An Overview of Levamisole Hydrochloride with Immuno-Stimulant Activity

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ABSTRACT
Levamisole, marketed as the hydrochloride salt under the trade name Ergamisol (R12564), is an anti helminthic and immunomodulator belonging to a class of synthetic imidazo-thiazole derivatives. It was discovered in 1966 at Belgium's Janssen pharmaceutica, where it was prepared initially in the form of its racemate called tetramisole. The two stereoisomers of tetramisole were subsequently synthesized, and the levorotatory isomer was given the name levamisole. Levamisole has been used in humans to treat parasitic worm infections, and has been studied in combination with other forms of chemotherapy for colon cancer, melanoma, and head and neck cancer. In some of the leukemic cell line studies, both levamisole and tetramisole showed similar effect. Effective studies need to be undertaken to study the full potential of levamisole in Immunostimulation activities and immuno synergestic effect of Levamisole with combination to natural polysaccharides.

Keywords-Levamisole, Immunostimulation, polysaccharide, Tetramisole

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Received 22 February 2014, Accepted 26 March 2014
INTRODUCTION

Levamisole, an anthelmintic agent with a wide range of immunomodulatory actions, has been used successfully as monotherapy and an adjunct to treatment in a variety of diseases. Levamisole is a promising agent for use in the immunotherapy of patients with deficient host defense mechanisms. Both in anergic patients and in experimental animals levamisole has been shown to stimulate cell mediated immunity probably through the enhanced maturation of cells. Because of its immunomodulating effects Levamisole has been used in a wide range of diseases with and without success. In dermatologic disease Levamisole has been successfully used in the treatment of parasitic, viral and bacterial infections including leprosy, collagen vascular diseases, inflammatory skin diseases and children with impaired immune a variety of reasons. It has also been used in combination with polymers for treating a number of dermatologic disorders, e.g. in combination with cimetidine for treating recalcitrant warts, with prednisolone for treating lichen planus, erythema multiforme and aphthous ulcers of the mouth. Adverse affects of levamisole are mild and infrequent and include rash, nausea, abdominal cramps, taste alteration, alopecia, arthralgia, and a flu-like syndrome. It can rarely cause agranulocytosis.

Drug Profile

Levamisole:
The levorotatory isomer of tetramisole

Synonym:
Levamisol, L-Tetramisole; dl-Tetramisol, Phenyl imidothiazole, Levamisolum, Levamisole hydrochloride.

Drug Category:
Adjuvants, Immunologic, Antinematodal Agents, Anti-rheumatic Agents

Dose: A single dose of 2.5 mg/kg has been recommended for antihelminthic therapy in humans and a dose of 50 mg, three times a day.

Proprietary Names:
Ergamisole, Nemicide, solaskil, Stimamizol, Tramisole, Worm-chek, Decaris –Vet, Nilverm,Ripercol, Narpenol, Levasole

CHEMISTRY

Chemical Name:
(-)-2, 3, 5, 6-Tetrahydro-6-phenylimidazo-[2, 1-b] thiazole monohydrochloride

Molecular formulae: C_{11}H_{13}ClN_{2}S
Chemical Structure:

![Chemical Structure of Levamisole Hydrochloride](image)

**Figure1- Structure of Levamisole Hydrochloride**

**Molecular weight**\(^1\): 240.7

**PHYSICOCHEMICAL PROPERTIES**

Description: A white crystalline stable powder\(^6\).

Taste: Metallic

Odour: Characteristic odour\(^7\).

Melting Point: 227°C to 229°C.

Solubility: Soluble in water, methanol, slightly soluble in ethanol, very slightly soluble in chloroform, insoluble in acetone\(^7, 8\).

Optical Rotation: \([\alpha]_D^{20} = -124^\circ \pm 2^\circ (c=0.9 \text{ in water})\)

PH: Levamisole solution has a pH of 3–4.5

UV maxima\(^7\): 213 nm

Heavy metals: maximum 20 ppm. 12 ml of solution S complies with limit test A. Prepare the standard using *lead standard solution (1 ppm Pb)* \(R^8\).

Loss on drying: maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 100-105°C for 4 h\(^7\).

Sulphated ash: maximum 0.1 per cent, determined on 1.0 g.

Dissociation Constant: pKa 8

Partition Coefficient: Log P (octanol/water) = 0.64

Storage: Levamisole to be stored in well-closed, light resistant container\(^10\)

**PHARMACOLOGY**

Levamisole acts as an anti parasitic, Immunomodulators and adjuvant in colorectal cancer. It appears to restore depressed immune function through stimulating antibody formation and enhance T-cell response by stimulating T-cell activation and proliferation. Anti parasitic action appears to be tied to its agonistic activity towards nicotinic receptors in nematode muscles resulting in spastic paralysis. The net effect is a paralyzing of the worm, which is then expelled live\(^6-10\).

