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Review

Possible Biological and Clinical Applications of Phenothiazines

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Abstract. *Phenothiazines have been used in many areas of medicine, mainly in psychopharmacology. These compounds are able to effectively inhibit dopamine, histamine, serotonin, acetylcholine, and α-adrenergic receptors; thus, their effect and side-effect profiles are extremely diverse. Besides their antipsychotic activity, phenothiazines have a significant antimicrobial effect as well, since they can enhance the bactericidal function of macrophages and inhibit efflux pumps. They are also able to eliminate bacterial resistance plasmids and destroy bacteria by their membrane-destabilizing effect. Their antiviral, antiprotozoal, antifungal, and antiprion activities have also been described. Phenothiazines have also been proven to destroy cancer cells and sensitize them to chemotherapy. Anti-angiogenesis and anticancer stem cell activities have also been reported, and they might be applied as adjuvants in the treatment of infections and tumors in the future. Finally, phenothiazines can also be effective in the treatment of neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease.*

Phenothiazines are unanimously one of the most versatile compounds from the view of biological activity. Since their discovery, new exploitable pharmacological properties have emerged from time to time; thus, they have an important role in many areas of medicine and beyond. This is why phenothiazines are basic compounds in pharmacology. The history of these compounds goes back to the second half of the 19th century, when a German chemist, Heinrich August Bernthsen began to study the structure of methylene blue, which was first synthesized in 1876 by Heinrich Caro. In 1885, two years after Bernthsen first managed to produce phenothiazine, he also succeeded in describing the structure of methylene blue (1). At the same time, Paul Ehrlich began to investigate the possible therapeutic use of methylene blue in malaria infections, which he first published in 1891 (2). Due to this, methylene blue was often prescribed for patients with malaria in the subsequent period (3).

Research conducted in the 1930s and '40s proved the potential wide use of phenothiazines. The insecticidal (4), antihelmintic (5, 6), and antibacterial (7) effects of the compound were detected; however, it was not widely used for these purposes. In the 1940s, another novel phenothiazine derivative, namely promethazine was brought to the forefront of attention; it was investigated by Paul Charpentier at the Rhone-Poulenc laboratory in Paris (8). It was shown that promethazine had an antihistamine effect (9), which was later used in the therapy of allergic diseases and in anesthesia (10, 11). A few years later in the same laboratory, chlorpromazine was discovered, which has strong anxiolytic and antipsychotic effect along with its antihistaminic effect (12). Psychiatry fully exploited this feature in psychotic patients (13), which resulted in the almost complete emptying of psychiatric hospitals in the 1950s. As a result, the discovery of chlorpromazine and the beginning of its use as an antipsychotic are considered the beginning of modern psychiatry and psychopharmacology.

Since then, several antipsychotic phenothiazine compounds have been used in clinical practice, although research conducted in the last couple of decades indicated that phenothiazine compounds could have an important role in other fields of medicine as well, such as in the treatment of tumorous, infectious, or neurodegenerative diseases (3).

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Principal Compounds with Phenothiazine Skeleton and Their Structure

Phenothiazine. As has been described above, methylene blue was the first compound synthesized with a phenothiazine skeleton. Only afterwards was phenothiazine synthesized by heating diphenylamine and sulfur by Bernthsen. Currently, the reaction is mostly performed by the cyclization of 2-substituted diphenyl sulfides (14) (Figure 1).

In general, compounds derived from the phenothiazine skeleton are characteristic of an alkyl side chain attached to a nitrogen in position 10, which is responsible for its specific properties, whereas the substituent on the carbon atom at position 2 has an impact on its efficacy. On carbon atom 2, mainly highly electronegative lipophilic substituents, such as halogens, give the highest efficacy, especially the trifluoromethyl group. The action profile of the compound is particularly influenced by the length of the alkyl side chain; thus, for example, a three-carbon atom alkyl chain should be present between the nitrogen at position 10 and the terminal nitrogen of the phenothiazine skeleton in order to achieve an antipsychotic effect, whilst in case of a carbon atom at position 2, there is an antihistaminergic activity rather than an antipsychotic effect (15). Efficacy increases if the terminal amino group is substituted with piperazine, and also if the hydrophobicity of the substituents is higher, due to their penetration across the blood–brain barrier.

