ........................................................................................................... 154
6. Conclusion .......................................................................................................... 154

References .................................................................................................................. 154

1. Introduction

Selective serotonin reuptake inhibitors (SSRIs) are amongst the most prescribed drugs worldwide [1]. Indications for these drugs are broad and comprise major depression, panic disorder, obsessive-
compulsive disorder and other less well-established indications such as obesity, eating disorders, post-traumatic stress disorder, social phobia and premenstrual disorder [2]. In comparison to other types of antidepressants, SSRIs have a more beneficial adverse effect profile with nausea, diarrhea, sexual dysfunction, headache, dizziness, agitation, insomnia and under certain circumstances increased suicide risk [2,3]. In addition to these well described side effects, there are indications from animal studies [4], in vitro studies [5], and some clinical observations in patients with depression [6] that SSRIs treatment may affect the cellular immune response.

Abnormalities in the proliferation, cytokine secretion and viability of peripheral blood lymphocytes have been observed when these cells were exposed to SSRIs. Moreover, the replication and viability of cancer cells are also affected by SSRIs. Several groups have attempted to reveal the underlying mechanism by which these effects are executed. An overview of the immunological effects of SSRIs on immune cells is provided, both in in vitro and in vivo situations, and special attention is paid to the role of serotonin (5HT) and its transporter in this effect.

The majority of research reports immunosuppressive effects of SSRIs such as decreased lymphocyte proliferation and reduced pro-inflammatory cytokine secretion [5,7]. Therefore, SSRIs have already been tested in several animal models of autoimmune disorders and graft-versus-host disease [8–14]. However, for fluoxetine an immunosuppressive as well as an immunostimulatory effect was described, depending on the concentration used and the degree of lymphocyte activation in vitro [15,16]. Similarly, the beneficial effect of fluoxetine in the treatment of lymphoma [17,18] was attributed to both a direct suppressive effect on the malignant cells and a stimulatory effect on anti-cancer immunity. Thus, it appears that under specific in vitro and in vivo conditions, fluoxetine can exert immunostimulatory effects, which seem applicable in the treatment of lymphoma. The potential applications of SSRIs in cancer were recently reviewed by Frick and Rapanelli [19]. As the doses of fluoxetine used in animal models of lymphoma and autoimmune disorders were in the same range (10–25 mg/kg) [11,12,18,20], the difference between immunostimulation and immunosuppression in vivo does not rely on the different dose used. Instead, the investigated disease and its underlying immunological mechanism appear to determine whether fluoxetine exerts either a stimulatory or a suppressive effect. For other SSRIs such as paroxetine and sertraline, only immunosuppressive effects have been described [5,8,13]. In this review we will focus on the immunosuppressive effect of SSRIs.

Based on the observed immunosuppressive effects, the application of SSRIs in autoimmune disorders and graft-versus-host disease was examined. Although several research groups have shown improvement in clinical scores in animal models of autoimmune diseases, discussion remains about the feasibility of using SSRIs as immunosuppressants in men, as the doses applied in animal studies are generally higher than the ones currently used in humans for the treatment of depression. Nevertheless, limited clinical evidence is available demonstrating the usefulness of SSRIs in autoimmune disorders.

2. Effects of SSRIs on immune cell functioning

SSRIs have been shown to alter several aspects of immune cell functioning. Not only the proliferation, but also cytokine secretion and viability of lymphocytes are affected when exposed to SSRIs. In addition to lymphocytes, cancer cells also seem to undergo changes when they are incubated with SSRIs [21–23]. Finally, recent evidence showed an effect of fluoxetine on neutrophil adhesion and recruitment to inflammatory sites, demonstrating that not only cellular but also innate immunity is impacted by SSRIs [24].

2.1. Proliferation

In the last decades, several research groups have demonstrated that micromolar concentrations of SSRIs are capable of altering lymphocyte proliferation. In vitro exposure to paroxetine, sertraline and fluoxetine has been shown to decrease the proliferation of mitogen-stimulated lymphocytes in a concentration-dependent manner [5,15,16,25–27]. An anti-proliferative effect has also been observed in Jurkat T cells [28]. Pellegrino and Bayer found that in vivo administration of fluoxetine to rats similarly decreased lymphocyte proliferation when induced by mitogens ex vivo [29,30]. The effect, however, seems to be dependent on the activation status of the cells. At suboptimal mitogenic Concanavalin A (ConA) concentrations, relatively low concentrations (0.1–1 μM) of fluoxetine have been found to stimulate T cell proliferation [15,18]. In contrast, at optimal ConA concentrations, 1 μM fluoxetine inhibited T cell proliferation and a maximal suppressive effect was reached at 10 μM [15]. Although in some situations low levels of fluoxetine seem to stimulate lymphocyte proliferation, the majority of research in general points to a negative immunoregulatory effect of SSRIs on lymphocytes. Our own data support the observation that SSRIs reduce T cell proliferation in a concentration-dependent manner at concentrations equal to or higher than 1 μM when stimulated with anti-CD3/CD28 beads [14]. In addition to fluoxetine, other clinically available SSRIs (paroxetine, sertraline, citalopram, and fluvoxamine) also appear to induce this anti-proliferative effect [14]. Although the SSRIs concentrations used in in vitro experiments are in the micromolar range, the anti-proliferative effect is concentration-dependent, indicating that this effect is specific and not due to general toxicity.