**Mechanism of action**
The mechanism of action of levamisole as an antiparasitic agent appears to be tied to its agonistic activity towards the L-subtype nicotinic acetylcholine receptors in nematode muscles. This agonistic action reduces the capacity of the males to control their reproductive muscles and limits their ability to copulate. The mechanism of action of Levasimol as an anticancer drug in combination with fluorouracil is unknown. The effects of levamisole on the immune system are complex. The drug appears to restore depressed immune function rather than to stimulate response to above-normal levels. Levamisoline can stimulate formation of antibodies to various antigens, enhance T-cell responses by stimulating T-cell activation and proliferation, potentiate monocyte and macrophage functions including phagocytosis and chemotaxis, and increase neutrophil mobility, adherence, and chemotaxis ⁸⁻¹²

**Indications and usage**

- Levamisole is an antihelminthic drug approved for use in human medicine as well as veterinary medicine.
- It is used as an immunomodulator in rheumatoid arthritis and colorectal cancer therapy.
- A single dose of 2.5 mg/kg has been recommended for antihelminthic therapy in humans and a dose of 50 mg, three times a day, for three days every other week for colon cancer therapy.
- Levamisole is used for adjuvant treatment in combination with fluorouracil after surgical rejection in patients with Dukes' stage C colon cancer.
- Levamisole is also used to treat malignant melanoma and head/neck cancer ¹³.

**Adverse drug reactions**

Significant adverse reactions of levamisole are agranulocytosis, skin rash, and febrile illness, rheumatoid arthritis, blood dyscrasia. Nausea, vomiting, diarrhea, mouth sores, loss of appetite, stomach pain, change in taste and smell, muscle aches, fatigue, dizziness & headache ⁵⁻¹¹.

**Drug interactions**

- Anticoagulant effect increases when levamisole combines with Anisindione, Dicumarol, Acenocoumarol, Warfarin ¹².
- Levamisole hydrochloride has been reported to produce "ANTABUSE"-like side effects when given concomitantly with alcohol ¹².
- Concomitant administration of phenytoin and Levamisole fluorouracil has lead to increased plasma levels of phenytoin. The physician is advised to monitor plasma levels of phenytoin and to decrease the dose if necessary.
- By taking levamisole and warfarin sodium, prolongation of the prothrombin time beyond the
therapeutic range found in patients, it is suggested that the prothrombin time should be monitored carefully, and the dose of warfarin sodium or other coumarin-like drugs should be adjusted accordingly, in patients taking both drugs.\textsuperscript{13}

**Pharmacokinetics**

Levamisole is rapidly absorbed from the GI tract and extensively metabolized in the liver and excreted mainly by the kidneys (70\% over 3 days). Its plasma elimination half-life is 4.4-5.6 hours. Due to short elimination half-life, blood levels of levamisole fall more rapidly and go unnoticed in toxicological examination. Data from few clinical studies indicate that the consumption of 50-200 mg/day of levamisole causes agranulocytosis in 0.08 – 5\% of the studied population (WHO, FAS 33).\textsuperscript{11-13}

**Table 1-Pharmacokinetics parameters of Levamisole at daily dose**

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Pharmacokinetic Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Absorption.</td>
<td>Rapidly absorbed from</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gastrointestinal tract</td>
</tr>
<tr>
<td>2</td>
<td>Fraction Bioavailable (%)</td>
<td>47– 74.2%</td>
</tr>
<tr>
<td>3</td>
<td>Elimination half-life (h)</td>
<td>4.4 – 5.6</td>
</tr>
<tr>
<td>4</td>
<td>Volume of distribution (lit/Kg)</td>
<td>2-6</td>
</tr>
<tr>
<td>5</td>
<td>Protein Binding (%)</td>
<td>20-25%</td>
</tr>
<tr>
<td>6</td>
<td>Clearance (lit/min)</td>
<td>0.19 – 0.26</td>
</tr>
<tr>
<td>7</td>
<td>$C_{\text{max}}$ (ng/ml)</td>
<td>82 – 100</td>
</tr>
<tr>
<td>8</td>
<td>$T_{\text{max}}$ (hr)</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>Steady state plasma concentration (mg/lit)</td>
<td>1</td>
</tr>
</tbody>
</table>