Methylene blue. Methylene blue has the greatest chain-breaking antioxidant activity of all the phenothiazine compounds, which is due to the fact that a hydrogen atom can easily leave the nitrogen atom in the reduced methylene blue (leucomethylene blue), and the resulting free radical of leucomethylene blue forms a particularly stable compound with mesomerism, which is methylene blue itself. Furthermore, the leucomethylene blue free radical can also act as an electron donor (Figure 2). Due to these characteristics, the compound has a remarkable antioxidant activity, which is utilized in many areas (3). Moreover, it has an inhibitory effect on nitric oxide synthase and guanylate cyclase (16).

Typical antipsychotics can be structurally grouped according to the structure of the side chain connected to the atom at position 10 (Table I). According to this, aliphatic, piperidine and piperazine type compounds can be distinguished (promethazine is not an antipsychotic, but it also has an aliphatic side chain).

Promethazine. Among phenothiazines, the structure of promethazine is most similar to that of histamine; thus, its histamine antagonistic effect can be derived from it as it inhibits histamine H1 receptors, and it also has an anticholinergic and a mild anti-dopaminergic activity (Figure 3) (17).

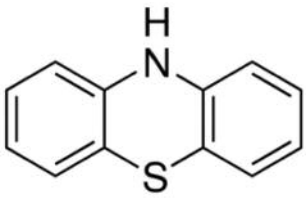

Figure 1. *The structure of phenothiazine.*

Chlorpromazine. The structure of chlorpromazine is most similar to that of dopamine; therefore, it is able to effectively inhibit dopamine receptors, and thus exert antipsychotic effects (18). Nevertheless, it has anti-serotoninergic, anti-muscarinic, antihistaminergic and α-adrenergic receptor inhibitory activity as well (Figure 4). *N*-Methyl-D-aspartate (NMDA) receptor inhibitory effects have also been described at high concentrations of chlorpromazine (19).

Levomepromazine. Levomepromazine is similar to chlorpromazine in its structure and pharmacodynamics, but considering its antipsychotic effect, levomepromazine is approximately half as potent as chlorpromazine; nonetheless, it causes fewer extrapyramidal side-effects (EPS) (Figure 4). It has the most powerful sedative effect of all antipsychotics, and it is widely used in palliative care for its powerful analgesic, sedative and anti-emetic effects (20).

Thioridazine. The pharmacodynamics of thioridazine is similar to that of chlorpromazine; however, its adverse effect profile is different since thioridazine causes fewer EPS, and instead, anticholinergic symptoms are predominant (Figure 4).

Due to the anti-bacterial and antitumor effects of thioridazine, it is still a focus of research.

Trifluoperazine. Trifluoperazine has higher affinity towards dopamine receptors and lower towards serotonin and adrenergic receptors; thus, it is generally better tolerated by patients, but it causes more EPS in the long term (Figure 4). In clinical practice, it is mainly used in the therapy of schizophrenia and in the treatment of anxious and agitated patients. Similarly to thioridazine, it is frequently applied for research purposes (20).

Pharmacodynamics

Phenothiazine derivatives are often referred to as 'dirty drugs' because they act on so many receptors. The pharmacodynamic effects of phenothiazine derivatives that are used in clinical practice, particularly for their antipsychotic effect, are detailed in the following.

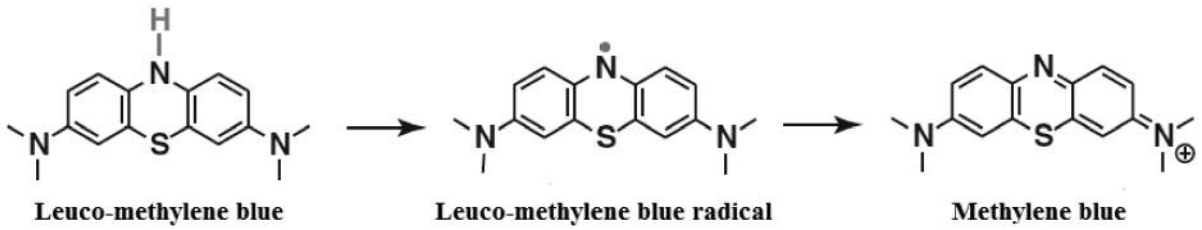

Figure 2. *The structure and the redox cycle of methylene blue.*

Table I. *Phenothiazine groups.*

Derivatives with aliphatic side chains	Chlorpromazine, promazine, triflupromazine, levomepromazine, (promethazine)
Piperidine derivatives	Thioridazine, mesoridazine
Piperazine derivatives	Fluphenazine, perphenazine, prochlorperazine, trifluoperazine