2.2. Cytokine secretion

Although investigated the most extensively, proliferation is not the only parameter affected by SSRIs. The functional ability of lymphocytes, under the form of cytokine secretion, is equally affected. For example, 20 μM citalopram decreased IL-2 and IFNγ secretion by mitogen-activated T cells [31]. Furthermore, paroxetine and sertraline (0–30 μM) have been demonstrated to reduce TNFα secretion by human anti-CD3 stimulated T lymphocytes [5]. Others showed that sertraline (0.01 and 1 μM) significantly decreases the IFNγ/IL-10 ratio in the supernatant of mitogen-stimulated whole blood [7,32]. Although these studies point in the same direction, showing a suppressive effect of SSRIs on the production of pro-inflammatory cytokines, it should be noted that these studies are not equal in terms of experimental setup. Whereas the first two studies used purified lymphocytes, Maes et al. used whole blood assays. In the latter model interactions between different types of blood cells are preserved, and this model is therefore believed to be more representative for the in vivo situation. Recently, Shenoy et al. demonstrated that not only peripheral blood lymphocyte but also thymocyte cytokine production is suppressed by citalopram [33]. Concentrations ranging from 25 to 250 μM citalopram completely suppressed anti-CD3 triggered IL2 production, severely reduced IL4 and partially suppressed IL17 production [33]. As is the case for the anti-proliferative effect of SSRIs, suppression of cytokine production is a concentration-dependent effect, confirming that it is not due to general cytotoxicity.

2.3. Apoptosis

Finally, SSRIs have been shown to induce apoptosis in lymphocytes. Whereas paroxetine and sertraline were found to decrease activated T cell viability with an IC50 of around 10 μM [5], other SSRIs exerted this effect only at tenfold higher concentrations. For citalopram, an IC50 of 180 μM was reported for pro-apoptotic action on naïve T cells [34]. According to our own research, this apoptotic effect is induced by all SSRIs used in clinical practice (paroxetine, fluoxetine, sertraline, fluvoxamine and citalopram), albeit in different concentration ranges [14]. Not only do SSRIs affect the function of healthy lymphocytes, but they also seem capable of reducing the viability of several cancerous immune cells. Amit et al. showed that paroxetine (IC50 = 18 μM) and
sertraline (IC$_{50} = 9.5 \mu M$) reduced the viability of Jurkat T cells [28]. In Burkitt lymphoma cells, SSRIs (fluoxetine IC$_{50} = 9.3 \mu M$, paroxetine IC$_{50} = 6.9 \mu M$ and citalopram IC$_{50} = 20.9 \mu M$) could induce extensive apoptosis [22]. Also other cancerous cell lines, such as colon cancer cells are sensitive to the anti-proliferative and pro-apoptotic actions of SSRIs [23]. In comparison with cancer cells, resting peripheral lymphocytes are much less sensitive to the effects of SSRIs [22]. In contrast, actively proliferating lymphocytes respond to SSRIs in a comparable way as cancerous immune cells [35]. Our own data support that there is a difference in sensitivity to the pro-apoptotic action of SSRIs between activated and resting T cells, and that activated T cells undergo apoptosis at significantly lower SSRI concentrations [14]. According to Schuster et al., this discrepancy between resting and activated lymphocytes is due to the intrinsic higher sensitivity of proliferating cells to undergo apoptosis [35]. However, since the exact mechanism by which SSRIs induce their effects has yet to be established, other possibilities explaining the different responses cannot be excluded. In resting tonsilar B cells, serotonin transporter (SERT) protein was undetectable or expressed in only small amounts. Upon activation with mitogens, B cells upregulated SERT [36]. The observation that proliferating B cells were more sensitive to SSRI-induced effects than were resting B cells [36] leads to the assumption that SERT expression is an important factor in the execution of the immunomodulatory effect of SSRIs.

