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MINI REVIEW

Pharmacology and Toxicology of Leflunomide

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ABSTRACT

Leflunomide (LEF), used for rheumatoid arthritis, inhibits dihydro-orotate dehydrogenase (DHODH) and tyrosine kinase (TK) enzymes and has anti-inflammatory, lymphocyte proliferation regulatory, immunosuppression and chondroprotective effects. The most common adverse effects are gastrointestinal disorders, weight loss, hypertension, skin infection, and neurological and hematological toxicity. It also produces hepatotoxicity and teratogenic effects on long term therapy. The Food and Drug Administration (FDA) categorized it as black box warning drug since 2010. Therefore, it is necessary to give toxicological informations to scientific communities. This review article is elaborately describes the toxicity of this drug during its long and short term therapies.

Keywords: *Leflunomide, Rheumatoid arthritis, Pharmacology, Toxicology*

Leflunomide (LEF, Fig 1) is used in rheumatoid arthritis (RA) since 1998 [1]. Its anti-RA activity is mainly due to its dihydro-orotate dehydrogenase (DHODH) and tyrosine kinase (TK) enzymes inhibitory activity [2]. On the other hand, this action is mediated through inhibition of T- and B-lymphocytes proliferation in vitro [3]. LEF induces tumor necrosis factors α (TNF-α) and interleukin 1β (IL-1β) factors like metalloproteinase (MMPs) and prostaglandin E2 (PGE2) during anti-RA activity [4]. Recently, it has been associated with acute hepatic failure in humans due to increase in the activity of liver cytochrome 2C9 (CYP2C9) enzyme [5]. CYP2C9 is the key enzyme which metabolizes LEF to its active form A771726 (melononitrilamide, major), 4-trifluoroaniline and other minor metabolites [6]. LEF is commonly treated as safe drug with minor side effects which include nausea, vomiting, diarrhea, dyspepsia, immunosuppressant and alopecia [7]. A recent study proposed that it elevates serum transaminase level up to 10% in human [8]. The Food and Drug Administration (FDA) recommended it as a black box warning drug in 2010 [9]. This article mainly reviewes above-mentioned topics shortly, followed by major discussions on toxicology of LEF.

PHARMACOKINETICS

After oral administration, LEF is absorbed from gastrointestinal tract at high rate and oral bioavailability attains up to 80% and 90% for human and rat, respectively. It is converted to major A771726 intermediate during first pass metabolism (Fig 2) which is pharmacologically more active than its parent drug. A771726 is 99.3% plasma protein bound (mainly with albumin) and volume of distribution is very less than its parent drug but has longer half-life (14-18 days). Both LEF and its metabolite are excreted through bile and urine. Another minor metabolite of LEF is 4-trifluoromethyl aniline which is detected in plasma at very low concentration and does not have any pharmacological effect but is responsible for clastogenecity [10,11].

MECHANISM OF ACTION

The pharmacological action of LEF is mediated through the inhibition of DHODH and TK enzymes. LEF inhibits de-novo pyrimidine synthesis by inhibiting DHODH enzyme which is a rate-limiting step in the

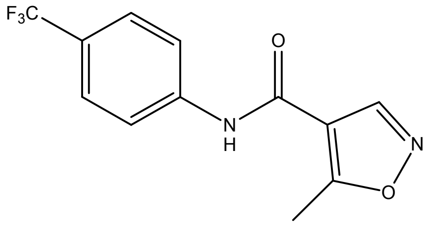

Fig 1. Structure of LEF

pyrimidine synthesis (Fig 3). This inhibition is also mediated through lower concentrations of A771726 and considered as a major mode of action [2]. It is postulated that lymphocytes activation mainly depends on this metabolic pathway for clonal expansion and terminal differentiation into effector cells. This pathway is important with respect to various physiological perspectives like nucleic acid synthesis, phospholipid synthesis and protein glycosylation [12]. LEF prevents the expansion of activated and autoimmune lymphocytes by interfering with cell cycle progression which is mediated by inadequate production of ribosomal uridine monophosphate (rUMP) and the sensor protein p53 [2].