Antidopaminergic effect. The most important feature of phenothiazine derivatives with antipsychotic activity is that they non-selectively inhibit all the five types of dopamine receptors. Their most important effect is their D2 (and D3) receptor-blocking activity, since it is correlated with the antipsychotic effect of the molecule (21). The highest density of D2 receptors in the brain is found in the striatum, *nucleus accumbens*, ventral tegmental area, and *substantia nigra*, and the therapeutic effect is due to the inhibition of D2 receptors in neurons originating from the ventral tegmental area, and projecting to the limbic system (mesolimbic dopaminergic pathway). As a result, phenothiazine-based antipsychotics can effectively reduce the positive symptoms of schizophrenia (hallucinations, delusions, and disorganized speech and behavior). Furthermore, dopamine receptor blockade of the medullary chemoreceptive trigger zone also results in an antiemetic effect. However, the adverse effects of the drug are also due to inhibition of D2 receptors. The inhibition of the dopaminergic nigro-striatal system leads to a wide variety of EPS, such as akathisia, tardive dyskinesia, parkinsonism, acute dystonic reactions, and other drug-induced dyskinesias (tic, chorea, or athetosis). The anti-dopaminergic effect on the tuberoinfundibular system causes hyperprolactinemia as a result of the termination of tonic inhibition of lactotrophic cells by dopamine. The D2 receptor blockade is proven to be in the background of neuroleptic malignant syndrome (hyperthermia, muscle rigidity, and autonomic dysregulation), which is a potentially fatal adverse effect (22).

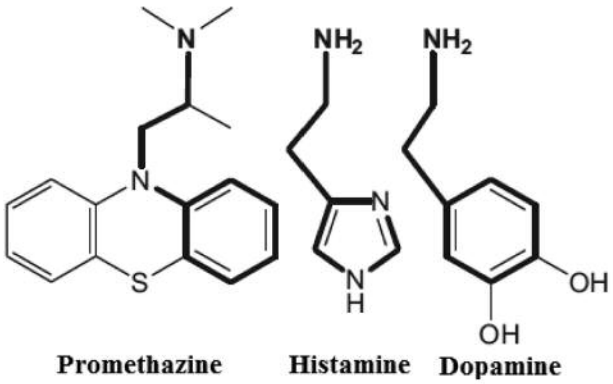

Figure 3. *Structural similarities between promethazine, histamine and dopamine, which explain the pharmacological properties of promethazine and other phenothiazine derivatives.*

Antihistaminergic effect. All phenothiazine derivatives used in clinical practice have an antihistamine effect, among which promethazine has the most significant one. It mainly involves inhibition of H1 receptors, and plays a role in the alleviation of allergic symptoms (17). In the nervous system, H1 receptors are found mainly in the tuberomammillary nucleus. This area is responsible for multiple tasks, among which regulation of the sleep–wake cycle, appetite and body temperature are the most essential. The inhibition of the histaminergic neurons in this nucleus is responsible for the sedative effects of antihistamines and antipsychotics, and for an increased appetite, moreover subsequent hypothermia or hyperthermia may also develop through the inhibition of body temperature regulation (23).

Antiserotonergic effect. Phenothiazine antipsychotics have high affinity for serotonin (5-hydroxytryptamine, 5-HT) receptors,