3. Potential mechanisms of action

Although the immunological effects of SSRIs have been described by several research groups, little is known about the mechanism underlying these effects. Initially, the inhibition of SERT and consequent rise in extracellular 5HT concentration were thought to be responsible for the anti-proliferative and pro-apoptotic action of SSRIs on lymphocytes. More recent research, however, provides several arguments against this assumption. Other research has focused on the participation of direct triggering or inhibition of signal transduction pathways in the immunological effects of SSRIs and on the pathways underlying the apoptotic action of SSRIs. Finally, some of the current views on antidepressant action in depression, such as modulation of membrane-associated lipid rafts or activation of the glucocorticoid receptor, may also be of importance in the immunomodulatory effects of SSRIs.

3.1. Involvement of 5HT and its transporter

Early work concerning the immunological effects of SSRIs assumed 5HT to be involved in the mechanism underlying the effects of SSRIs on lymphocytes. It was postulated that SSRIs increased the extracellular 5HT concentration by blockage of 5HT uptake through SERT, which has been shown to be present on the cell surface of lymphocytes (Fig. 1A) [26,37]. Pellegrino and Bayer demonstrated that elevation of 5HT levels through the administration of the 5HT precursor 5-hydroxytryptophan results in a decreased lymphocyte proliferation [29]. Also, when 5HT synthesis was inhibited in vivo, SSRIs were no longer capable of suppressing lymphocyte proliferation [29]. Lesioning of serotonergic neurons in vivo resulted in the same inability of SSRIs to decrease lymphocyte proliferation [29]. Thus, if no 5HT was present, SSRIs were not able to increase the extracellular 5HT concentration and no effect on proliferation was observed. Also, fluoxetine and sertraline, two SSRIs with distinct chemical structures but with the same capacity to block SERT, were found to exert similar anti-proliferative effects on lymphocytes whereas dopamine and noradrenaline reuptake inhibitors did not [29]. These findings suggest an important role of 5HT in the anti-

![Fig. 1. Possible mechanisms underlying the anti-proliferative and apoptotic effects of SSRIs on lymphocytes. A) Inhibition of 5HT uptake through SERT results in increased binding of 5HT on 5HT receptors, thereby reducing lymphocyte proliferation. The inability to take up 5HT itself might as well cause decreased lymphocyte proliferation. B) SSRIs increase cAMP levels, thereby activating the PKA pathway. SSRIs inhibit translocation of PKC, ultimately resulting in reduced lymphocyte proliferation and/or SSRIs increase Ca$^{2+}$ influx, causing reduced T cell proliferation in response to optimal mitogen concentrations. C) SSRIs induce activation of the apoptotic cascade, with activation of caspase 3 and MAPK, generation of reactive oxygen species (ROS), upregulation of Fas and downregulation of bcl-2 and c-myc.](image-url)
proliferative effect of SSRIs. In addition, 5HT itself has been shown to induce apoptosis in Burkitt lymphoma cells [38], and the pro-apoptotic action of SSRIs thus might as well be explained through the elevation of extracellular 5HT levels.

In contrast, 5HT has been identified as an important factor in the process of T cell activation [39–42]. Several research groups have shown that antagonism of 5HT receptors, as well as inhibition of 5HT synthesis, results in impaired T cell activation and proliferation. Both 5HT-1A [39], 5HT-1B [40,41] and 5HT-7 [41] receptors have been suggested to be involved in this process. Alternatively, it has been suggested that not the 5HT receptors, but the uptake of 5HT through SERT accounts for the mitogenic effect of 5HT [43]. Internalization of 5HT through SERT would lead to proliferation of the cells. Consequently, the anti-proliferative effect of SSRIs could be explained by the inhibition of 5HT uptake. These observations point to a stimulatory effect of 5HT on the activation and proliferation of lymphocytes. Taken together, the optimal activation of lymphocytes seems to require certain levels of 5HT, and both too low and too high concentrations result in sub-optimal lymphocyte activation, proliferation and viability. The role of 5HT in immune functioning has been described in more detail elsewhere [44–46].

On the other hand, several arguments have come up recently that refute the involvement of 5HT and SERT in the immunosuppressive effect of SSRIs. First, acetylation of fluvoxamine suppressed the capability of the compound to inhibit 5HT uptake, but did not impair the anti-proliferative effect [35]. Nevertheless, acetylation of paroxetine resulted in an increase of the IC50 from 6.5 μM to 93.3 μM [35] and thus decreased the ability of paroxetine to suppress proliferation. Whereas the anti-proliferative effect of paroxetine shifted 15-fold by acetylation, the affinity for SERT decreased over 1000-fold demonstrating that both effects are not entirely dependent on each other [35]. However, it should also be noted that isomerization of fluvoxamine from the trans to the cis form canceled its capability to suppress in vitro neural cell proliferation, as well as its ability to block 5HT uptake [47].