The second mode of action of LEF is the inhibition of TK enzymes. Both LEF and A771726 inhibit the action of epithelial growth factor receptor followed by $p56^{lck}$, $p59^{fyn}$ and Janus Kinase 1 and 3. A771726 action is more pronounced on platelet-derived growth factor receptor than epidermal growth factor receptor [13]. The inhibition of TK is due to decreased production of soluble inflammatory mediators like cytokines and normally-secreted antibodies. A771726 inhibits T cell and B cells signaling in the G_0/G_1 phase of the cell cycle [14].

The other mechanisms of A771726 are the increased production of MMPs and tissue inhibitor metalloproteinase-1 (TIMP-1) and up-regulation of IL-1 and TNF-α. It has been postulated that imbalance between MMPs and TIMP-1 leads to matrix destruction. It has been found that A771726 has tendency to inhibit proinflammatory and matrix degradative factors over the anti-inflammatory and MMPs inhibitors [15].

PHARMACOLOGY

The pharmacological actions of LEF are mainly due to its active metabolite A771726 which has anti-inflammatory, lymphocyte proliferation regulatory, immunosuppression and chondroprotective effects.

Anti-inflammatory effect

Synovial cell inflammations occur during activation of B cells, CD4+ and CD8+ T lymphocytes via stimulation of plasma cells, macrophages, mast cells and synovial fibroblasts through production of inflammatory mediators like TNF-α and IL-1. A771726 regulates T cells progression through regulating the cell cycle by inhibiting the denovo pyrimidine ribonucleotide biosynthesis in the late G_1 phase of the cell cycle [12]. The anti-inflammatory effect of A771726 is dose-dependent on human cultured macrophages [3]. A771726 depletes the pyrimidine pool which down regulates the glycosylation of adhesion molecule, further reducing cell to cell contact activation and pooling of inflammatory cells during inflammatory reaction [16].

Regulation of lymphocyte proliferation

At low doses, A771726 has been shown to regulate lymphocyte proliferation both *in vitro* [17] and *in vivo* [18] in dose dependent manner. The enzyme DHOH is used by rapidly proliferating cells involved in the pathogenesis of RA. Blocking of DHOH enzyme by

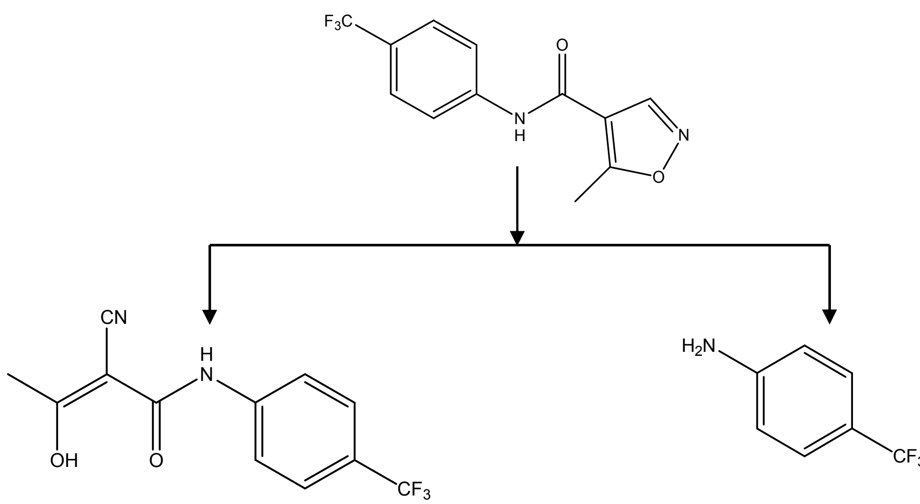

Fig 2. Biotransformation of LEF to its active metabolite A771726 and 4-trifluoromethyl aniline