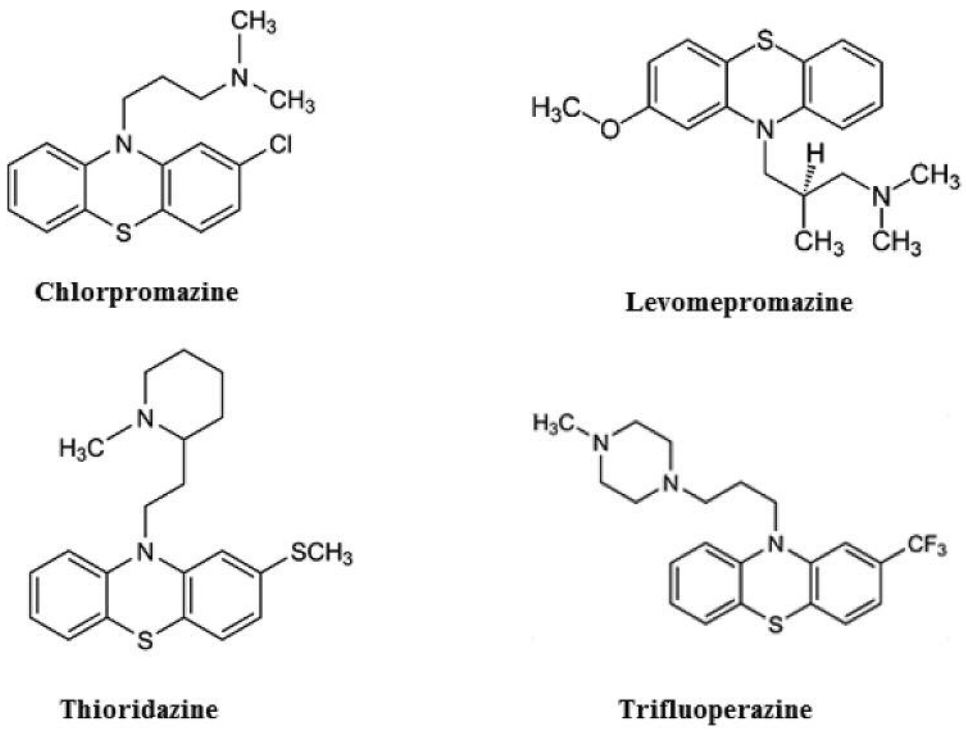

Figure 4. *The structure of chlorpromazine, levomepromazine, thioridazine and trifluoperazine.*

the most important of which is the inhibition of 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors in achieving the antipsychotic effect (24); however, it is crucial mainly in the case of atypical antipsychotics. For phenothiazine antipsychotics, the inhibition of serotonin receptors results in anxiolytic and antidepressant activity, it reduces aggression and the risk of developing EPS, but leads to weight gain, changes in blood pressure, and the development of ejaculatory disorders (25).

Alpha-adrenergic receptor-blocking effect. Phenothiazine-type antipsychotics can inhibit both α1 and α2 adrenergic receptors. The inhibition of α1 receptors is mainly responsible for the sympatholytic side-effects such as hypotension, orthostatic hypotension, reflex tachycardia, dizziness, myosis, and sexual dysfunction. The inhibition of α2 receptors may have an antidepressant effect (25).

Anticholinergic effect. Phenothiazine antipsychotics have special affinity for M1 and M2 muscarinic acetylcholine receptors. The inhibition of M1 receptors can be related to the blocking activity of the autonomic ganglia, which results in autonomic symptoms, such as dry mouth, constipation, urinary retention, dry and warm skin, nausea, mydriasis, closed-angle glaucoma, nasal congestion, priapism, and orthostatic hypotension. Phenothiazines can effectively reduce sea sickness because of their central antimuscarinic effect (22).

Antiglutaminergic effect. It has recently been reported that at low concentration, phenothiazine derivatives stimulate glutaminergic transmission on NMDA receptors, and at high concentration, they inhibit it (19).

Antimicrobial Effect

Antibacterial effect. The antimicrobial effect of phenothiazines has been the subject of numerous studies to date, which supports the idea that phenothiazine therapy can be an effective option in the treatment of certain bacterial infections. Such an effect of phenothiazine derivatives can manifest as a direct antibacterial effect and also as an effect reducing or inhibiting antibiotic resistance, in the background of which several possible mechanisms have already been described. Among these mechanisms, one of the most important is the inhibition of bacterial multidrug resistance (MDR) efflux pumps. Since bacterial MDR proteins prevent antibiotics from achieving an appropriate bactericidal concentration in the cytoplasm, they are largely responsible

for the development of resistance to antibiotics, and by inhibiting these pumps, bacteria can be re-sensitized to the corresponding antibiotics. Phenothiazines inhibit the binding of calcium to calcium-dependent proteins, such as calmodulin, and they can inhibit MDR efflux pumps using energy derived either from ATP hydrolysis or proton motive force. As a result, MDR pumps are inhibited, and the antibiotic drug is able to accumulate in the cytoplasm of the bacterium (26). Besides the efflux pump inhibitory effect, phenothiazine can also inhibit bacterial replication as it inhibits DNA-based processes and replication by intercalating DNA bases. It may be potentially of major importance in case of infections caused by multi-resistant bacteria, such as multidrug resistant *Mycobacterium tuberculosis*, methicillin resistant *Staphylococcus aureus*, and other infections caused by Gram-positive pathogens, but it also appears to be essential during therapy of infections caused by some Gram-negative rods, such as *Shigella, Salmonella*, and *Escherichia coli* (26-28). The antibacterial effect of phenothiazines, such as chlorpromazine and thioridazine, was achieved at *in vitro* concentrations (25 μg/ml) highly exceeding the clinically relevant plasma concentration (0.5 μg/ml); however, it has been shown that phenothiazines are able to reach up to 100-fold concentration in macrophages and cause destruction of phagocytosed bacteria. Thus, a therapeutic effect can be achieved at a plasma concentration which is lower than that used in antipsychotic therapy (29).