Second, the concentrations needed for the inhibition of 5HT uptake are in the nanomolar range, while those exerting an anti-proliferative effect are in the micromolar range [16,35]. Although Ferriere et al. found specific binding of 3H-paroxetine in fish lymphocytes to be in the nanomolar range (0–10 nm), micromolar concentrations were needed to substantially inhibit 5HT uptake in these cells [48]. Thus, the anti-proliferative action of SSRIs in the micromolar range might be explained by the substantial inhibition of 5HT uptake in this concentration range, notwithstanding specific binding of SSRIs to SERT already occurs in the nanomolar range.

Third, it was put forward that HEK293 cells, which were assumed not to express SERT, were still sensitive to the effects of SSRIs and thus these effects could not be mediated by SERT inhibition [35]. To this end, it should be noted that Chamba et al. found SERT expression in wild-type HEK293 cells both on mRNA and protein levels [49], suggesting that these cells might yet encounter SSRI-induced effects through SERT inhibition.

Cloonan et al. pointed out that not all SSRIs induced a pro-apoptotic effect (citalopram did not induce apoptosis in any of the tested cell lines), whereas they all do inhibit 5HT uptake through SERT. Further, the same group also showed that 5HT was not able to prevent the induction of cell death by SSRIs, and that 5HT itself, amongst other SERT ligands, could not induce apoptosis in the tested malignant cell lines [50]. In addition, SSRIs did not induce more extensive cell death in cells expressing higher levels of SERT [50]. Whereas Pellegrino et al. reported that in vivo administration of noradrenaline and dopamine reuptake inhibitors in rats did not affect lymphocyte proliferation, Diamond et al. found that antidepressants, inhibiting the reuptake of noradrenaline (reboxetine, desipramine) or not inhibiting the reuptake of any monoamine (trimipramine), were still capable of inhibiting in vitro T cell proliferation, as well as TNFα secretion [51].

Interestingly, it has been hypothesized that binding of monoamines on SERT can itself induce signal transduction pathways [38]. Possibly, binding of SSRIs on SERT induces the same changes in signal transduction pathways. Furthermore, 5HT uptake has been demonstrated to influence signal transduction directly through ‘serotonylation’ of small GTPases [52]. Thus, SSRIs might affect signal transduction through restriction of available 5HT for serotonylation.

Recently, a modified SERT knock-in mouse strain (SERT I172M) was developed that expresses a modified SERT protein with normal 5HT recognition and transport, but with a decreased sensitivity for antidepressants, including fluoxetine and citalopram [53]. The pruning question whether or not SERT is involved in the immunomodulating effects of SSRIs might be answered using this SERT I172M mouse model [46].

3.2. Effects on signal transduction pathways

Regardless of the blockade of SERT, further downstream events leading to SSRI-induced decreases in proliferation have been investigated by studying the interference of SSRIs with signal transduction pathways, such as the cAMP and phosphoinositol systems. SSRIs have been demonstrated to interfere with the activation of the cAMP-dependent protein kinase A (PKA) pathway and the activation of protein kinase C (PKC), as well as with the influx of Ca2+ (Fig. 1B).

cAMP has been shown to be an important regulator of immune responses by inhibition of T cell proliferation [54]. Consequently, an increase in cAMP in response to SSRIs could explain the anti-proliferative action of SSRIs on lymphocytes. At optimal concentrations of ConA, fluoxetine induced a rise in intracellular cAMP concentration [15,16]. Citalopram similarly elevated cAMP levels in T cells stimulated with phytohemagglutinin [31]. However, Kenis et al. did not find any increase in cAMP in peripheral blood mononuclear cells exposed to 0.01–1 μM paroxetine [55]. The same group further examined the involvement of cAMP and PKA activation in the immunoregulatory effect of fluoxetine and concluded that the cAMP-dependent PKA pathway was probably not involved in the fluoxetine-induced suppression of the IFNγ/IL-10 ratio, but the activation of PKA might contribute to the reduction in TNFα secretion [56].

On the other hand, PKC activation stimulates lymphocyte proliferation [16] and SSRI-mediated suppression of PKC translocation to the cell surface may account for the anti-proliferative effect. Translocation of PKC was inhibited by fluoxetine at optimal mitogenic concentrations [16], which might contribute to the observed anti-proliferative effect.