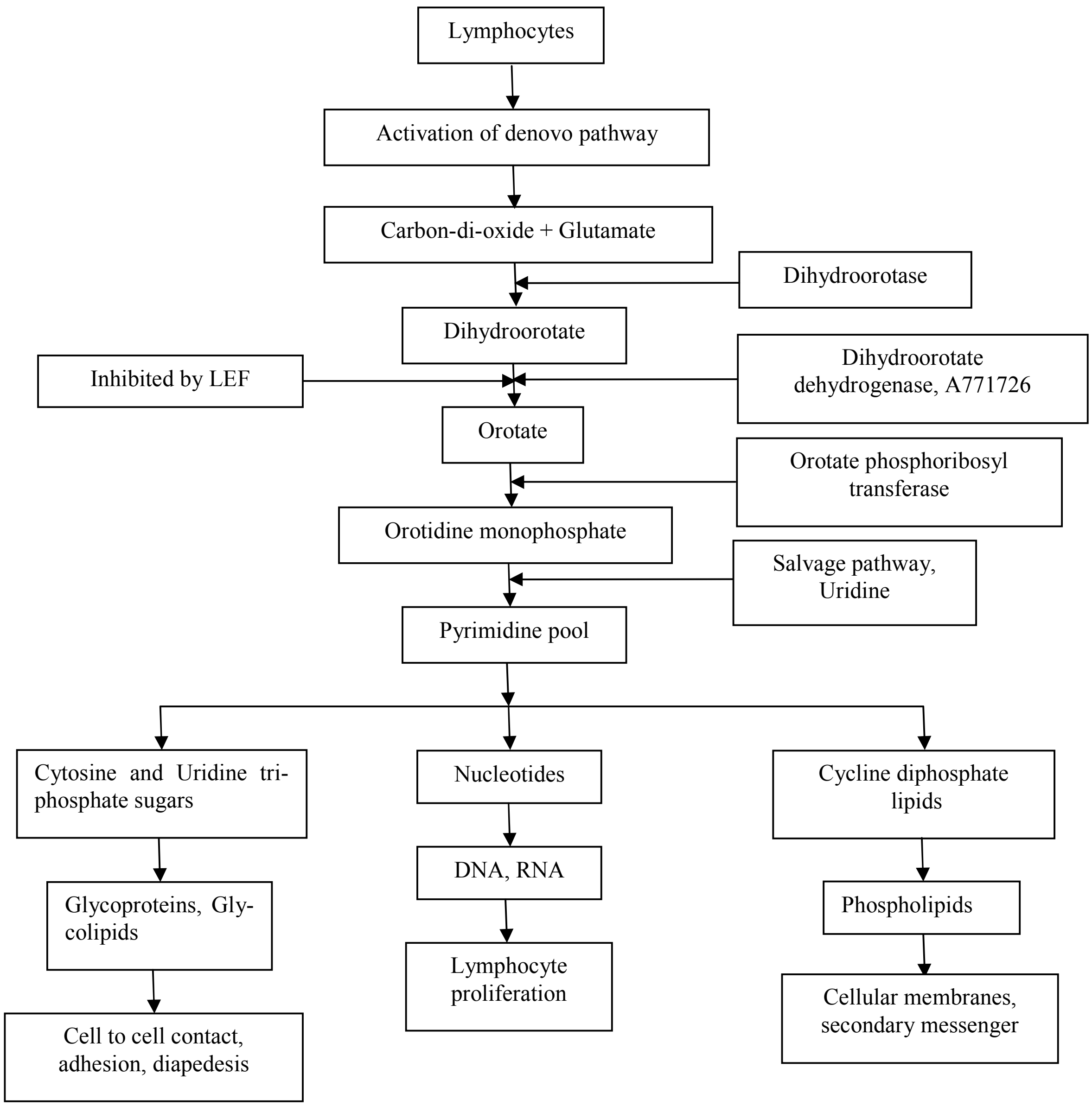

Fig 3. Mechanism of De novo Pyrimidine Biosynthesis and DHOH Inhibition by LEF

LEF reduces the pyrimidine biosynthetic pathways via interrupting T-lymphocyte clonal expansion between G1 and S phase. Cherwinski *et al*. (1995) investigated that the addition of A771726 to mitogen-stimulated human blood lymphocytes causes change in the proportion of stimulated cells [18]. The *de novo* pyrimidine synthesis inhibitor LEF controls the cell cycle through the p53 and p53 $^{WAF-1}$ pathways as well [13].

Another potential explanation for therapeutic effect of LEF is the reduction in the number and/or reactivity of T cells, involved in the pathogenesis of chronic inflammatory diseases. This hypothesis is supported from LEF studies on the T-cell-driven immune responses in animal models of autoimmunity, including collagen type II and other models of arthritis [19].

Immunosuppression

LEF is a novel immunosuppressive agent which is used for treatment of autoimmune diseases and transplant rejections [20]. Suppressing TNF-α and IL-1β produced during cell to cell contact activation between T lymphocytes and monocytes and direct inhibition of human stellate cell (HSCs) collagen synthesis are the two possible mechanisms of immunosuppressive action of LEF [21]. LEF has demonstrated to prevent and reverse acute allograft rejection by suppressing immune system [22]. Chong *et al*. (1999) revealed that *in vivo*

Gastrointestinal side effects are the most common and least harmful side effect associated with LEF treatment which includes diarrhea (27%), nausea (13%), vomiting and dyspepsia (10%). Klinik *et al*. (2008) investigated the diarrhea, lymphocytic colitis and weight loss actions on long term therapy of LEF [30]. On discontinuation of therapy, diarrhea ceases within few days and lymphocytic colitis is no longer evident after 3 months. The gastrointestinal side effects may arise due to induction of COX-2 and inducible nitric oxide synthetases which lead to inhibition of PGE_2 synthesis [31].