**Metabolism & Excretion**

A single oral labeled levamisole dose in humans was eliminated in urine (60\%) and feces (4\%) within 24 hours. Urinary products included unchanged drug (3.2\%) and free (1.6\%) and conjugated (11\%) p-OH-levamisole. Aminorex has been identified as a minor urinary metabolite in the horse; reported urine concentrations of levamisole and aminorex following oral or subcutaneous levamisole administration ranged from 210–2754 or 20–330 μg/L, respectively.\textsuperscript{12-16}

**Reported Immunostimulant Activities**

SOPPI et al. have studied the in vivo effect of levamisole on the PHA and responses of chicken peripheral blood lymphocytes and on the in vivo antibody response to a thymus dependent antigen (BSA) and to a thymus independent antigen (Brucella abortus). Levamisole (0-25 mg/kg) increased significantly both the PHA and Con A responses of chicken blood lymphocytes. The antigens were given at the time of enhanced mitogenic responses and a significant increase was observed in both IgM and IgG antibodies to BSA. In contrast, no effect was obtained on antibody
responses to Brucella abortus organisms. The results show that levamisole is able to enhance both humoral and cellular immune responses in normal chickens. The effect is probably mediated by the activation of the T cell function and effects only antibody responses to thymus dependent antigen. These findings confirm and extend the observations regarding the ability of levamisole to modulate immune responses.\cite{17}

Wang et al. have explored the effects of liniment levamisole on cellular immune functions of patients with chronic hepatitis B. The levels of T lymphocyte subsets and mIL-2R in peripheral blood mononuclear cells (PBMCs) were measured by biotin-streptavidin (BSA) technique in patients with chronic hepatitis B before and after the treatment with liniment levamisole. After one course of treatment with liniment levamisole, the levels of CD3+, CD4+, and the ratio of CD4+/CD8+ increased as compared to those before the treatment but the level of CD8+ decreased. The total expression level of mIL-2R in PBMCs increased before and after the treatment with liniment levamisole. Liniment levamisole may reinforce cellular immune functions of patients with chronic hepatitis B.\cite{18}

Jaya et al. have determined the immunomodulatory effect of dietary levamisole in Asian catfishsh (Clarias batrachus), fish were fed four different diets for 10 days: a formulated diet as control and the same diet supplemented with 50, 150 or 450 mg levamisole kg\textsuperscript{-1} feed. The serum bacterial agglutination titre against Aeromonas hydrophila as a measure of specific immunity, serum haemagglutination titre, natural haemolytic complement activity (ACH50), myeloperoxidase and lysozyme activities, total protein level and oxidative radical production by neutrophils as a measure of non-specific immunity as well as disease resistance against a hydrophila challenge to separate vaccinated and non-vaccinated groups were evaluated at 0, 1, 2 and 3 weeks after last administration of levamisole. Levamisole supplement at the lowest level (50 mg kg\textsuperscript{-1}) significantly enhanced oxidative radical production and serum myeloperoxidase (MPO) content immediately after 10 days of feeding, which reached peak values after 3 and 2 weeks of feeding respectively. Haemolytic complement and haemagglutination titre were significantly enhanced after 3 and 1 weeks respectively.\cite{19}

Tekade et al. have studied in birds treated with Cyclophosphamide @ 150 mg/kg body weight I/V on 21st day of age produced marked immunosuppression. Administration of A. racemosus, Sida cordifolia in combination with Levamisole was the more effective in producing immunomodulatory effect in immunosuppressed birds.\cite{20}

Sajid et al. have determined the immunomodulatory effect of dietary intake of levamisole in the common carp, specimen were fed diets containing 0 (control), 125, 250 and 500 mg levamisole.
kg-1 of dry diet for a period of 70 days. Experimental stock was challenged intra-peritoneally with *Aeromonas hydrophila* on 30th and 58th day. Hematobiochemical parameters were determined on day 0, 57 and day 70. The total erythrocyte count, haemoglobin content, hematocrit value, total serum protein, albumin and globulin content were significantly (P<0.05) enhanced in levamisole supplemented groups particularly in 250 mg kg-1 group; however the TLC was significantly (P<0.05) higher in control (infected) group. Lysozyme activity and NBT assay were significantly stimulated in levamisole supplemented groups displaying the highest value in 250 mg kg-1 group on day 57.

**Discussion**

Levamisole exert its immunopotentiating activity in patients with chronic diseases. As an immune stimulant, levamisole connected with receptors of thymopentin on the surfaces of immunologic cells and induces inhibited T lymphocytes into immunologically competent cells. According to clinical experiments, levamisole has therapeutic effectiveness and boosts immunity in a variety of infectious diseases and some cancers. This review helps in further research to search for a new immunosynergestic molecule by taking levamisole as a model immunostimulating drug.

**REFERENCES**

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