Further, cytosolic Ca2+ influx is an important factor in lymphocyte activation and subsequent proliferation [57]. Thus, SSRIs might interfere with lymphocyte proliferation through interference with Ca2+ influx. Edgar et al. demonstrated that fluoxetine exerted similar effects on mitogen-induced T cell proliferation as calcium ionophores [15]. At sub-optimal mitogen concentrations, both fluoxetine and calcium ionophores stimulated T cell proliferation, whereas at optimal mitogen concentrations, both compounds inhibited T cell proliferation [15]. Thus, when suboptimal mitogen concentrations are used, fluoxetine might induce an influx of extracellular Ca2+ that enhances T cell proliferation. In T cells exposed to optimal mitogen concentrations, however, fluoxetine causes an excessively high Ca2+ concentration resulting in impaired proliferation [15].

In conclusion, interference with the cAMP and phosphoinositol systems can explain some of the effects of SSRIs on lymphocytes, but the exact mechanism behind the immunomodulating effects of SSRIs remains unresolved and therefore requires further investigation.

3.3. Induction of the apoptotic cascade

SSRIs have been found to induce apoptosis in lymphocytes and cancer cells. The pathways involved in this apoptotic effect have been investigated extensively. Xia et al. showed that the decrease in cellular
viability was due to the induction of apoptosis, and was accompanied by extensive DNA fragmentation [34]. In lymphocytes exposed to citalopram, the anti-apoptotic genes c-myc and bcl-2 were downregulated and Fas membrane expression was increased [58]. In cancer cells, the process involves caspase-3 activation, as was demonstrated in both Jurkat cells [28] and acute myeloid leukemia HL-60 cells [59]. Early in the apoptotic cascade triggered by SSRIs in HL-60 cells, reactive oxygen species are formed, and this precedes the change in mitochondrial trans-membrane potential [60]. Further, Taler et al. showed an activation of the MAPK death signaling pathway and suppression of the anti-apoptotic protein bcl-2 in mitogen-activated rat splenocytes [27]. In conclusion, several well-known mechanisms leading to apoptosis are involved in the process by which SSRIs reduce cellular viability of lymphocytes (Fig. 1C).

3.4. Unexplored mechanisms

In addition to the abovementioned targets that have been investigated in lymphocytes to a greater or lesser extent, other mechanisms explaining the antidepressive action of SSRIs might as well account for their immunological effects. Amongst others, it was suggested that SSRIs might directly influence mitochondrial pathways, as was demonstrated for clomipramine in human glioma cells [61,62]. Furthermore, it was proposed that SSRIs could affect cell dynamics through e.g. phospholipid binding and lysosomal trapping, given their lipophilic and amphiphilic nature [61]. In this respect, SSRIs have been found to accumulate in membrane-associated lipid rafts in HEK293 and N1E-115 neuroblastoma cells [63]. Moreover, antidepressants have been shown to enhance G protein Gox migration from lipid rafts and thereby facilitate adenylyl cyclase activity and cAMP formation in C6 glioma cells [64]. As a result, signal transduction post G protein-coupled receptor activation is enhanced. The observed rise in cAMP after SSRI treatment of T lymphocytes activated with mitogens as described by Edgar et al. [15,16] and Xia et al. [31] might relate to the effects of SSRIs on lipid rafts. Given the presumed importance of lipid rafts in TCR clustering during T cell activation [65], SSRIs might disturb T cell function either directly via disturbance of lipid raft integrity or indirectly via enhanced G protein signaling.

Another possible mechanism is the upregulation of the glucocorticoid receptor (GR). Antidepressants have been shown to increase GR expression, promote GR nuclear translocation and enhance GR function in mouse fibroblasts [66,67]. As glucocorticoids have strong immunosuppressive effects, it is possible that SSRIs exert their immunosuppressive effects on T lymphocytes through GR modulation. However, these suggestions have not been investigated in lymphocytes and further research will be necessary to clarify whether the immunomodulating effects of SSRIs are mediated through any of the abovementioned mechanisms.

4. Animal studies of SSRIs as immunosuppressants

As it became more and more clear that SSRIs induced significant changes in immune cells, the possibility to use SSRIs in immune related pathologies was investigated. Two distinct possibilities have already been described: first, SSRIs were suggested as drugs to suppress unwanted immune responses in autoimmune diseases. Second, SSRIs were proposed as immunosuppressants to inhibit allogeneic T cell responses after transplantation. An overview is given in Table 1.