mechanism of immune suppression is complex and is affected by at least four factors which include type and vigor of immune responses, availability of uridine for salvage by proliferating lymphocytes, species being investigated and concentration of serum A771726 [23]. Its immunosuppressive action is also due to the inhibition of TK enzymes. LEF inhibits p59fyn and p56lck activity *in vitro* during TK assay. It is also well documented that *src* related kinases are involved in signal transduction of hematopoietic cells. LEF inhibit the activity of these kinases in dose-dependent manner [24].

Chondroprotective effect

Several studies reported that TNF-α and IL-1β are the two important key proinflammatory cytokines which mediate cartilage degradation in patients with RA and osteoarthritis. TNF-α and IL-1β participates in these processes. They stimulate chondrocytes and synoviocytes to produce matrix proteases, chemokines and eicosanoids such as prostaglandins and leukotrienes. IL-1β induces large scale apoptosis in chondrocytes in association with mitochondrial dysfunction and depletes the cellular energy production. TNF-α causes increased production of latent metalloproteinase (collagenase and caseinase) and peptidoglycan release leading to matrix degradation [25,26].

A771726 also inhibits stromal-cell-medium-induced cell growth and leads to a down regulation of adhesion molecule of bone marrow stromal cells and growth factors like insulin growth factor-1 (IGF-1) and cytokines such as IL-6 through activating Akt pathway [27]. Siemasko *et al.* (1996) demonstrated that LEF inhibits B cell antibody production by directly acting on B cells and thus antigen-antibody reaction is prohibited leading to inhibition of rheumatoid factor formation and ultimately synovial tissue degradation is prevented [28].