Another important feature of phenothiazines is their plasmid-eliminating effect. The research about this effect has been mainly on promethazine, which clearly demonstrated its ability to inhibit the replication of bacterial resistance plasmid (R-factor), thereby reducing or even eliminating resistance to antibiotics. Inhibition of the replication of the R-factor, *i.e.* the polyresistance factor, occurs because promethazine binds to and complexes with the guanine- and cytosine-rich regions of plasmid DNA (30), thus inhibiting replication. However, inhibition of plasmid conjugation by preventing formation of sex pili is also a possible mechanism (31). Moreover, inhibition of other plasmid-related functions was also observed in the presence of promethazine, which is due to the fact that it is also able to inhibit plasmids with other functions, such as the virulence plasmid, in addition to the R-plasmid. Taking advantage of this feature, a synergistic effect has been described while using promethazine in combination with gentamicin for the treatment of recurrent urogenital infections caused by *E. coli*, and the therapy was also successful in cases with gentamicin-resistant pathogen (32). Besides the anti-plasmid mechanism, the inhibition of bacterial adhesion to the urothelium may also be responsible for the above-described findings. Furthermore, antibacterial activity of phenothiazines can be related to their membrane-destabilizing effect, since it has been observed that phenothiazine compounds increase the membrane permeability of certain bacteria, and as a result, it is easier for antibiotics to penetrate through the membrane (33). Nevertheless, as a result of the disturbed ion gradient, several intracellular processes may be disturbed, and the motility of the bacteria may also be inhibited (34).

Antiprotozoal effect. Ehrlich described the antimalarial effect of methylene blue as early as in the 1910s; however, due to the spread of chloroquine-resistant *Plasmodium* strains, phenothiazines may regain their important role in the therapy of patients with malaria. Several studies have already confirmed the antimalarial effect of chlorpromazine both *in vitro* and *in vivo*; direct bactericidal effect and ability to inhibit the resistance to chloroquine have also been described (35). Most recently, it was detected that chlorpromazine is capable of effectively destroying *Naegleria fowleri* both *in vitro* and *in vivo*. The prognosis of patients with meningoencephalitis caused by *N. fowleri* is very poor, with a mortality of 95% even with the administration of adequate therapy. At present, amphotericin B is considered to be an adequate therapy for naegleriasis; however, it has been revealed that the survival rates were 35% higher in mice infected with *Naegleria* and then treated with chlorpromazine than in the those treated with amphotericin B (36).

Antiviral effect. There have also been many achievements regarding the antiviral effect of phenothiazines. Mainly the antiviral effect of chlorpromazine has been studied so far, in the background of which there might be several mechanisms, for example, inhibiting the binding of the virus to its receptor on the plasma membrane, blocking endocytosis of the virus by inhibiting the required calcium-dependent processes, and inhibiting the DNA replication by intercalating DNA bases (27). Most of the published studies were on the antiviral activity of chlorpromazine *in vitro*. These articles reported that chlorpromazine is able to inhibit the replication of the genetic material in hepatitis B virus (37), SV40 (38), and arenavirus (39); viral budding in measles (40), Sindbis and vesicular stomatitis virus (41), and infection with HIV (42), human herpes virus (43), and John Cunningham viruses (44). Although these effects were achieved at clinically-irrelevant concentrations, the observation that phenothiazines have a potential antiviral effect provided the idea for developing several phenothiazine derivatives that are able to exert antiviral effect at lower plasma concentrations.

Antifungal effect. It has already been reported that phenothiazine compounds are able to inhibit the growth of certain fungi (*Candida* species, *Cryptococcus neoformans*) (45). Besides antifungal activity, synergistic effects have been described when administered in combination with certain antifungal agents, such as amphotericin B or ketoconazole (46). The mechanism behind the fungicidal

effect of phenothiazines is not yet fully understood, but is probably due to the membrane-destabilizing effect and the inhibition of calcium–calmodulin system, which have also been observed in bacteria (47). In recent years, an antifungal effect against filamentous fungi has also been reported, namely in case of *Aspergillus, Scedosporium*, and *Zygomycetes* species (48). In the therapy of *Zygomycetes*-induced rhinocerebral zygomycosis, the synergistic effect of trifluoperazine co-administered with amphotericin B seems to be promising (49).