4.1. Autoimmune diseases

The potentially beneficial effects of SSRIs in autoimmune diseases have been tested in animal models of multiple sclerosis, rheumatoid arthritis, contact hypersensitivity reaction, inflammatory bowel diseases, septic shock and allergic asthma. In experimental autoimmune encephalomyelitis (EAE), a murine model of multiple sclerosis (MS), venlafaxine, paroxetine and sertraline were tested and both venlafaxine and sertraline were able to ameliorate the clinical symptoms of the disease (tail limpness, paraparesis, and hindlimb and forelimb paralysis) [13,68]. Paroxetine did not affect the clinical progression of EAE [13]. However, animals were treated with only 5 mg/kg paroxetine, which may have failed to induce high enough plasma concentrations to reach an immunomodulatory effect. Cytokine secretion was also investigated and sertraline decreased the secretion of IFNγ, TNFα and IL-12 as well as the viability and in vitro proliferation of EAE splenocytes [13]. Histological examination of venlafaxine-treated animals revealed decreased central nervous system inflammation and infiltration of inflammatory cells in the brain and spinal cord [68]. Venlafaxine also reduced pro-inflammatory cytokine secretion (IL12 p40, IFNγ, and TNFα) and diminished mRNA expression of inflammatory genes [68]. In a similar multiple sclerosis model in rats, fluoxetine has recently been shown to promote remission of EAE [69]. Fluoxetine-pretreated rats recovered faster and clinical scores during remission were lower than those found in vehicle-treated animals [69]. Spinal cord demyelination and inflammatory foci were reduced and IFNγ production was suppressed [69].

In a murine model for rheumatoid arthritis (RA), fluoxetine and citalopram were tested and both SSRIs were able to reduce clinical scores (based on the occurrence of erythema, swelling and joint deformity with ankylosis) [12]. Fluoxetine additionally improved paw thickness and significantly reduced IL12 secretion, whereas citalopram did not [12]. Histological examination of the affected joints revealed reduced inflammation, cartilage and bone erosion in fluoxetine-treated

<table>
<thead>
<tr>
<th>SSRI</th>
<th>Pathology</th>
<th>Animal model/species</th>
<th>Beneficial effect</th>
<th>Reference(s)</th>
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<td>EAE, murine</td>
<td>No</td>
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<td>Fluoxetine</td>
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<td>Septic shock</td>
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<td>Rheumatoid arthritis</td>
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<td>Yes</td>
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<td></td>
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<td>DSS, murine</td>
<td>Yes</td>
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<td>Yes, moderately</td>
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<td>Multiple sclerosis</td>
<td>EAE, murine</td>
<td>Yes</td>
<td>Sacre et al. [12]</td>
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animals and a tendency towards reduced inflammatory cell infiltration, pannus formation and joint deformation in citalopram-treated mice [12]. Further, a beneficial effect of sertraline has been demonstrated in a rat model of RA [8]. The decrease in clinical symptoms was accompanied by an increase in IL10 secretion, and a decrease in TNFα and COX2 levels [8].

Recently, the effect of fluoxetine on murine contact hypersensitivity (CS) reaction of the skin has been studied [10]. CS is a T-cell mediated immune reaction that was successfully suppressed by fluoxetine as determined by the reduction in swelling of the ear to which the contact allergen was applied. The weight of axillary lymph nodes was decreased and the production of IL10, an anti-inflammatory cytokine, was increased by fluoxetine [10].

Inflammatory bowel disease is another example of an immunological disorder that might benefit from SSR1 treatment. This disease is caused by a dysregulation of the gastro-intestinal immune system and is considered to be the result of an altered immune response to luminal antigens. In a dextran sulfate sodium (DSS)-induced murine model for colitis, fluoxetine showed to improve the disease activity index, consisting of a composite score for weight loss, stool consistency and gross rectal bleeding [9]. Histological examination of the proximal and distal colon showed less infiltration of inflammatory cells and reduced impairment of the glandular architecture in fluoxetine-treated animals versus controls. Another study demonstrated that fluoxetine and desipramine attenuate acetic acid-induced experimental colitis in rats [70]. In addition to a reduction of colonic damage, fluoxetine and desipramine suppressed serum cytokine levels (TNFα, IL1β) that were induced by experimental colitis [70].

Finally, in a lipopolysaccharide-induced murine model of septic shock, preventive administration of fluoxetine diminished the expression of TNFα and the mortality rate [11]. In a rat model of allergic asthma, fluoxetine reduced lung inflammation and infiltration of inflammatory cell types [11]. Fluoxetine reduced not only the number of lymphocytes, but also the number of macrophages, neutrophils and eosinophils [11]. In vitro, fluoxetine dose-dependently inhibited the release of TNF-α from LPS-treated monocytes [11].