TOXICOLOGY

LEF was licensed for the treatment of rheumatoid arthritis in 1998. It is known to have minor side effects like diarrhea, weight loss, dyspepsia, skin rashes, alopecia, hypertension as well as predisposition to infection and peripheral neuropathy [29]. It has major side effects such as hepatotoxicity and acute pancreatitis.

Gastrointestinal side effects

Most cases of liver toxicity are seen within 6 months of treatment when multiple risk factors like hepatotoxin are present with previous liver diseases. Manifestation of liver toxicity ranges from mild jaundice to severe permanent hepatitis, severe liver necrosis and liver cirrhosis [32-34]. In most cases, it increases serum concentration of hepatic transaminases which returns to normal level within 4-6 weeks after discontinuation of therapy or when dose is reduced from 20 mg/day to 10 mg/day [35]. Elevation of hepatic enzyme level is possibly related to CYP2C9 polymorphism [36]. An *in vitro* study on cultured hepatocyte cells of rat showed that LEF and its major metabolite A771726 are cytotoxic to these cells but data demonstrated that metabolic process of LEF is a detoxification process rather than initiating events leading to toxicity [37].

Researcher proposed that LEF-induced hepatotoxicity is due to CYP2C9 polymorphism but mechanism of toxicity has not been yet elucidated. It seems to be dose-dependent, predictable and possibly avoidable with careful maintenance of concentration below 10 mg/ml.It is also reversible with temporary discontinuation of drug or a reduction in dose [35].

Weight loss

14-26% of weight loss was recorded in 7.1% of LEF-treated population. This weight loss was found in the patients on combination therapy with methotrexate which might be associated with diarrhea and other gastrointestinal side effects. Coblyn *et al*. (2001) proposed that it may be either due to interference of LEF with oxidative phosphorylation and ATP generation in the mitochondria or like other flavin-linked enzymes, DHOH may nonspecifically inhibit the mitochondrial electron transport chain by uncoupling oxidative phosphorylation [38].

Hypertension

Hypertension has been mentioned as common side effect of LEF treatment. In phase II clinical trial, blood pressure is elevated in 10.6% of patients receiving 25 mg LEF. In an American phase III study, a mean increase in systolic and diastolic blood pressure of 2.2% and 1.9% respectively, was found in 2.1% patients. As the heart rate also rises during LEF treatment, it has been assumed that hypertension may be caused by an increased sympathetic drive [39].

Dermatologic toxicity

In general, LEF is well tolerated in RA patients but life-threatening Stevens Johnson, alopecia, acne eruptions, hair discoloration, maculopapular rashes, toxic epidermal nacrolysis and nail discoloration are seen in less than 1% patients [40,41]. A study by Smolen *et al*. (1999) showed that LEF cause rash (10%)

and alopecia (8%) during phase II clinical trial [42]. These features suggest drug hypersensitivity syndrome (DHS) which have a characteristic pattern of events like delayed onset of reaction, fever, widespread and long lasting skin rash and internal organ involvement. The pathogenesis of DHS is not well understood in these cases but it has been proposed that there may be partially-inherited increased susceptibility to the toxic effects of oxidative drug metabolites which causes immunological reaction by either forming hapten or by danger signaling [43,44].

Neurologic toxicity

Rare cases of peripheral neuropathy, brain abscess, aseptic meningitis, cytoid macular edema and severe axonal sensorimotor polyneuropathy were seen on long term use of LEF in RA [45]. Cases of toxic neuropathy have been observed during treatment of rheumatoid arthritis with leflunomide. Their occurrence seems to be associated with known risk factors [46]. A study conducted in the south India reported that there were significant higher incidences of peripheral neuropathy in patients on LEF (10%) compared with those on methotrexate (0.8%) [47]. Peripheral neuropathy was thought to be due to triggering of severe vasculitis by LEF [47,48].