Anti-prion activity. Until now no potent medication which could prolong patient survival to some extent has been found against the protein-based infectious agent of Creutzfeld–Jakob disease, Gerstmann–Sträussler–Scheinker syndrome, and Kuru. However, it has been proven in infected mouse neuroblastoma cell cultures that chlorpromazine is able to inhibit the formation of aberrant prion proteins together with another heterocyclic compound, which is quinacrine (50). The anti-prion effect of these two compounds observed in cell cultures has not been evident *in vivo*, which may be due to the fact that the drug was unable to reach appropriate concentrations in the brain (51). Further studies and research are needed to fully exploit the potentials of these two compounds.

Antitumor Effect

Due to their wide biological activity, phenothiazines also have an important role in cancer research. They can exert antitumor activity through several mechanisms, the most important of which are the processes inducing apoptosis, such as the inhibition of DNA-repair mechanisms and signal transduction pathways, but their direct DNA-damaging and membrane-destabilizing effects are also outstanding. It is also important to emphasize the ability of phenothiazines in inhibiting MDR tumor resistance, and their anti-angiogenesis effect; however, they are also able to exert antitumor activity through several further processes.

Apoptosis-inducing effect. The importance of inhibiting DNA repair mechanisms lies in the fact that if DNA damage remains intact at transition points of the cell cycle, the appropriate checkpoint proteins, such as p53, initiate the internal death signal, thereby inducing apoptosis. DNA-dependent protein kinase, which is a serine/threonine kinase, plays an important role in repairing damage to the DNA chain. Phenothiazines have been shown to be able to inhibit the DNA-dependent protein kinase *in vitro* (52), as a result of which the effectivity of DNA repair is lower; however, they are also purported to interfere with further DNA-repair processes (53). This assumption is based on the observation that phenothiazines reduce endonuclease activity in human fibroblast cells, which plays an important role in the DNA-repair mechanisms (54). However, phenothiazines can exert an effect on checkpoint proteins not only directly but indirectly as well. Translationally controlled tumor protein (TCTP) is an important regulatory protein in the cell cycle; it stimulates ubiquitination, and thus the degradation of p53 protein. However, it has been shown that thioridazine is able to inhibit TCTP and increase the expression of p53 protein, and thus its activity in inducing apoptosis (55). It may have practical importance, for example, in case of tumors with high TCTP expression (56). Furthermore, thioridazine is able to block the so-called Tousled-like kinases, which are responsible for the chromatin rearrangement in the S-phase of the cell cycle, and the inhibition of which consequently leads to genomic instability and apoptosis (57). Similar processes may exist in the background of the observations that thioridazine reduces the expression of cyclin D1 and cyclin-dependent kinase 4 (CDK4), and increases the expression of CDK inhibitor proteins, such as p16 and p27. Cyclin D1 and CDK4 play an important role in the G_1/S transition, while p16 and p27 inhibit G_1/S and G_2/M transitions. It has been shown that thioridazine reduces the expression of various anti-apoptotic and antiproliferative proteins, such as B-cell lymphoma-2 (BCL2), survivin, and cellular myelocytomatosis oncogene (c-MYC) (58). It was mentioned earlier that phenothiazines inhibit the binding of calcium to many calcium-dependent enzymes, such as calmodulin. As a result, many calcium-mediated signal transduction pathways can be disturbed, which may result in apoptosis and the inhibition of proliferation (59). The blockade of the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT) pathway has the same consequence, and it can be effectively inhibited by thioridazine; the resulting cytotoxic effect has been demonstrated in cervical and endometrial tumor cells (60). It has also been shown that tumor cells having a mutation in their B-type rapidly accelerated fibrosarcoma (*BRAF*) gene at codon 600 exhibit increased sensitive to the antiproliferative effect of phenothiazines, and this phenomenon has been confirmed in melanoma cells *in vitro* (61). Furthermore, it is also likely that these apoptosis-inducing processes could be even more effective if supplemented by radiotherapy (62).

Some recent studies have also confirmed that the use of phenothiazines leads to apoptosis and cell death. Some examples of these are the following: the apoptosis-inducing effect of phenothiazines described above has been confirmed by different studies on lymphatic and leukemia cells. In these experiments, thioridazine was the main focus of interest; however, trifluoperazine and chlorpromazine presented similar antitumor activity at clinically relevant concentrations. It is important to note that the induction of apoptosis in leukemia cells was achieved while healthy