The abovementioned studies demonstrate the potential use of SSRIs in a wide variety of autoimmune diseases. Nonetheless, the data are limited and further research is needed to evaluate which SSRI, which dose and which dosage regimen are optimal for each individual pathology. To date, most preclinical evidence of immunosuppression exists for fluoxetine (Table 1). Whereas fluoxetine is definitely a suitable candidate to proceed to clinical testing, it is worthwhile to screen the effect of other SSRIs in autoimmune disorders as well, as these might show to be equally or even more effective. Other autoimmune disorders, such as diabetes mellitus type 1, lupus erythematosus, autoimmune thyroid diseases and others might as well benefit from SSRI treatment and studies exploring the potential use of SSRIs in these disorders should be encouraged.

4.2. Graft-versus-host disease

Unwanted immune activation not only occurs in autoimmune disorders, but also in transplantation. For instance, allogeneic T cells present in the graft can cause graft-versus-host disease (GvHD) after hematopoietic stem cell transplantation. As in autoimmune diseases, the possibility to prevent or suppress this major complication with SSRIs has been investigated. Fluoxetine (20 mg/kg) was able to reduce clinical symptoms of acute GvHD in a MHC-matched, minor histocompatibility mismatched bone marrow transplantation model [14]. In comparison with vehicle-treated controls, fluoxetine reduced the occurrence of ruffled fur, hunched posture, lethargy, diarrhea, weight loss and inflammation of the eyes after transplantation of T cell-enriched bone marrow in lethally irradiated mice. In addition, fluoxetine increased the percentage survival of mice six months after transplantation in comparison to controls. In the peripheral blood of fluoxetine-treated mice, a significantly lower percentage of alloreactive T cells could be detected, demonstrating that fluoxetine was able to reduce alloreactive T cell proliferation, and/or increase apoptosis of these cells [14].

Concern has been raised that SSRIs might elevate prolactin levels and thereby negatively influence graft fate [71]. Fluoxetine appears to have an overall beneficial effect on GvHD without affecting the efficiency of the stem cell transplantation [14].

Whereas corticosteroids form the golden standard therapy for acute GvHD, these drugs can induce severe side effects such as increased infection risk. Cushing syndrome, diabetes, osteoporosis and myopathy [72]. In comparison, SSRIs have a more beneficial side effect profile with nausea, diarrhea, sexual dysfunction, dizziness, agitation and insomnia [3]. Furthermore, steroid treatment results in complete remission in less than half of the patients [73], indicating that new treatment options are highly necessary.

In the past decades, it has become more and more clear that hematopoietic stem cell transplantation is a successful therapy for leukemia not only because of the replacement of the blood forming compartment, but also because of the anti-leukemia effect that is executed by the graft [74]. However, GvHD and graft-versus-leukemia effect often go hand-in-hand and are at least in part mediated by the same effector cells and target antigens [74]. Whereas fluoxetine has been shown to suppress GvHD, the impact of this drug on graft-versus-leukemia effect has not been investigated. Therefore, further research will be necessary to evaluate whether the anti-leukemia effect is maintained during SSRI therapy.

5. From bench to bedside

Although the beneficial effects of SSRIs in divergent autoimmune diseases and cancer were demonstrated by several research groups, discussion remains on whether SSRIs are suitable candidates for immunomodulation in the clinic. The main concern relates to the plasma concentrations that are believed to be necessary in order to achieve an immunosuppressive effect with SSRIs. Although these concerns are valid, limited evidence is already available that SSRIs have a beneficial effect in MS and RA patients [75,76].

5.1. Plasma concentrations

There is a considerable difference between the SSRI concentrations that are reported to exert immunosuppressive effects in vitro, and the ones found in plasma of depressed patients. Concentrations used in vitro for immunosuppressive effects range from 1 to 20 μM for paroxetine, fluoxetine and sertraline and even higher for the other SSRIs. These concentrations are considerably higher than plasma concentrations found in depressed patients, which range from 10 to 600 ng/ml or 0.03 to 1.6 μM [77,78].

However, various factors contribute to the reasoning that SSRIs might still be suitable for immunomodulation in vivo. First, SSRI concentrations might vary considerably between organs and lymphocytes may be exposed to high enough SSRI concentrations in peripheral compartments instead of in the blood. Uhr et al. determined plasma and organ concentrations of SSRIs after subcutaneous injection in mice and found 10-fold higher concentrations in spleen compared to plasma [79]. Thus, lymphocytes might be exposed to SSRI-concentrations high enough for immunomodulation in the spleen while plasma concentrations can be kept low.