A study by Barak *et al*. (2004) reported LEF produced cytoid macular oedema in a 67 year old white patient of RA [49]. Other drugs like lanatoprost and hypotensive lipids also cause cytoid macular oedema by triggering the biosynthesis of endogenous prostaglandins by the drug, thus causing disruption of the blood–aqueous barrier and the creation of cystoid macular oedema. Hence similar mechanism was also presumed in the case of LEF [50].

Hematologic toxicity

LEF is an immunosuppressive agent used in RA and other disorders. It is thought to suppress bone marrow thus inhibit lymphocyte proliferation leading to hematologic adverse events like Leucopaenia, thrombocytopaenia, anaemia, granulocytopenia, pancytopenia , leukocytosis, aplastic and haemolytic anaemia , eosinophilia and lymphopaenia [51].

A771726 is an active metabolite of LEF which inhibits DHOH leading to impaired pyrimidine synthesis. Halting of T-lymphocyte proliferation and mylosuppression are thought to be major mechanism of pancytopenia. Direct injury of both proliferating and quiescent haematopoietic cells may impair DNA replication and trigger apoptosis [52]. On discontinuation of LEF therapy, these symptoms got subsided. Cholestyramine therapy caused dramatic reduction in the symptoms as it washes out LEF [53].

Opportunistic infection

LEF and its active metabolite A771726 are immunosuppressive agents. Their immunosuppressive action is mediated by suppressing TNF-α and IL-1β produced during cell to cell contact activation between T lymphocytes and monocytes and direct inhibition of human stellate cell (HSCs) collagen synthesis [21]. This immunosuppressive action of LEF increases the chances of opportunistic infection, pulmonary tuberculosis, brain abscess, fatal sepsis, postsurgical osteomyelitis and Propionibacterium acnes endophthalmitis [45].

Teratogenicity

In oral embryotoxicity and teratogenicity studies in rats and in rabbits, LEF was embryotoxic (growth retardation, embryolethality) and teratogenic (in rats, malformations of the head, rump, vertebral column, ribs, and limbs; in rabbits, malformations of the head and bilateral dysplasia of the spine of the scapula) [54]. Teratogenic potential was also established in the clinical study of ARAVA hence it was contraindicated in the pregnant and nursing women since 1998 [55]. In 2007, FDA added previously a boxed warning regarding teratogenic potential in pregnant women [56].

LEF has been reported to cause teratogenicity in rats, rabbits, and mice [57]. Leflunomide inhibits the enzymatic activity of protein tyrosine kinases and of DHODH, which is involved in pyrimidine nucleotide de novo synthesis. DHODH inhibition by LEF causes G_0 to S phase arrest of cell cycle leading to inhibition of DNA synthesis and thus pyrimidine. Inhibiton of uridine synthesis was thought to be responsible for the embryotoxic and teratogenic effects [54,58,59].

Pulmonary toxicity

Since LEF is an immunosuppressant drug because it leads to the susceptibility to opportunistic infection by bacteria, viruses and fungi. During the launch of drug in 2003 at Japan, adverse pulmonary events were reported with accelerated interstitial lung disease, leading to several deaths. Fatal interstitial pneumonia was reported in about 30% of patients [60,61] Pulmonary abscess, pulmonary hypertension, *Mycobacterium abscessus* infection, pulmonary tuberculosis, rheumatoid lung nodulosis and osteopathy, atypical pneumonia and pulmonary aspergillosis are seen on long term therapy of LEF either alone or in combination with MTX [45]. Cases of ILD are more when patients switched from MTX to LEF or preexisting medical history of ILD [62].

A study at Spain demonstrated that there are four fold more chances of increased risk of tuberculosis in RA patients using LEF [63]. Active metabolite of LEF inhibits T cell and B cell signaling, thus inhibiting proinflammatory cytokines. It also inhibits TNF-α-induced NF-kB activation. Optimal activity of TNF-α and other cytokines is crucial for host defense mechanism. Studies on animal models have shown that inhibition of TNF-α increases the frequency and reactivation of tuberculosis [64,65].

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