lymphocytes remained intact; therefore phenothiazines also show some degree of selective toxicity (63). Similar antitumor effects have been demonstrated in MDR melanoma cells *in vitro*, as well as *in vivo* after injecting these cells into mice. In this analysis, the apoptosis-inhibiting effect of thioridazine was clearly observed *in vitro*; while *in vivo*, studying the number of tumor foci in the mouse lungs and their survival rates, thioridazine was shown to be effective against this tumor type (64). During *in vitro* experiments, in which MDR mouse T-lymphoma cells were treated with thioridazine, along with the apoptosis-inducing effect, the agent was also effective in the inhibition of P-glycoprotein (ABCB1 protein), which is the most studied MDR ATP-binding cassette transporter of tumor cells (65). It has been described that a newly synthesized 2,7-diazaphenothiazine derivative, 10-[4-(4-methylpiperazin-1-yl)but-2-ynyl]-2,7-diazaphenothiazine, exerted pronounced anticancer activity against T47D human ductal breast epithelial tumor cells. Furthermore, against T47D cells this 2,7-diazaphenothiazine derivative had stronger anticancer properties than cisplatin. According to the analysis of the *BLC2*/BCL2-associated X protein (*BAX*) gene-expression ratio in T47D cells mitochondrial apoptosis was induced after 24 hours of treatment in the presence of the derivative (66). In addition, photodynamic therapy is a method for inducing tissue damage with light irradiation of a drug selectively retained in malignant tissue, for example in case of the photosensitizing drug methylene blue (67).

Inhibition of efflux pumps. Efflux pumps play an important role in the chemotherapy resistance of cancer cells as well as in the antibiotic resistance mechanisms of bacteria. There are three main types of MDR proteins in humans, out of which the most important role is played by ABCB1, previously called MDR1 or P-glycoprotein, and ABCC1 and ABCC2 from the ABCC family, also known as MRP1 and MRP2 (68). Overexpression of these proteins may be observed in chemotherapy-resistant tumor cells, and as in case of bacteria, phenothiazines also have a beneficial effect as efflux pump inhibitors. It has also been confirmed by various experiments where, for example, thioridazine made resistant sarcoma sensitive to doxorubicin again by the inhibition of P-glycoprotein (69). Multidrug-resistant KBV20C human oral squamous carcinoma can be adapted to eribulin at 500-fold higher than that used to treat their drug sensitive parental counterparts indicating that eribulin may be effectively pumped out by ABCB1. Thioridazine is able to sensitize eribulin-resistant KBV20C cells, moreover, the combined effect of eribulin and thioridazine resulted in G_2 arrest (70). In another study, ABCB1 of MDR mouse T-lymphoma cells was also inhibited by thioridazine (71).

These observations raise the possibility of using resistance-modifying compounds in the future as adjuvants to chemotherapy, for example, efflux pump inhibiting phenothiazines. The dose of chemotherapeutic agents could be reduced as well as their adverse effects, whereas the efficacy of the therapy could be increased since the chemotherapeutic agents themselves also induce the excessive expression of MDR efflux pumps of tumor cells. We should bear in mind, though, that several further investigations are required before these compounds can be used in clinical practice as MDR proteins are not only found in tumor cells, but also play an essential role in the normal functioning of the blood–brain barrier, the kidneys, and the liver. The inhibition of these physiological proteins involves serious potential side-effects, especially if administered in combination with chemotherapeutics. These potentially adverse effects could be eliminated by using tumor cell-specific efflux pump inhibitors; however, this option is still to be investigated (62).

Inhibition of angiogenesis. Among phenothiazines, thioridazine can also block the angiogenesis-stimulating effect of tumors. Tumors need increased blood supply for their intense metabolism, which they achieve by secreting vascular endothelial growth factor (VEGF) and similar substances. These substances exert an effect on the endothelial cells, which results in small vessels entering the tumor. The inhibition of these substances and the associated signal pathways may play an important role in the inhibition of tumor growth, especially in the more vascularized tumors. *In vivo* experiments in mice have shown that the expression of VEGF and hypoxia-inducible factor 1α (HIF1α) as well as phosphorylation of VEGF receptor 2 are decreased in ovarian tumors. Antibodies to VEGF have been shown to be effective in the treatment of certain tumors; thus, it is suspected that this property of phenothiazine derivatives may be utilized during the therapy of the disease in the future (58).

Anticancer stem cell activity. Further studies with thioridazine have also revealed that this compound is able to selectively inhibit cancer stem cells (CSC) without inhibiting somatic stem cells. A possible mechanism behind the anti-CSC effect could be that thioridazine antagonizes dopamine receptors that are expressed on CSCs (72). Similar observations have been made in the case of glioblastoma stem cells, since thioridazine is able to induce autophagy and selectively destroy glioblastoma stem cells, and thus the glioblastoma itself. This activity is exerted without affecting healthy neurons and glial cells (73). It has also been shown that thioridazine can suppress the proliferation and invasion of colon CSCs and thioridazine can interfere with mitochondrial membrane potential and can up-regulate the relative expression of proapoptotic genes *BAX* and *caspase-3* (74).