Second, evidence exists that doses currently used in patients already exert immunomodulatory effects. For instance, Reed and Glick reported reactivation of herpes simplex virus in patients receiving high doses of SSRIs [6]. A case of recurring sinusitis was reported in a patient suffering from obsessive–compulsive disorder and treated with high doses of venlafaxine [80]. Thus, the concentrations needed to obtain an immunosuppressive effect in vitro might not correlate with those exerting an immunosuppressive effect in vivo.
Third, the doses that exert immunomodulatory effects in some of the animal experiments give rise to plasma concentrations within the same range as concentrations found in patients. Chronic daily administration of 10 to 18 mg/kg fluoxetine orally given to mice gives rise to plasma concentrations within the same range as those found in patients (100–700 ng/ml) [81]. Several of the animal experiments analyzing the effect of fluoxetine on autoimmune disease and cancer used doses below 20 mg/kg/day and reported significant changes in immune function and symptoms [12,17,18]. Others reported doses below 20 mg/kg/day to already exert small changes in immune function, but higher doses were needed in order to reach significance [12].

Finally, if higher dosing would be necessary, this may be achieved without severe adverse effects. Barbev and Bristow concluded from a comprehensive literature search that SSRI doses up to thirty times the normal daily dose do not induce any side effects or only minor effects. Only at doses exceeding 75 times the normal daily doses, more severe adverse effects occurred [82]. Doses two to three times higher than the ones used for the treatment of depression are already being subscribed for other disorders, such as obsessive compulsive disorder without unacceptable side effects [83]. Although to our knowledge, no data are available on plasma concentrations in OCD patients, one might expect these to be much higher, given the non-linear kinetics of most SSRIs. This was confirmed for fluoxetine in mice, where a chronic dose of 25 mg/kg per day gave rise to a plasma concentration 3.15 times higher than the plasma concentration obtained after a chronic dose of 18 mg/kg (the latter dose gives rise to a plasma concentration within the same range as those found in patients) [81].

Nevertheless, one aspect that needs further attention is the potentially increased risk to commit suicide under treatment with SSRIs. There is limited evidence that antidepressant treatment might elevate the risk of suicide in depressed patients, especially at the start of treatment [2]. When using SSRIs as immunosuppressors in patients suffering from autoimmune disorders or GVHD, in particular when concomitant depression is present, the potentially increased risk of suicide should be considered. In undepressed patients, this seems less to be an issue, as the increased suicide risk with antidepressants is associated with the underlying depression [2].

Thus, although immunoregulatory application of SSRIs will probably require higher doses than the ones currently used for the treatment of major depressive disorder, there are indications that achieving the needed plasma concentrations may be feasible without competing against unacceptable side effects.

5.2. Clinical evidence supporting the human use of SSRIs as immunosuppressors

Although clinical evidence for SSRI use in autoimmune diseases is scarce, two studies have been conducted that demonstrate the usefulness of SSRIs in MS and RA. In undepressed patients with relapsing MS, fluoxetine (20 mg/day) reduced the occurrence of new enhancing lesions, as measured by an MRI scan [75]. The beneficial effect was attributed to an anti-inflammatory effect of fluoxetine on astrocytes, rather than a suppressive effect on peripheral lymphocytes. The peripheral effects on immune cells, however, were not investigated.

In RA patients, a clinical trial was performed to evaluate the efficacy of paroxetine and amitriptyline for concurrent depression [76]. In order to differentiate between both possibilities, studies in non-depressed RA patients should be conducted.

Furthermore, a patient suffering from RA was found to be in remission when treated with citalopram for concurrent depression and discontinuation of citalopram treatment resulted in reoccurrence of the rheumatic symptoms [84]. Although it can be argued that the mental state of a patient influences his perception of rheumatic symptoms, this case report mentions a significant improvement of DAS 28 score, which is an objective measure of disease severity. Therefore, it seems to indicate that there is a direct link between SSRI treatment and severity of RA symptoms in this patient.

This limited evidence is encouraging for the use of SSRIs in autoimmune diseases, even with doses in the same range as the ones used for the treatment of depression. However, more extensive studies are needed to evaluate the immunosuppressive potential of SSRIs and the doses needed to achieve an optimal effect in each individual pathology.

6. Conclusion

SSRIs clearly exert immunological effects that potentially could be used to alter immune responses in autoimmune pathologies and graft-versus-host disease. Whereas the impact of SSRIs on proliferation, cytokine secretion and apoptosis of lymphocytes has already been well characterized, the underlying mechanism still needs further investigation. Although discussion remains about whether or not SSRIs can be administered in high enough doses to exert the immunosuppressive effects, they are an interesting new treatment option and further research in the area should be encouraged.

References

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