Potential clinical use in oncology. In the light of the above results, it can be concluded that phenothiazines and similar compounds are likely to play an important role in the treatment of oncology patients. Currently, the most frequently used chemotherapeutic agents in tumor therapy are not sufficiently selective; therefore, they not only damage cancer cells, but also harm other healthy tissues and cells, resulting in various side-effects. Serious efforts have been made for a long time to develop even more selective therapeutic tools to fight cancer. However, this selectivity can be achieved by adding adjuvant compounds which sensitize cancer cells to currently used chemotherapeutics and radiotherapy. Thus, current therapies can be more effective and selective, and the side-effects of these chemotherapeutics can be eliminated, since using a suitable adjuvant could reduce their dosage while achieving the same effect. In addition, other pharmacological effects of phenothiazines could also be used in the treatment of patients with cancer. Currently, the most uncomfortable side-effects of chemotherapeutics are nausea and vomiting, which might be alleviated effectively by use of phenothiazines. Phenothiazines may also be useful in the palliative therapy of patients with cancer, for example, as analgesics, and they may also play a role in the psychological treatment of these patients (62).

Potential Role in the Treatment of Neurodegenerative Diseases

As early as in 1992, research was conducted in mouse models to reduce post-ischemic brain damage with phenothiazine pre-treatment. This observation has been attributed to the redox properties of phenothiazine, since it has been shown to inhibit lipid peroxidation and to have cytoprotective effects on neurons. Research was started in this context in the 2000s, but as a result of the theories on the pathomechanism of neurodegeneration. It was observed that oxidative stress caused by reactive oxygen species played an important role in neurodegeneration. Since phenothiazine and methylene blue are among the most potent antioxidant compounds, it is evident that they may have role in the treatment of neurodegenerative processes. For example, phenothiazine and methylene blue have been observed to be able to prevent dopaminergic neuro-degeneration in *Caenorhabditis elegans* models of Parkinson's disease (75). There are ongoing studies related to the possible treatment of Alzheimer's disease with phenothiazines, especially with methylene blue. Several theories have been made to explain the pathomechanism of Alzheimer's disease, according to which the main triggers are abnormal aggregation and plaque-like deposition of β-amyloid proteins, leading to oxidative stress and abnormal phosphorylation and intracellular aggregation of tau proteins, which make up the so-called neurofibrillary bundles. Methylene blue is able to intervene in these pathological processes at several points. Along with the effect of reducing oxidative stress, methylene blue has also been shown to effectively inhibit pathological phosphorylation and aggregation of tau proteins in mouse models as well as the up-regulation of protein degradation systems (proteasome and autophagy). However, this agent was effective only during prophylactic treatment, and did not exert its effect in cases that had already developed (76). Furthermore, it is also suspected that methylene blue can interfere with β-amyloid misfolding and consequent extracellular aggregation, which reflects the aforementioned anti-prion activity of the agent as β-amyloid behaves similarly to prion proteins. Methylene blue has also been proven *in vitro* to reduce oligomerization of β-amyloids and to stimulate their fibrillization, resulting in a less toxic structure (77). Methylene blue is an antioxidant, since the molecule is able to bind reactive oxygen species and to stabilize changes in the proteins of the mitochondrial respiratory chain by redox cycling between complexes I and IV (3).

Concluding Remarks

The diverse biological and pharmacological properties of phenothiazines have been known since a long time, and they are widely exploited especially in the field of psychiatry. However, recent research has shown that these compounds may have an important role in many other areas of medicine in the future.

The spread of antibiotic resistance is increasingly challenging in the treatment of infectious diseases. Phenothiazines, either alone or in combination with antibiotics, may be promising alternatives to overcoming antibiotic resistance, since they are able to interfere with the resistance mechanisms and other cellular functions at several points.

It has also been proven that phenothiazines are able to eliminate cancer cells and sensitize them for chemotherapy by multiple mechanisms; thus, it is believed that these compounds will also be used as adjuvants in oncology in the near future.

Finally, there is a promising perspective in the use of phenothiazines during the treatment of neurodegenerative diseases which currently have poor prognosis, such as Alzheimer's disease and Parkinson's disease.

To sum up, a new Renaissance may soon arrive for this overlooked group of compounds according to recent scientific research.

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